BMC Pregnancy and Childbirth

RESEARCH

Open Access

Proteomic analysis of plasma total exosomes and placenta-derived exosomes in patients with gestational diabetes mellitus in the first and second trimesters

Jing Lin[1†], Danqing Zhao[2†], Yi Liang[3], Zhiyuan Liang[1], Mingxian Wang[1], Xiaoxiao Tang[1], Hongbin Zhuang[1], Hanghang Wang[1], Xiaoping Yin[2], Yuhan Huang[1], Li Yin[2] and Liming Shen[1,4*]

Abstract

Gestational diabetes mellitus (GDM) is the first spontaneous hyperglycemia during pregnancy. Early diagnosis and intervention are important for the management of the disease. This study compared and analyzed the proteins of total plasma exosomes (T-EXO) and placental-derived exosomes (PLAP-EXO) in pregnant women who subsequently developed GDM (12–16 weeks), GDM patients (24–28 weeks) and their corresponding controls to investigate the pathogenesis and biomarkers of GDM associated with exosomes. The exosomal proteins were extracted and studied by proteomics approach, then bioinformatics analysis was applied to the differentially expressed proteins (DEPs) between the groups. At 12–16 and 24–28 weeks of gestation, 36 and 21 DEPs were identified in T-EXO, while 34 and 20 DEPs were identified in PLAP-EXO between GDM and controls, respectively. These proteins are mainly involved in complement pathways, immunity, inflammation, coagulation and other pathways, most of them have been previously reported as blood or exosomal proteins associated with GDM. The findings suggest that the development of GDM is a progressive process and that early changes promote the development of the disease. Maternal and placental factors play a key role in the pathogenesis of GDM. These proteins especially Hub proteins have the potential to become predictive and diagnostic biomarkers for GDM.

Keywords Biomarker, Exosome, Gestational diabetes mellitus, Mechanism, Proteomics

†Jing Lin and Danqing Zhao contributed equally to this study.

*Correspondence:
Liming Shen
slm@szu.edu.cn
[1]College of Life Science and Oceanography, Shenzhen University, Shenzhen 518071, P. R. China
[2]Department of Obstetrics and Gynecology, Affiliated Hospital of Guizhou Medical University, Guiyang 550004, P. R. China
[3]Department of Clinical Nutrition, Affiliated Hospital of Guizhou Medical University, Guiyang, P.R. China
[4]Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen 518055, P. R. China

Introduction

Gestational diabetes mellitus (GDM) is a common pregnancy complication that refers to spontaneous hyperglycemia during pregnancy [1–3]. GDM affects approximately 14% of pregnancies worldwide [1, 4], and has become an important public health problem and one of the hotspots in obstetrics research today [5, 6]. However, so far, the etiology and pathophysiology of GDM have not been fully clarified [7]. Major risk factors associated with GDM include advanced maternal age, obesity, and excessive weight gain during pregnancy [2, 8]. Metabolic changes in early pregnancy are characterized by insulin sensitivity, which gradually transitions to insulin resistance in the second and later trimesters [9–11].

GDM poses risks to the mother, fetus and newborn, resulting in the risks of short-term and long-term diseases, such as macrosomia, dystocia, obesity, type II diabetes and cardiovascular disease [12–18]. On the other hand, studies have reported that a combination of diet and exercise interventions during pregnancy may reduce the risk of GDM [19]. Consequently, it is particularly important to carry out early diagnosis and intervention for GDM patients [2, 20, 21]. However, the most appropriate diagnostic strategy and treatment for GDM remain controversial [7]. The diagnostic method is mainly based on the 75 g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation [2]. This method is cost-effective only when GDM patients receive postpartum counseling and care to prevent type II diabetes [22].

Exosomes are small membranous vesicles released by the fusion of multivesicular endosomes with the plasma membrane [23–27]. It is secreted by a variety of cells and carry contents such as proteins and RNA, reflecting the function and body state of the secreting cells. It is found in various body fluids and plays a role in the transport of substances and the exchange of information [23, 24, 28, 29], and is closely linked to the reproductive process [28]. Interestingly, studies have shown that the concentration of exosomes in the plasma of pregnant women is more than 50 times that of non-pregnant women, and the number increases significantly more than twofold with gestational age [30, 31]. At 6 weeks of gestation, the placenta releases exosomes into the maternal circulation [30]. Placenta-derived exosomes have a syncytiotrophoblast-specific protein, placental alkaline phosphatase (PLAP), and PLAP-positive exosomes only exist in the circulation of pregnant women [32]. Exosomes can carry the information of the placenta and circulate in the mother's body with the blood flow, play a variety of functions, and play an important role in the pathogenesis of GDM [30, 33]. Further studies confirmed that exosomes present in the plasma of pregnant women with GDM are biologically active and can modulate pro-inflammatory cytokines released by endothelial cells [30]. Therefore, the study of exosomes may provide new knowledge for deciphering the pathophysiology of GDM in mothers and infants, and provide valuable biomarkers for disease prediction and monitoring [34].

Several studies have investigated the exosomal miRNA profiles of GDM patients [34–36], however, there are limited studies on proteomics [37]. The longitudinal proteomic studies of GDM blood exosomes in early and mid-pregnancy have not been reported. Isobaric labeling for relative and absolute quantification (iTRAQ) is an efficient proteomic method and has been used in many studies [38, 39], including the studies of serum proteomics in GDM patients [40, 41]. In this study, the total plasma exosomes (T-EXO) and placental derived exosomes (PLAP-EXO) from GDM patients and healthy controls at 12–16 weeks and 24–28 weeks of gestation were studied by iTRAQ quantitative proteomics.

Materials and methods

Plasma sample selection and collection

The study flow diagram is shown in Fig. 1. In this prospective cohort study, pregnant women who presented for the first time in 12–16 weeks of gestation at the Affiliated Hospital of Guizhou Medical University (Guiyang, China) were recruited, and plasma samples were collected and stored [42]. All subjects underwent a 75 g OGTT at 24–28 weeks of gestation. Diagnosis of GDM according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) standard [43]. Plasma samples from GDM patients and controls were collected at 24–28 weeks again. There were no significant differences between GDM and controls in maternal age, pregnancy, parity, body mass index (BMI), gestational age at delivery, gestational age at sample collection, and gestational age at 75 g OGTT. Fasting blood glucose and glucose concentrations at 1 and 2 h OGTT were significantly higher in the GDM group than in the control group (Supplementary Table 1). The study protocol was approved by the Human Research Ethics Committee of the Affiliated Hospital of Guizhou Medical University. Informed consent was obtained from all participants.

Plasma samples from pregnant women who subsequently developed GDM (12–16 weeks, early stage), pregnant women with GDM (24–28 weeks, middle stage), and their corresponding healthy pregnant women were analyzed and divided into four groups: pregnant women who subsequently developed GDM (12–16 weeks, GDM-E, $n=16$) and healthy controls (12–16 weeks, CON-E, $n=12$); pregnant women with GDM (24–28 weeks, GDM-M, $n=16$) and healthy controls (24–28 weeks, CON-M, $n=12$). Equal amounts of plasma from 4 individuals in the same group were mixed to form an experimental sample. Thus, GDM-E and GDM-M groups

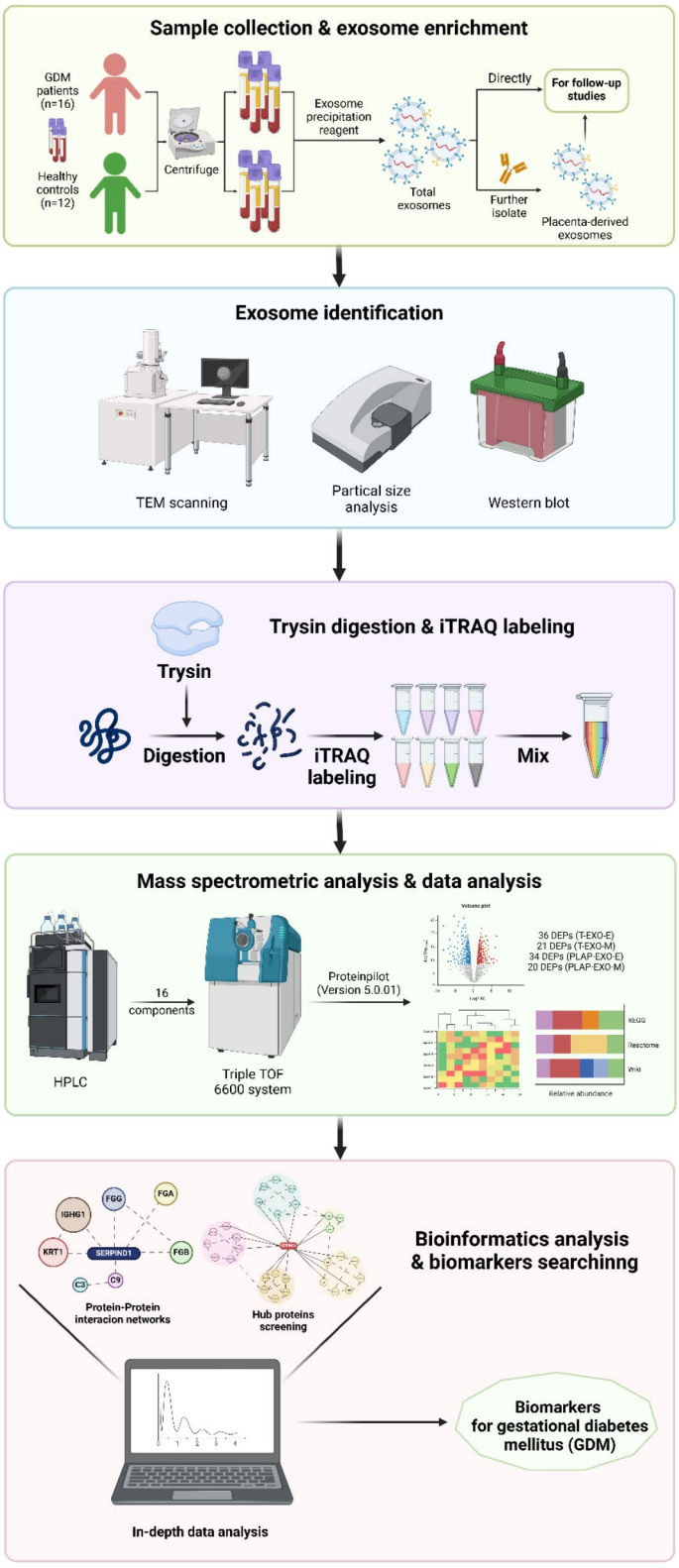

Fig. 1 Flow diagram of study identification

had 4 mixed samples respectively, and CON-E and CON-M had 3 mixed samples respectively.

Enrichment of total exosomes and placenta-derived exosomes

Minute™ Hi-Efficiency Exosome Precipitation Reagent (EI-027, Invent Biotech, USA) was used to extract plasma exosomes according to the manufacturer's instructions. First, add 1.2 mL phosphate-buffered saline (PBS) to 600 µL of mixed plasma, and centrifuge at 2000 × g for 10 min to remove impurities. Then add an appropriate amount of exosome precipitation reagent, mix and incubate at 4 °C for 1 h. At 4 °C, 10,000 × g, centrifuge for 15 min, remove all the supernatant and redissolve the precipitate with PBS to obtain a total exosome suspension.

An appropriate amount of total exosome suspension was taken as a sample for subsequent experiments, the rest was used to extract placenta-derived exosomes. Add 5µL of placental alkaline phosphatase polyclonal antibody (PLAP, PA5-112357, Invitrogen, USA), 3% bovine serum albumin (BSA), and protease inhibitors to exosome suspension, and mix them gently at 4 °C for 1 h. Then add protein A/G Sepharose rapid purification resin (Yeasen, China) at 4 °C and mix gently for 1 h. Centrifuge at 400 × g for 10 min at 4 °C, then remove the supernatant, and the precipitate was exosome-antibody-resin complex. 0.05 mM Glycine-HCl (pH=3) was added to the pellet at 4 °C for 15 min. Centrifuge at 1500 × g for 10 min at 4 °C, and aspirate the supernatant as placenta-derived exosomes (PLAP-EXO).

Extraction and identification of exosomes

Exosomes were identified by using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blot analysis. Before TEM detection, the exosome solution (T-EXO) was dropped on the front of formvar/carbon film-coated grids (Beijing Zhongjingkeyi Technology Co., Ltd, China), settled for 5–10 min, and the excess solution was sucked off. It was stained using 1% uranyl acetate staining solution (Beijing Zhongjingkeyi Technology Co., Ltd) and then detected by transmission electron microscopy (HT7700, HITACHI, Japan). NTA was performed using a nanoparticle size analyzer (ZetaPlus, Brookhaven, USA). Two exosome-specific proteins, ALIX (Programmed cell death 6-interacting protein) and CD63 (Lysosomal-associated membrane protein 3) [44], were selected for Western blot analysis. Exosomes are lysed by RIPA lysis buffer (Beyotime, China) and the proteins were extracted [45]. Western blot analysis was performed as previously described [46, 47].

Trypsin digestion and iTRAQ labeling

The exosomal proteins were obtained by lysis with RIPA buffer. For each sample, 150 µg proteins were reduced, alkylated, desalted and digested, and then labeled with the iTRAQ reagents (AB Sciex) [39, 47, 48]. Two sets of iTRAQ tags were used, where the tag 113 of each set was an equal mixture of all samples as reference samples (113-1, 113-2). The GDM-E group was labeled with 114-1, 115-1, 116-1, and 117-1, and the CON-E group was labeled with 118-1, 119-1, and 121-1. The GDM-M group was labeled with 114-2, 115-2, 116-2, and 117-2, and the CON-M group was labeled with 118-2, 119-2, and 121-2. The two sets of samples were separately mixed and lyophilized. The samples were then injected into an Agilent high-performance liquid chromatography (HPLC; Agilent Technologies, Santa Clara, CA) system with a high pH RP column (Durashell, C18, 250 mm × 4.6 mm, 5 µm; Bonna-Agela Technologies, Inc., Wilmington, DE) to remove the excess labeling reagents and salts [39, 47, 48]. Finally, the peptides were eluted, combined into 16 fractions, lyophilized, and stored at −80 °C.

Mass spectrometric analysis

The 16 fractions were reconstituted with LC-MS buffer (2% acetonitrile, 0.1% formic acid, 98% ddH_2O), and 4µL of each fraction was submitted to a Triple TOF 6600 system equipped with a NanosprayIII source (AB Sciex, Foster City, CA) [49]. The data was obtained with a 2.4 kV ion spray voltage, 35 psi curtain gas, 12 psi nebulizer gas, and an interface heater temperature of 150 °C. Automatic collision energy and automatic MS/MS accumulation were employed to activate smart information-dependent acquisition (IDA). The identification and quantification of proteins were analyzed using Proteinpilot (Version 5.0.01, AB Sciex) with the Paragon Algorithm against the UniProt "complete proteome"human proteins database. Based on 95% confidence level, protein identification required at least one unique peptide per protein, while protein quantification required two quantified peptides.

Protein expression characteristics analysis and DEPs identification

Principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) were performed using SIMCA software (V14.1, Sartorius Stedim Data Analytics AB, Umea, Sweden). The PLS-DA model was evaluated by 200 permutation tests. The value of the regression line of Q2 intersecting the vertical axis was less than zero, and there was no overlap between R2 and Q2, indicating that the model was not overfitted [50]. Differentially expressed protein (DEP) were identified using OMICSBEAN (http://www.omicsbean.cn/), based on 95% confidence, compared with the control, the proteins with a fold change (FC)≥1.2 were identified as

up-regulated proteins, and those with a fold change ≤ 0.83 were identified as down-regulated proteins.

Bioinformatics analysis

The OMICSBEAN database, STRING (https://string-db.org/), and Cytoscape software (V 3.8.2; https://cytoscape.org/) were used for bioinformatics analysis, which included gene ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes), Reactome, and Wiki pathways analysis, as well as protein−protein interaction (PPI) network analysis. GO analysis included biological process (BP), cellular component (CC), and molecular function (MF). Hub proteins were analyzed by Cytohubba software using the Bottleneck algorithm.

Results

Exosome identification results

As shown in Fig. 2A-C, clear exosome particles could be detected by TEM. Two exosomal marker proteins, CD63 and ALIX, could be detected in the exosomal protein extracts. Paticle size analysis showed that the isolated exosomes were mainly distributed between 50 and 200 nm.

Overview the protein expression profile of T-EXO

By iTRAQ analysis, a total of 924 and 883 proteins were identified and 505 proteins and 487 proteins were quantified in 12–16 weeks group and 24–28 weeks group, respectively. Among these, 352 quantified proteins were overlapping in these two groups. Compared with the exosomal proteins database (ExoCarta) [51], 314 quantified proteins in the 12–16 weeks group and 286 quantified

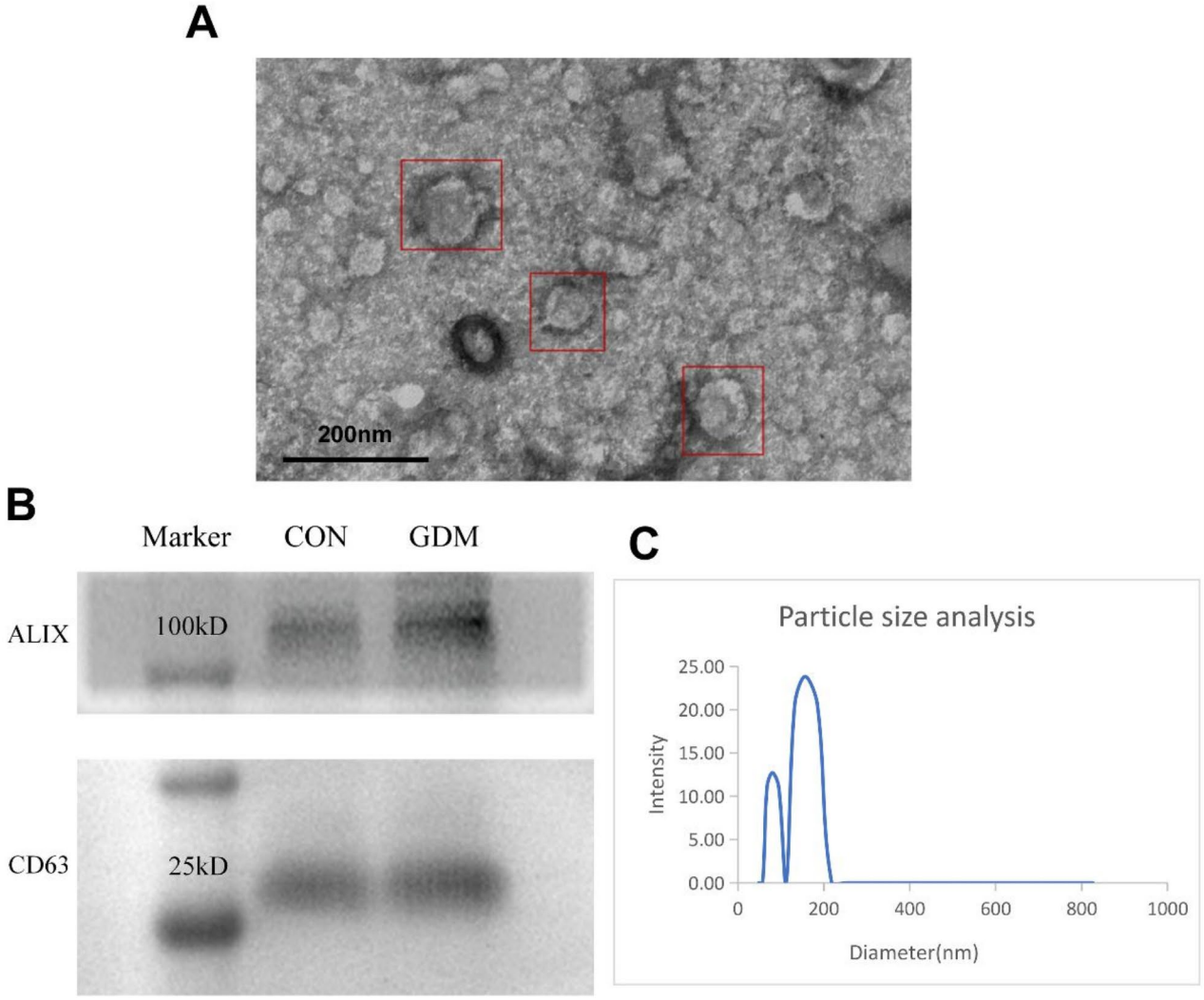

Fig. 2 Exosome validation. (**A**) Transmission electron microscopy (TEM) analysis. (**B**) Western blot analysis detection of CD63 and ALIX proteins. (**C**) Nanoparticle tracking analysis (NTA)

proteins in the 24–28 weeks group could be matched to the database. These quantified T-EXO proteins and their matching information with the ExoCarta database are listed in Supplementary Table 2 and Figure S1. PCA analysis showed that the two periods were clearly separated, however, although there was a separation trend between the GDM group and the CON group in the same period, there was overlap between them (Fig. 3A). In the PLS-DA plot, GDM and CON group were well separated in both periods (Fig. 3B). The intercept of the vertical axis of the Q2 value in the permutation tests was <0, indicating that the constructed model was feasible (**Figure S2A**).

Identification and bioinformatics analysis of T-EXO's DEPs

A total of 36 DEPs were identified in the T-EXO between GDM-E and CON-E groups. Among them, 19 DEPs were up-regulated and 17 DEPs were down-regulated (Fig. 3C; Table 1). Likewise, 21 DEPs were identified between GDM-M and CON-M groups, and 11 DEPs were up-regulated and 10 DEPs were down-regulated (Fig. 3D; Table 1). Cluster analysis showed that they could distinguish GDM patients and controls in corresponding period (Figs. 3E and 4F). There were no common DEPs in these two periods. Among the 57 DEPs of total exosome in both periods, 44 matched the ExoCarta database. Interestingly, 39 DEPs have been reported to be altered in the blood or blood exosomes of pregnant women who subsequently developed GDM and/or GDM patients in previous studies (Table 1).

As shown in Fig. 4A and Supplementary Table 3, at 12–16 weeks, the BPs associated with these DEPs of T-EXO included platelet degranulation, regulation of immune system process, regulation of humoral immune response, defense response, vesicle-mediated transport, positive regulation of tumor necrosis factor production, regulation of complement activation, immune system process, inflammatory response, and acute inflammatory response, etc. CCs included extracellular space, extracellular region, blood microparticle, extracellular organelle, and extracellular exosome, etc. (Fig. 4B, Supplementary Table 4). KEGG pathways included complement and coagulation cascades, and ECM-receptor interaction, while Reactome pathways included platelet degranulation, hemostasis, complement cascade, laminin interactions, immune system, cell surface interactions at the vascular wall, and extracellular matrix organization etc. (Fig. 4C, Supplementary Tables 5 and 6). The PPI network is showed in the Fig. 4D, the results were agreed with the above analysis.

At 24–28 weeks, the DEPs of T-EXO were mainly involved in immune, endocytosis, and phagocytosis (Fig. 5A and Supplementary Table 7), and belonged to the extracellular part, including extracellular exosome (Fig. 5B and Supplementary Table 8). PPI network showed that the DEPs were associated with extracellular exosome, complement activation, complement and coagulation cascades, ECM-receptor interaction, cholesterol metabolism, type II diabetes mellitus, insulin secretion, PPAR signaling pathway, neutrophil activation, immune effector process, leukocyte degranulation, and leukocyte mediate immunity (Fig. 5C).

A noteworthy phenomenon at 24–28 weeks was the emergence of metabolism-related DEPs, including cholesteryl ester transfer protein (CETP) related to lipid metabolism, adiponectin (ADIPOQ) related to type II diabetes mellitus and PPAR signaling pathway, and ryanodine receptor 2 (RYR2) related to insulin secretion. Transthyretin (TTR) and insulin-like growth factor-binding protein complex acid labile subunit (IGFALS) were associated with thyroid hormone synthesis and growth hormone synthesis, secretion and action, respectively.

Moreover, the top 10 hub proteins related to the DEPs of T-EXO at 12–16 weeks and 24–28 weeks were enriched and shown in Fig. 5D and E, respectively. At 12–16 weeks, they included CRP, PF4, VCAM1, ITGB1, THBS1, HRG, ORM2, HPX, ORM1, and SERPINC1, which were associated with extracellular exosome, coagulation, inflammation, immunity, and platelet degranulation. At 24–28 weeks, the top 10 hub proteins included CFH, CETP, ADIPOQ, FBLN1, KPRP, FLG2, HRNR, TTR, AFM, and IGFALS. They were associated with extracellular exosome, complement and coagulation cascades, lipoprotein particle, and retinol transport and transthyretin.

Overview of protein expression profile of placenta-derived exosomes (PLAP-EXO)

By iTRAQ analysis, at the 12–16 weeks, a total of 538 proteins were identified in the PLAP-EXO, of which 221 were quantified. At the 24–28 weeks, 589 proteins were identified, of which 246 proteins were quantified. Among these quantified proteins, 166 proteins overlapped in these two periods, 148 quantified proteins in the 12–16-week group and 174 quantified proteins in the 24–28-week group could be matched to ExoCarta database. These quantified PLAP-EXO proteins and their matching information with ExoCarta database are listed in Supplementary Table s2 and Figure S1. Compared with T-EXO, 176 proteins were common in the early stage (**Figure S3A**) and 196 proteins were common in the middle stage (**Figure S3B**) of pregnancy.

PCA result showed that the two periods were clearly separated. There was a separation trend between the two groups (GDM group and CON group) in the same period (Fig. 6A). In PLS-DA plot, the separation trend of GDM group and CON group was more obvious, and GDM-E and CON-E groups could be well distinguished (Fig. 6B). The intercept of the vertical axis of the Q2 value in the

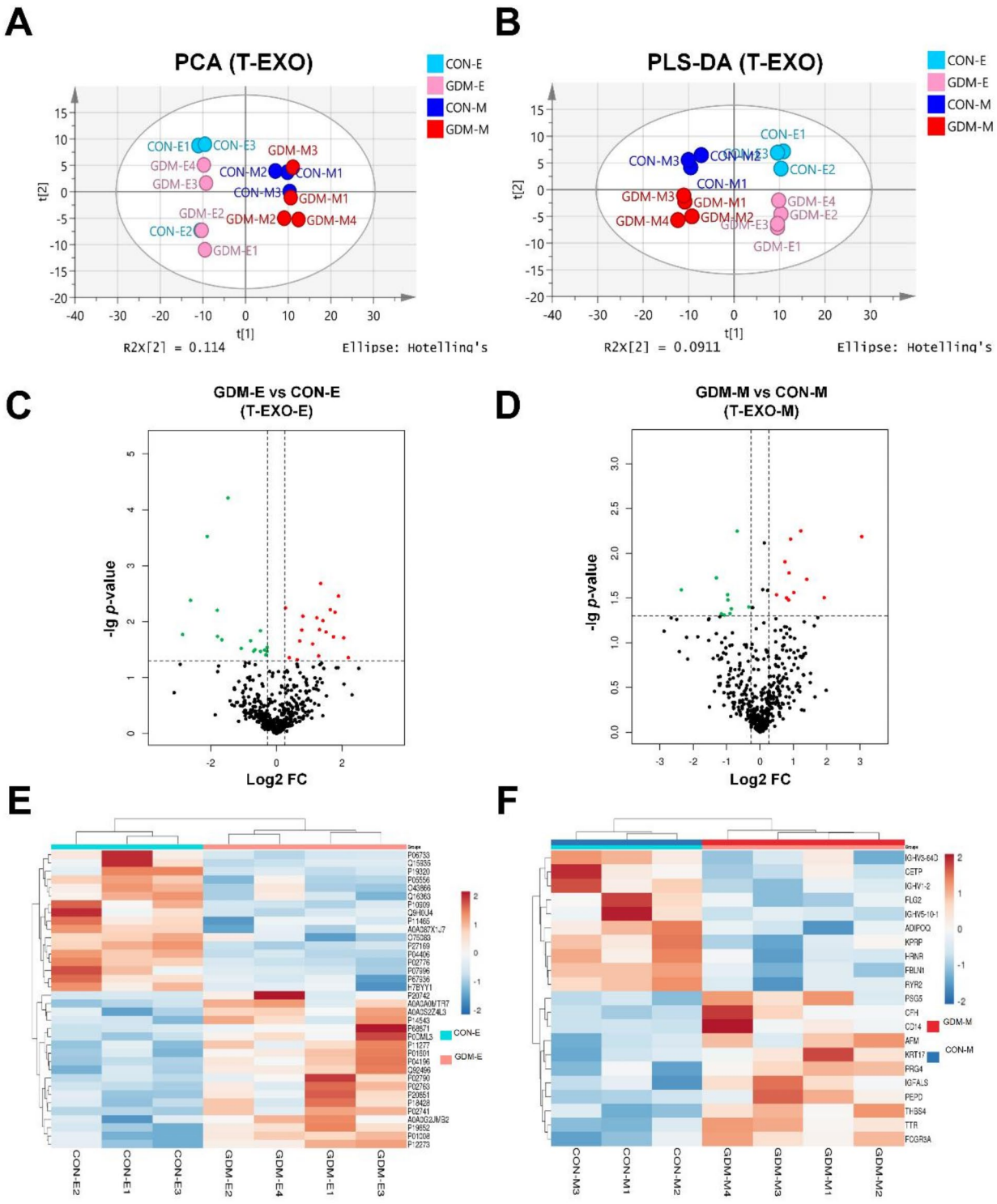

Fig. 3 Characterization of total exosomal protein expression and identification of DPEs at 12–16 and 24–28 weeks of gestation. (**A**) PCA plot. (**B**) PLS-DA plot. (**C**) Volcano plot depicting the distribution of proteins in the GDM-E/CON-E group. (**D**) Volcano plot depicting the distribution of proteins in the GDM-M/CON-M group. (**E**) Cluster analysis of DEPs between GDM-E and CON-E. (**F**) Cluster analysis of DEPs between GDM-M and CON-M. (**C** and **D**), green dots indicate down-regulated protein, and red dots indicate up-regulated protein

Table 1 Differentially expressed protein between GDM and control of total plasma exosomes by iTRAQ analysis

No.	Protein name	Accession number	Gene name	12–16 weeks of gestation		24–28 weeks of gestation		Reference
				Fold change*	*p* value	Fold change*	*p* value	
1	Adiponectin (-)	Q15848	ADIPOQ	0.51	0.278	**0.54**	0.047	[52, 53]
2	Afamin (+)	P43652	AFM	1.90	0.255	**2.02**	0.028	[54, 55]
3	Alpha-1-acid glycoprotein 1 (+)	P02763	ORM1	**2.85**	0.015	1.48	0.505	[56]
4	Alpha-1-acid glycoprotein 2 (+)	P19652	ORM2	**3.45**	0.007	1.72	0.400	[56]
5	Alpha-enolase (-)	P06733	ENO1	**0.71**	0.015	0.19	0.125	/ [c]
6	Antithrombin-III (+)	P01008	SERPINC1	**2.35**	0.008	1.43	0.439	[57]
7	C4b-binding protein beta chain (+)	P20851	C4BPB	**1.64**	0.022	1.67	0.066	[58]
8	CD5 antigen-like (-)	O43866	CD5L	**0.48**	0.030	0.43	0.286	[58]
9	Cholesteryl ester transfer protein (-)	P11597	CETP	1.49	0.559	**0.45**	0.047	[59]
10	Chorionic somatomammotropin hormone 2 (+)	P0DML3	CSH2	**3.13**	0.006	0.98	0.695	[60, 61]
11	Clusterin (-)	P10909	CLU	**0.29**	0.006	1.18	0.448	[60, 62]
12	Complement factor H (+)	P08603	CFH	2.01	0.172	**1.83**	0.017	[52, 52]
13	Complement factor H-related protein 4 (+)	Q92496	CFHR4	**1.21**	0.006	1.02	0.977	[52]
14	C-reactive protein (+)	P02741	CRP	**3.71**	0.003	0.22	0.152	[63]
15	E3 ubiquitin-protein ligase RNF213 (+)	A0A0A0MTR7	RNF213	**2.68**	0.010	/ [c]	/ [c]	/ [c]
16	Fibulin-1 (-)	P23142	FBLN1	0.86	0.474	**0.62**	0.006	[63]
17	Filaggrin-2 (-)	Q5D862	FLG2	1.25	0.678	**0.79**	0.040	/ [c]
18	Glutathione peroxidase 3 (-)	A0A087 × 1J7	GPX3	**0.58**	0.022	1.08	0.790	/ [c]
19	Glyceraldehyde -3-phosphate dehydrogenase (-)	P04406	GAPDH	**0.36**	0.000	1.18	0.414	[57, 58]
20	Hemoglobinsubunit beta (+)	P68871	HBB	**4.16**	0.020	1.17	0.816	[58, 64]
21	Hemopexin (+)	P02790	HPX	**2.15**	0.025	1.04	0.377	/ [c]
22	Histidine-rich glycoprotein (+)	P04196	HRG	**2.55**	0.002	1.83	0.147	[65]
23	Hornerin (-)	Q86YZ3	HRNR	0.90	0.735	**0.48**	0.049	[66]
24	Immunoglobulin heavy constant alpha 2 (+)	A0A0G2JMB2	IGHA2	**1.55**	0.048	0.54	0.107	[67]
25	Immunoglobulin heavy variable 1–2 (-)	P23083	IGHV1-2	0.87	0.611	**0.40**	0.019	/ [c]
26	Immunoglobulin heavy variable 3-64D (-)	A0A0J9YX35	IGHV3-64D	0.96	0.657	**0.55**	0.042	/ [c]
27	Immunoglobulin heavy variable 5-10-1 (-)	A0A0J9YXX1	IGHV5-10-1	3.55	0.067	**0.20**	0.026	/ [c]
28	Immunoglobulin kappa variable 1D-16 (+)	P01601	IGKV1D-16	**1.75**	0.008	3.08	0.065	/ [c]
29	Insulin-like growth factor-binding protein complex acid labile subunit (+)	P35858	IGF130ALS	0.98	0.937	**1.73**	0.031	[52]
30	Integrin beta-1 (-)	P05556	ITGB1	**0.32**	0.021	0.67	0.631	[68]
31	Keratin, type I cytoskeletal 17 (+)	Q04695	KRT17	1.31	0.717	**1.41**	0.029	/ [c]
32	Keratinocyte proline-rich protein (-)	Q5T749	KPRP	1.81	0.318	**0.51**	0.033	/ [c]
33	Laminin subunit alpha-4 (-)	Q16363	LAMA4	0.72	0.034	/ [c]	/ [c]	[61, 69]
34	Lipopolysaccharide-binding protein (+)	P18428	LBP	**2.44**	0.041	1.14	0.607	[70, 71]

Table 1 (continued)

No.	Protein name	Accession number	Gene name	12–16 weeks of gestation		24–28 weeks of gestation		Reference
				Fold change*	*p* value	Fold change*	*p* value	
35	Low affinity immunoglobulin gamma Fc region receptor III-A (+)	H0Y755	FCGR3A	/ [c]	/ [c]	**1.68**	0.012	/ [c]
36	Monocyte differentiation antigen CD14 (+)	P08571	CD14	1.12	0.141	**1.82**	0.033	[58]
37	Nidogen-1 (+)	P14543	NID1	**1.32**	0.044	0.64	0.524	[69]
38	Platelet factor 4 (-)	P02776	PF4	**0.16**	0.004	0.44	0.238	[57]
39	Pregnancy zone protein (+)	P20742	PZP	**4.58**	0.044	0.93	0.412	[52]
40	Pregnancy-specific beta-1-glycoprotein 2 (-)	P11465	PSG2	**0.80**	0.039	1.10	0.883	[61]
41	Pregnancy-specific beta-1-glycoprotein 5 (+)	E7EQY3	PSG5	1.55	0.057	**2.64**	0.020	[61]
42	Prolactin-Inducible protein (+)	P12273	PIP	**2.49**	0.014	0.75	0.112	[53]
43	Proteoglycan 4 (+)	Q92954	PRG4	4.16	0.131	**3.79**	0.031	[57]
44	Ras-related protein Rab-1B (-)	Q9H0U4	RAB1B	**0.78**	0.032	/ [c]	/ [c]	/ [c]
45	ropomyosin 1 (Alpha), isoform CRA_m (-)	H7BYY1	TPM1	**0.82**	0.034	/ [c]	/ [c]	[72]
46	Ryanodine receptor 2 (-)	Q92736	RYR2	/ [c]	/ [c]	**0.51**	0.029	[52]
47	Serum paraoxonase/arylesterase 1 (-)	P27169	PON1	**0.23**	0.000	1.19	0.581	[52, 57]
48	Spectrin beta chain, erythrocytic (+)	P11277	SPTB	**3.34**	0.019	0.83	0.706	/ [c]
49	Thrombospondin-1 (-)	P07996	THBS1	**0.29**	0.018	2.05	0.089	[73]
50	Thrombospondin-4 (+)	P35443	THBS4	0.61	0.111	**1.89**	0.007	[53, 74]
51	Transthyretin (+)	P02766	TTR	0.70	0.388	**2.34**	0.006	[58, 75]
52	Tropomyosin alpha-4 chain (-)	P67936	TPM4	**0.62**	0.034	/ [c]	/ [c]	/ [c]
53	Vascular cell adhesion protein 1 (-)	P19320	VCAM1	**0.14**	0.017	0.88	0.198	[76, 77]
54	Vitamin K-dependent protein S (+)	A0A0S2Z4L3	PROS1	**1.71**	0.014	1.65	0.106	[65, 78]
55	WD repeat-containing protein 1 (-)	O75083	WDR1	0.83	0.029	/ [c]	/ [c]	/ [c]
56	Xaa-Pro dipeptidase (+)	A0A494C165	PEPD	/ [c]	/ [c]	**8.27**	0.007	/ [c]
57	Zinc finger protein 77 (-)	Q15935	ZNF77	**0.64**	0.032	0.53	0.303	/ [c]

a) (+), protein increased in abundant; (-), protein decreased in abundant

b) The bolded fonts indicated differentially expressed proteins, whose *p*-value is less than 0.05

c) /, not been reported

replacement test is <0, indicating that the constructed model is feasible (**Figure S2B**).

Identification and bioinformatics analysis of PLAP-EXO's DEPs

In PLAP-EXO, 34 DEPs were identified between GDM-E and CON-E, of which 19 were up-regulated and 15 were down-regulated in the GDM-E group (Fig. 6C; Table 2). Meanwhile, 20 DEPs were identified between GDM-M and CON-M, of which 8 were up-regulated and 12 were down-regulated in the GDM-M group (Fig. 6D; Table 2). These DEPs were able to distinguish GDM patients and controls in corresponding periods (Fig. 6E and F). One DEP was common in the early and middle stages, i.e., IGKV3-7. In comparison with the DEPs of T-EXO, there were 3 common DEPs in the early stages of pregnancy, that is, HPX, SERPINC1, and CLU. Among which, the expression trend of CLU was opposite in the two exosomes, while HPX and SERPINC1 were consistent. Among the 54 DEPs of PLAP-EXO in two periods, 34

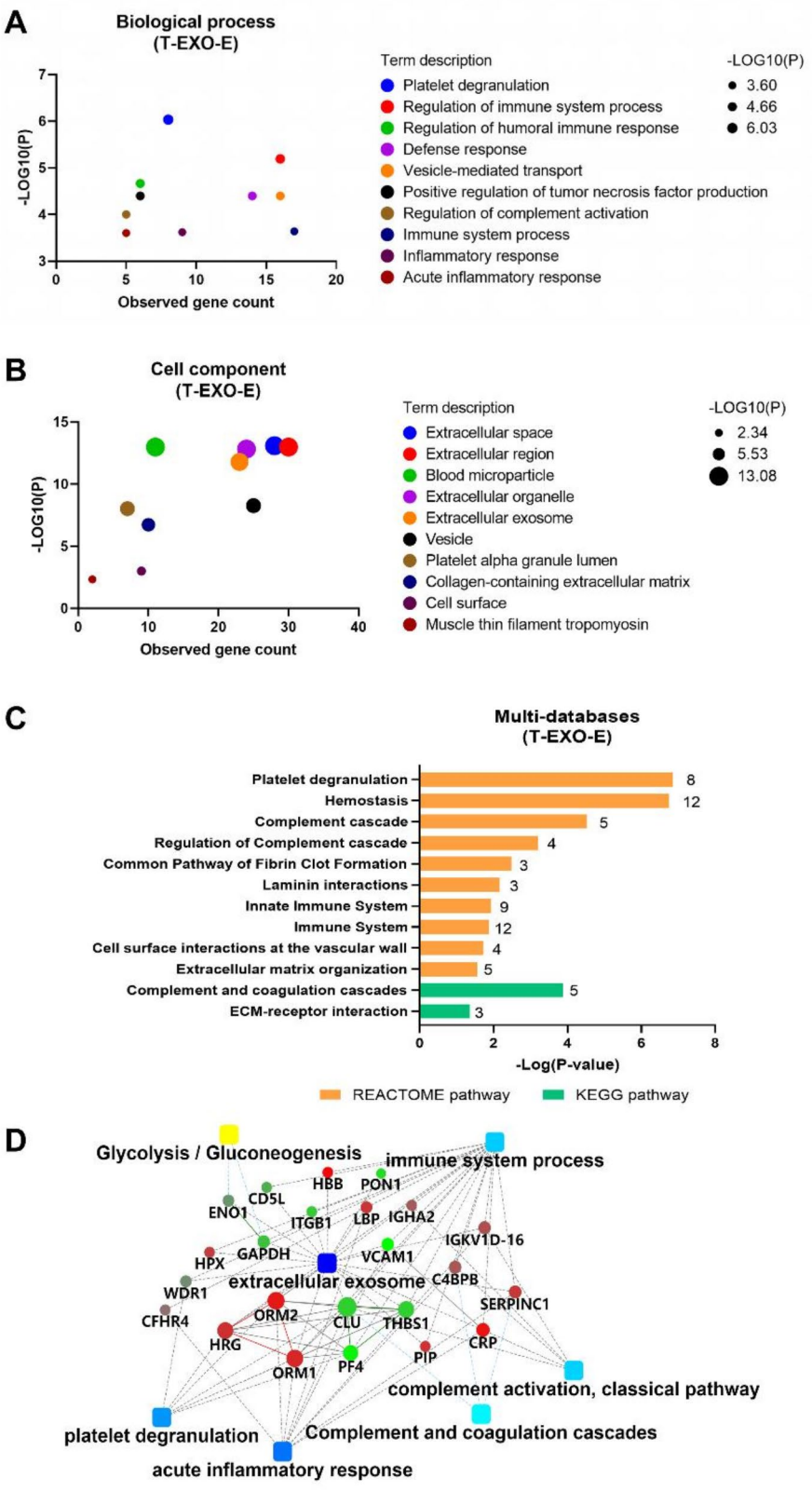

Fig. 4 Results of GO, KEGG and protein-protein interactions analyses of differentially expressed proteins in total exosomes at 12–16 weeks of gestation. (**A**) The top 10 biological processes associated with DEPs. (**B**) The top 10 cell components associated with DEPs. (**C**) The KEGG and Reactome pathways associated with DEPs. (**D**) Results of PPI analysis of total exosomal differential expressed proteins in early pregnancy

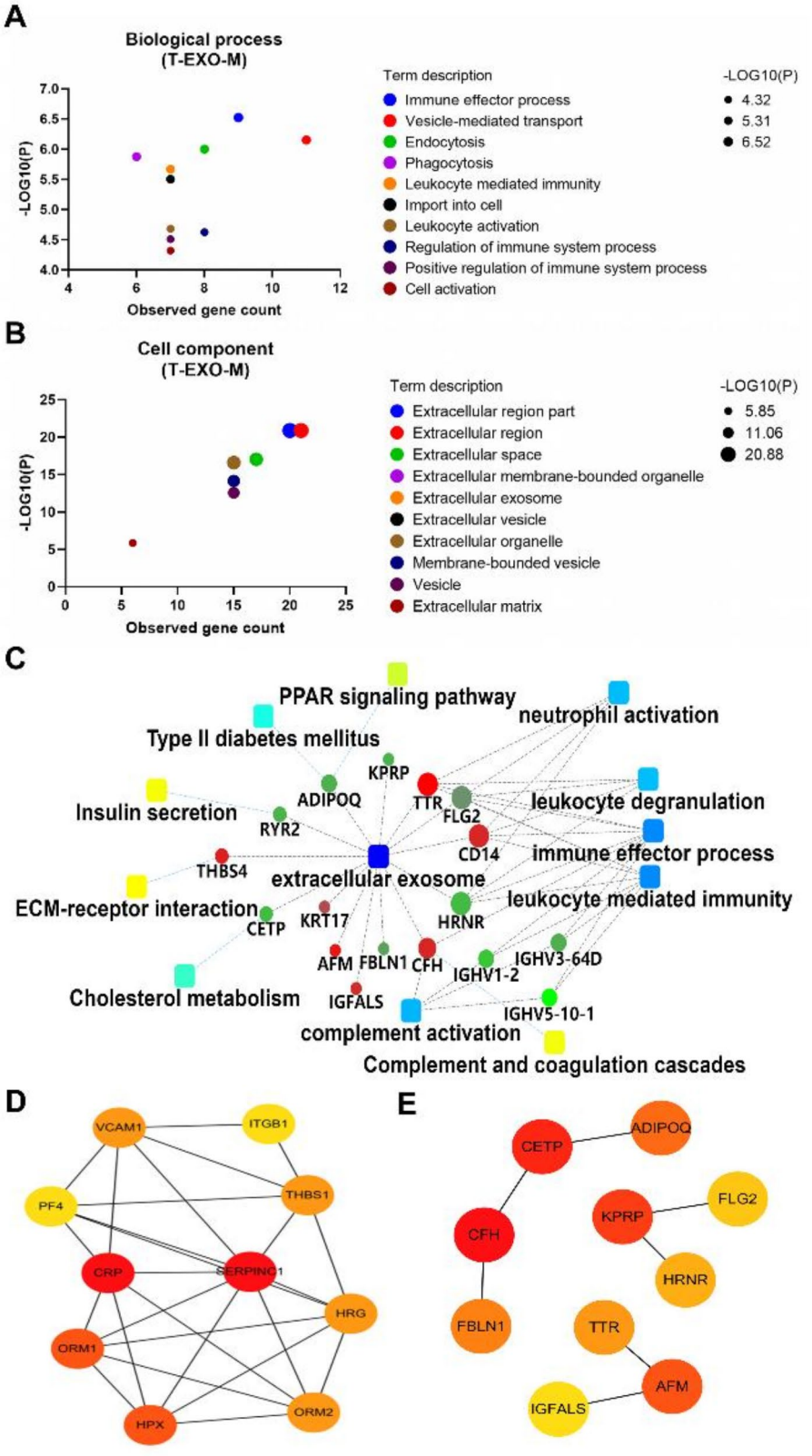

Fig. 5 Results of GO, protein-protein interactions and hub proteins analyses of differentially expressed proteins in total exosomes at 24–28 weeks of gestation. (**A**) The top 10 biological processes associated with DEPs. (**B**) The top 10 cell components associated with DEPs. (**C**) Results of PPI analysis of total exosomal differential expressed proteins at mid-pregnancy. (**D**) The top 10 hub proteins related to the DEPs of T-EXO at 12–16 weeks. (**E**) The top 10 hub proteins related to the DEPs of T-EXO at 24–28 weeks

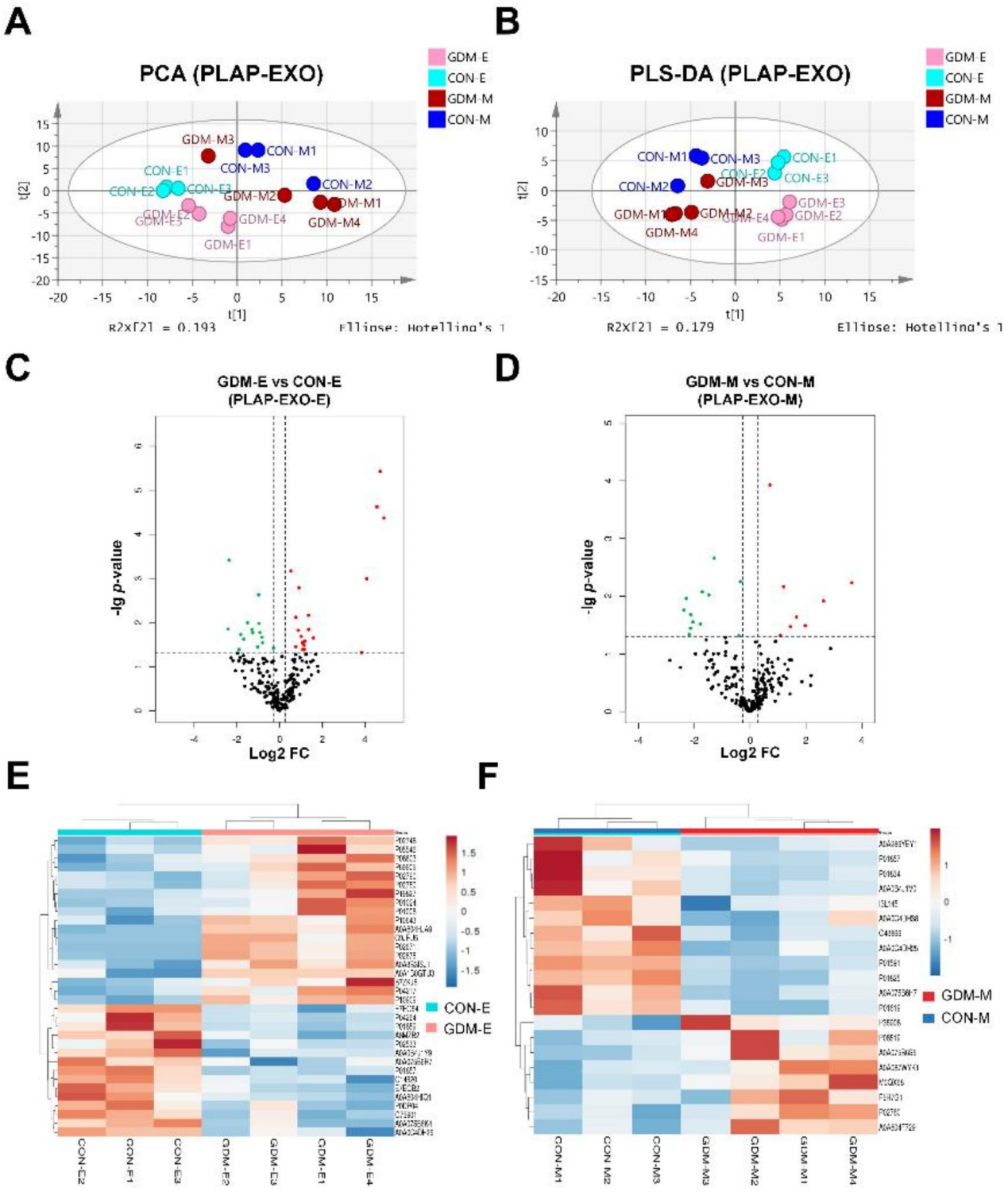

Fig. 6 Characterization of placenta-derived exosomal protein expression and identification of DPEs at 12–16 weeks and 24–28 weeks of gestation. (**A**) PCA plot. (**B**) PLSDA plot. (**C**) Volcano plot depicting the distribution of proteins in the GDM-E/CON-E group. (**D**) Volcano plot depicting the distribution of proteins in the GDM-M/CON-M group. (**E**) Cluster analysis of DEPs between GDM-E and CON-E. (**F**) Cluster analysis of DEPs between GDM-M and CON-M. (**C** and **D**), green dots indicate down-regulated protein, and red dots indicate up-regulated protein

Table 2 Differentially expressed protein between GDM and control of placenta-derived exosomes by iTRAQ analysis

No.	Protein name	Accession number	Gene name	12–16 weeks of gestation*		24–28 weeks of gestation*		Reference
				Fold change	*p* value	Fold change	*p* value	
1	Alpha-1B-glycoprotein (+)	P04217	A1BG	**1.84**	0.015	0.94	0.890	[65]
2	Antithrombin-III (+)	P01008	SERPINC1	**2.21**	0.033	1.08	0.744	[65, 79]
3	Apolipoprotein(a) (+)	P08519	LPA	1.23	0.735	**3.93**	0.033	[80–82]
4	CD5 antigen-like (-)	O43866	CD5L	0.64	0.281	**0.29**	0.031	/ [c]
5	Clusterin (+)	P10909	CLU	**1.44**	0.001	1.13	0.736	[52, 60, 83]
6	Complement C3 (+)	P01024	C3	**2.12**	0.041	1.15	0.062	[84]
7	Complement component C7 (+)	P10643	C7	**1.70**	0.008	1.04	0.774	[65]
8	Complement component C8 beta chain (+)	F5H7G1	C8B	/ [c]	/ [c]	**2.12**	0.048	[52, 57, 65]
9	Complement component C9 (+)	P02748	C9	**1.88**	0.002	0.94	0.849	[65]
10	Complement factor H (+)	P08603	CFH	**2.01**	0.021	1.23	0.458	[52, 57]
11	Doublecortin domain-containing protein (+)	A0A804HJA9	DCDC1	**16.84**	0.001	/ [c]	/ [c]	/ [c]
12	Fibrinogen alpha chain (+)	P02671	FGA	**26.17**	0.000	0.99	0.957	[52, 57]
13	Fibrinogen beta chain (+)	P02675	FGB	**23.44**	0.000	1.05	0.761	[52]
14	Fibrinogen gamma chain (+)	C9JEU5	FGG	**29.65**	0.000	1.55	0.204	[52]
15	Hemoglobin subunit alpha (+)	P69905	HBA1	**2.99**	0.023	1.23	0.406	[65, 85, 86]
16	Hemopexin (+)	P02790	HPX	**2.08**	0.029	1.10	0.838	[87, 88]
17	Heparin cofactor 2 (+)	P05546	SERPIND1	**1.69**	0.036	1.06	0.940	[65]
18	Hyaluronan-binding protein 2 (-)	Q14520	HABP2	**0.56**	0.022	1.55	0.141	/ [c]
19	Immunoglobulin heavy constant alpha 1 (-)	A0A286YEY1	IGHA1	1.43	0.431	**0.24**	0.028	[67]
20	Immunoglobulin heavy constant gamma 1 (-)	P01857	IGHG1	**0.19**	0.000	**0.22**	0.046	/ [c]
21	Immunoglobulin heavy constant gamma 2 (-)	P01859	IGHG2	**0.50**	0.002	0.34	0.090	/ [c]
22	Immunoglobulin heavy variable 1–3 (-)	A0A0C4DH29	IGHV1-3	**0.58**	0.029	2.16	0.324	/ [c]
23	Immunoglobulin heavy variable 3–15 (-)	A0A0B4J1V0	IGHV3-15	/ [c]	/ [c]	**0.23**	0.036	/ [c]
24	Immunoglobulin heavy variable 3-43D (-)	P0DP04	IGHV3-43D	**0.49**	0.036	/ [c]	/ [c]	/ [c]
25	Immunoglobulin heavy variable 3–72 (-)	A0A0B4J1Y9	IGHV3-72	**0.42**	0.017	/ [c]	/ [c]	/ [c]
26	Immunoglobulin heavy variable 4–59 (-)	P01825	IGHV4-59	/ [c]	/ [c]	**0.36**	0.009	/ [c]
27	Immunoglobulin heavy variable 5–51 (-)	A0A0C4DH38	IGHV5-51	0.53	0.388	**0.75**	0.049	/ [c]
28	Immunoglobulin J chain (-)	P01591	JCHAIN	0.66	0.459	**0.41**	0.002	[52]
29	Immunoglobulin kappa constant (-)	P01834	IGKC	0.57	0.232	**0.19**	0.017	[89]
30	Immunoglobulin kappa variable 1–27	A0A075B6S5	IGKV1-27	/ [c]	/ [c]	**12.53**	0.006	/ [c]
31	Immunoglobulin kappa variable 3–20 (-)	P01619	IGKV3-20	0.83	0.584	**0.23**	0.021	/ [c]
32	Immunoglobulin kappa variable 3D-20 (-)	A0A0C4DH25	IGKV3D-20	0.62	0.487	**0.20**	0.011	/ [c]

Table 2 (continued)

No.	Protein name	Accession number	Gene name	12–16 weeks of gestation*		24–28 weeks of gestation*		Reference
				Fold change	*p* value	Fold change	*p* value	
33	Immunoglobulin lambda variable 3–10 (-)	A0A075B6K4	IGLV3-10	**0.41**	0.014	/ [c]	/ [c]	/ [c]
34	Inter-alpha-trypsin inhibitor heavy chain H1 (+)	P19827	ITIH1	**2.57**	0.014	1.31	0.599	[52]
35	ITIH4 protein (+)	B7ZKJ8	ITIH4	**2.27**	0.026	0.87	0.772	[52, 57, 90]
36	Keratin, type I cytoskeletal 14 (-)	P02533	KRT14	**0.27**	0.041	1.24	0.724	[91]
37	Keratin, type II cytoskeletal 1 (-)	P04264	KRT1	**0.19**	0.014	1.25	0.767	[65]
38	Keratin, type II cytoskeletal 2 epidermal (+)	P35908	KRT2	0.32	0.181	**2.73**	0.034	/ [c]
39	Kinesin-like protein KIF16B (+)	A0A1B0GTU3	KIF16B	**14.30**	0.048	/ [c]	/ [c]	/ [c]
40	Lactotransferrin (-)	E7EQB2	LTF	**0.28**	0.019	/ [c]	/ [c]	[52]
41	Leucine-rich alpha-2-glycoprotein (+)	P02750	LRG1	**2.24**	0.041	0.86	0.722	/ [c]
42	N-acetyltransferase 5 (ARD1 homolog, S. cerevisiae), isoform CRA_a (-)	A8MZB2	NAA20	**0.31**	0.024	/ [c]	/ [c]	/ [c]
43	Pregnancy-specific beta-1-glycoprotein 3 (+)	M0QX68	PSG3	/ [c]	/ [c]	**6.18**	0.012	[61]
44	Pregnancy-specific beta-1-glycoprotein 9 (+)	A0A087WYK1	PSG9	1.05	0.884	**3.16**	0.023	[61]
45	Probable non-functional immunoglobulin (-) kappa variable 3–7	A0A075B6H7	IGKV3-7	**0.82**	0.038	**0.30**	0.008	/ [c]
46	Protein AMBP (+)	P02760	AMBP	1.31	0.207	**1.64**	0.000	[92]
47	Pyruvate kinase (+)	A0A804F729	PKM	/ [c]	/ [c]	**2.30**	0.007	/ [c]
48	Ras association domain-containing protein 9 (-)	O75901	RASSF9	**0.53**	0.017	/ [c]	/ [c]	/ [c]
49	Sacsin (-)	A0A804HIQ1	SACS	**0.35**	0.010	/ [c]	/ [c]	/ [c]
50	Serine protease 1 (-)	E7EQ64	PRSS1	**0.51**	0.011	/ [c]	/ [c]	/ [c]
51	Sex hormone-binding globulin (-)	I3L145	SHBG	/ [c]	/ [c]	**0.78**	0.006	[93]
52	Vitamin K-dependent protein S (+)	A0A3B3ISJ1	PROS1	**2.56**	0.007	/ [c]	/ [c]	[52]

a) (+), protein increased in abundant; (-), protein decreased in abundant

b) The bolded fonts indicated differentially expressed proteins, whose *p*-value is less than 0.05

c) /, not been reported

matched the ExoCarta database. Similar to total exosomes, 28 DEPs of PLAP-EXO have also been reported to be dysregulated in the blood or blood exosomes of pregnant women who subsequently developed GDM and/or GDM patients in previous studies (Table 2).

At 12–16 weeks, the BPs related to DEPs of PLAPT-EXO included complement activation, humoral immune response, fibrinolysis, vesicle-mediated transport, platelet degranulation, regulated exocytosis, and regulation of blood coagulation, etc. (Fig. 7A). CCs included blood microparticle, extracellular space, extracellular exosome, collagen-containing extracellular matrix, secretory granule lumen, vesicle, and platelet alpha granule lumen, etc. (Fig. 7B). Pathway analysis showed that they were associated with complement and coagulation cascades, common pathway of fibrin clot formation, innate immune system, platelet degranulation, hemostasis, terminal pathway of complement, regulation of insulin-like growth factor (IGF) transport and uptake by insulin-like growth factor binding proteins (IGFBPs), etc. (Fig. 7C). Detailed information of GO and pathway analysis are listed in Supplementary Tables 9–13. As shown in Fig. 7D, the result of PPI network analysis was similar to the GO and pathways analysis.

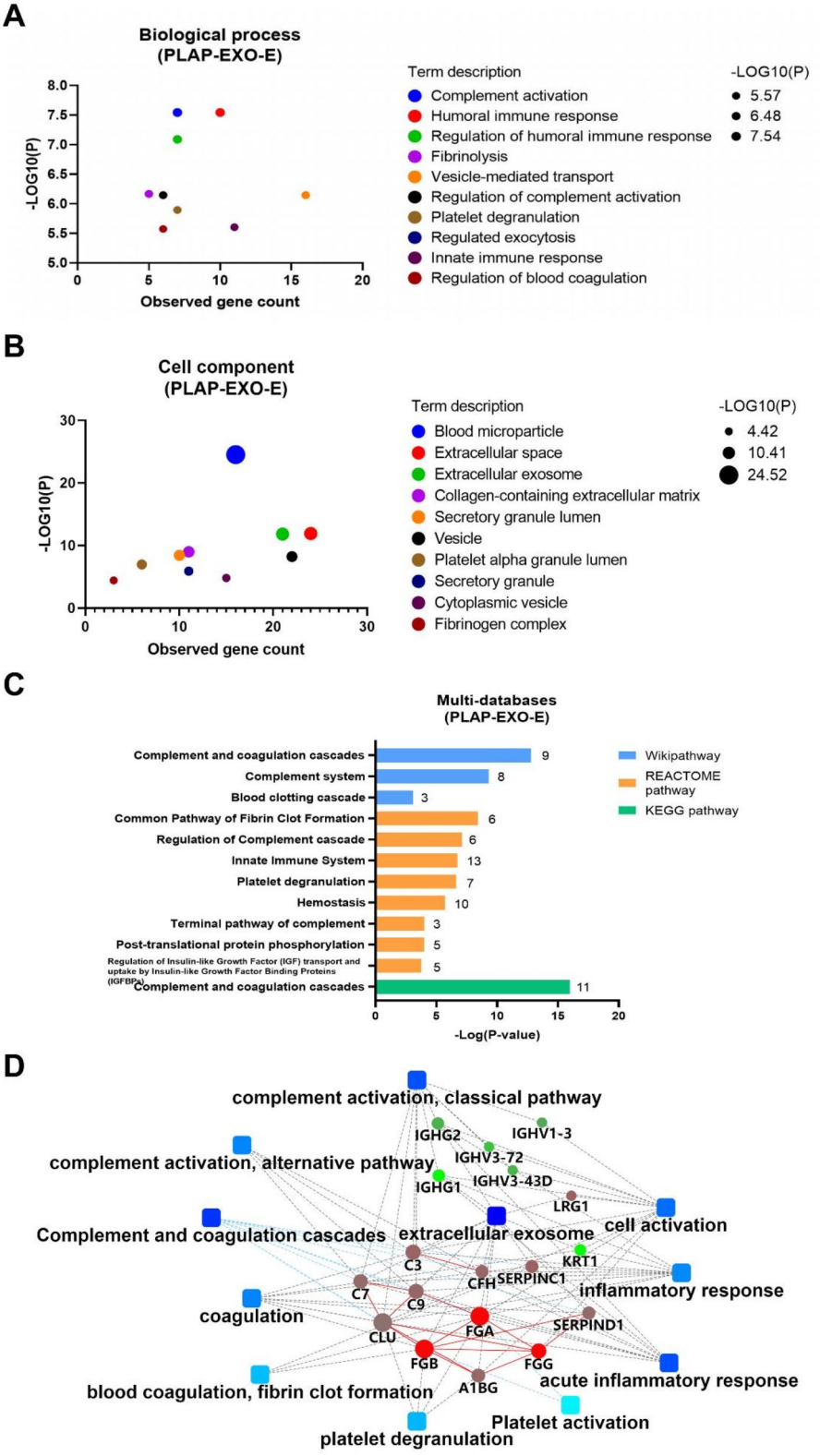

Fig. 7 Results of GO, KEGG and protein-protein interactions analyses of differentially expressed proteins in placenta-derived exosomes at 12–16 weeks of gestation. (**A**) The top 10 biological processes associated with DEPs. (**B**) The top 10 cell components associated with DEPs. (**C**) The KEGG, Reactome and Wiki pathways associated with DEPs. (**D**) Results of PPI analysis of placenta-derived exosomal differentially expressed proteins in early pregnancy

At 24–28 weeks, the DEPs of PLAP-EXO were involved in immune, complement active, protein activation cascade, and endocytosis (Fig. 8A and Supplementary Table 14), and immunoglobulin complex, extracellular region, and extracellular exosome (Fig. 8B, and Supplementary Table 15). They were enriched in extracellular exosome, and associated with complement activation, inflammatory response, type II diabetes mellitus, glycolysis/gluconeogenesis, and glucagon signaling pathway (Fig. 8C).

In addition, at the 12–16 weeks, hub proteins associated with DEPs of PLAP-EXO included HPX, C9, FGG, FGB, GLU, FGA, C3, SERPINC1, ITIH4, and A1BG, which were involved in complement and coagulation cascades, and platelet degranulation and activation, innate immune system, and regulation of IGF transport and uptake by IGFBPs (Fig. 8D). At the 24–28 weeks, hub proteins included AMBP, IGJ, CD5L, PSG3, and IGHV3-15, which were mainly related to immunity (Fig. 8E).

Discussion

In this study, we extracted total exosomes and placenta-derived exosomes from the plasma of GDM pregnant women and healthy pregnant women in early and mid-gestation. Characteristic identification, database matching, and bioinformation analysis confirmed that exosomes were successfully extracted. On this basis, a comparative proteomics study was carried out to obtain DEPs of exosomes between cases and controls.

At 12–16 weeks of gestation, the DEPs of T-EXO and PLAP-EXO were mainly involved in the complement and coagulation cascade, immune system process, inflammatory response, and platelet degranulation. These processes or pathways associated with mechanisms have been reported to be closely related to the pathogenesis of GDM [94–96] and diabetic complications [97]. The complement-related proteins include complement proteins or complement regulatory proteins or receptor proteins, and are involved in complement activation (classical and / or alternative pathways) and regulation. Most of them were up-regulated, including C4BPB, CLU, CFHR4, and CRP in total exosomes, and C3, C7, C9, and CHF in placenta-derived exosomes. Among them, C3 plays a central role in the activation of the complement system [98]. Elevated C3 levels have been reported to be associated with obesity, dyslipidemia, inflammation, insulin resistance and liver dysfunction [98, 99] as well as diabetes and GDM [100–102]. C7 levels has been observed to be elevated in the peripheral blood of GDM patients [103]. CFH is an alternative complement pathway inhibitor. Its levels are negatively correlated with insulin sensitivity [104] and may be elevated in GDM patients with insulin resistance [41]. CFHR4 (CFH-related proteins 4) is a complement in the form of CFH cofactor-enhancing activity that modulates activity. It binds to the central complement component C3b [105, 106]. Besides, the expression of CLU was decreased in total exosomes and increased in placenta-derived exosomes. Clusterin precursor has been observed to be decreased in the serum of pregnant women with GDM [107]. CLU has various functions and is a complement inhibitor, it and vitronectin inhibit the C5b-8 complex insertion into membrane attack complex [108, 109]. Another complement-related protein, CD5L, is a key regulator of lipid synthesis and regulates inflammatory response, which has been observed to be decreased in the plasma of type 2 diabetes mellitus (T2DM) patients [110, 111]. Together, these results suggested that complement may play a critical role in the pathogenesis of GDM.

However, the involvement of the complement system appears to be complex. Up- or down-regulation of complement-related proteins, leading to over-activation or deficiency of complement, may lead to the development of disease. At the same time, complement regulatory proteins have a regulatory role in the complement activation process. For example, in this study, complement proteins C3, C7 and C9 were increased in placental exosomes, while two complement regulatory proteins that inhibit complement activation, CFH and CLU, were increased and down-regulated, respectively. In contrast, in our previous study, serum levels of C7, C9 and CFH were down-regulated in pregnant women who subsequently developed GDM [40, 41]. Therefore, further studies are needed to elucidate the function of complement proteins in pregnancy and GDM. The results imply that under-involvement of the complement system as well as over-involvement of the complement system leads to pathophysiology and that an appropriate balance is important [108].

GDM is not only a metabolic disease but is also a low-grade inflammatory response [112–114]. In the present study, at 12–16 weeks, five DEPs (CRP, LBP, ORM1, ORM2, SERPINC1) related to inflammatory response were found to be altered in T-EXO. SERPINC1 is common among T-EXO and PLAP-EXO. In particular, elevated levels of CRP was observed in the T-EXO. CRP is an inflammatory marker released by the liver in response to cytokine stimulation, are associated with increased risk of type II diabetes, myocardial infarction, stroke, and peripheral vascular disease [115, 116]. Elevated maternal CRP during pregnancy is associated with pregnancy complications [117], including GDM [40, 114, 118, 119].

At 12–16 weeks, immune system processes were also significantly enriched. In the DEPs of T-EXO, they were associated with the innate immune system, whereas in PLAP-EXO DEPs, they are associated with innate and adaptive immune responses. Indeed, complement, immune, and inflammation are interrelated and contribute to the pathophysiological mechanism of GDM [73,

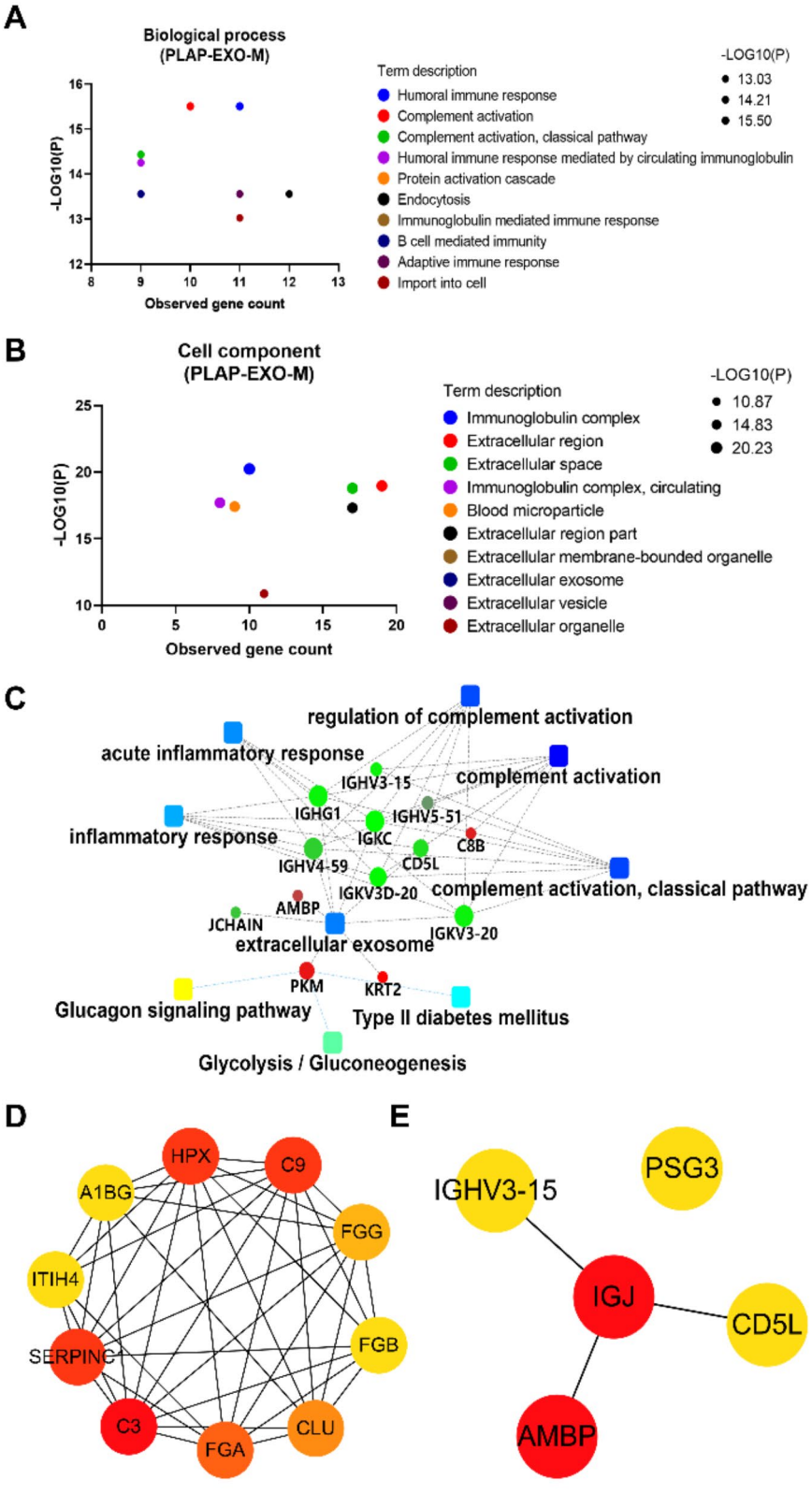

Fig. 8 Results of GO, protein-protein interactions and hub proteins analyses of differentially expressed proteins in placenta-derived exosomes at 24–28 weeks of gestation. (**A**) The top 10 biological processes associated with DEPs. (**B**) The top 10 cell components associated with DEPs. (**C**) Results of PPI analysis of placenta-derived exosomal differential expressed proteins at mid-pregnancy. (**D**) The top 10 hub proteins related to the DEPs of PLAP-EXO at 12–16 weeks. (**E**) The top 10 hub proteins related to the DEPs of PLAP-EXO at 24–28 weeks

99, 120–122] and T2DM [123]. As a regulator of both the innate and the adaptive immune system, complement system represents an important part of this inflammatory response. In this study, some of the DEPs related to them were overlapped. For example, CRP, CLU and CD5l were associated with all three. LBP, an acute-phase glycoprotein associated immune and inflammatory response, was found to be up-regulated in the T-EXO. Likewise, one of the hallmarks of the inflammatory response is increased activation and recruitment of immune cells. In the DEPs of T-EXO, some DEPs were related to leukocyte migration and leukocyte mediated immunity, while in the DEPs of PLAP-EXO, some were associated with neutrophil degranulation, leukocyte mediated immunity, and myeloid leukocyte activation. Interestingly, significant neutrophil infiltration has been observed in the placenta of GDM patients [124, 125]. Therefore, our results support that impaired immune and inflammatory homeostasis may contribute to GDM, which can be achieved by affecting insulin resistance (IR) and ß-cell function [126].

Compared to normal pregnancy, the hypercoagulable state of GDM is further enhanced [41, 127–129]. Here, several DEPs were associated with coagulation. Of which, SERPINC1 was found to be increased in the T-EXO, and FGA, FGB, FGG, and SERPIND1 were up-regulated in PLAP-EXO in early pregnancy. Interestingly, FGA, FGB, and FGG were significantly up-regulated in the serum of pregnant women who subsequently developed GDM or with GDM in our previous studies [40, 41]. Another study observed that fibrinogen concentrations were significantly elevated in the pregnant women with GDM [130]. The hypercoagulable state may be primarily caused by hyperglycemia. GDM may lead to blood coagulation due to increased platelet activation, increased coagulation factor synthesis (including fibrinogen), and decreased fibrinolytic activist [130]. On the other hand, some proteins that inhibit blood coagulation have also changed, such as SERPINC1, SERPING1, and PROS1. SERPING1 is a plasma proteinase C1 inhibitor. SERPINC1 is the most important serine protease inhibitor and regulates the coagulation cascade and inhibits thrombin activity. They have been reported to be associated with diabetes [131, 132]. PROS1 is an anticoagulant plasma protein. SERPINC1 and PROS1 was up-regulated in the T-EXO and PLAP-EXO, while SERPIND1 was up-regulated in the T-EXO of women who subsequently developed GDM. Similar to our previous observation [40], it may be a feedback inhibition reaction to the complement and coagulation cascade, and play a role in regulating the complement and cascade process.

Studies have shown that patients with T2DM have abnormal platelet activation [133] and their coagulation system is in a hypercoagulable state [134]. Increased fibrinogen leads to platelet aggregation, a step-in platelet activation [135, 136]. We found that some DEPs at 12–16 weeks in the T-EXO and PLAP-EXO were associated with platelet activation/degranulation. Platelet degranulation is related to its activation state, usually an increase in mean platelet volume (MPV), indicating that it is more active. Therefore, higher MPV indicates higher prothrombotic status [137]. Interestingly, an increase in MPV has been related to diabetes and GDM [138–142].

At 24–28 weeks, the DEPs were also mainly enriched in extracellular exosome, and related to complement, immunity and inflammation. Notably, immunoglobulins comprised the majority of DEPs linked to these biological processes, with the majority exhibiting down-regulation in both T-EXO and PLAP-EXO. The most DEPs associated with complement, immunity and inflammation were immunoglobulins, and most of them were down-regulated in the T-EXO and PLAP-EXO. This similar to T2DM [111], the role of immunity in GDM is bimodal: immunity may contribute to the pathology of T2DM, by contrast, the suppression of immunity is one of the major consequences of T2DM. Besides, several other DEPs are also of interest. CD14 was observed to be increased in the T-EXO. It is a monocyte and macrophage marker, has been shown to be increased in placental and omental adipose tissue during inflammation [143]. GDM pregnancies had an increased percentage of circulating CD14+cells and higher levels of soluble CD14+ (sCD14+) in serum [144]. Moreover, CFH was up-regulated in the T-EXO of pregnant with GDM, CD5L was down-regulated and C8B was up-regulated in the PLAP-EXO of pregnant with GDM.

Of note, at 24–28 weeks, the DEPs related to metabolism and GDM and/or diabetes were enriched, including adiponectin, afamin, CETP, IGFALS, RYR2, and TTR in the T-EXO, and apolipoprotein, PKM, and SHBG in the PLAP-EXO. Adiponectin is an important adipokine that controls fat metabolism and insulin sensitivity [145, 146], and is associated with GDM [147], even extending beyond pregnancy [145]. Similar to the previous study [148], it was decreased in the T-EXO of patients with GDM in this study. Afamin belongs to the albumin family and acts to bind and transport vitamin E and may be involved in oxidative stress and anti-apoptosis. Serum levels of afamin were significantly elevated in GDM patients before and during pregnancy [149]. IGFALS, an acid-labile subunit of insulin-like growth factor that binds to insulin-like growth factor-binding proteins, thereby controlling insulin-like growth factors essential for placental development and growth bioavailability [150]. It was found to be increased in the serum of pregnant with GDM in our previous study [40]. TTR is a thyroid hormone-binding protein that, in addition to transporting thyroxine, plays a role in glucose and lipid metabolism, and insulin resistance [151]. The serum TTR

concentration in GDM women is significantly higher than that in non-GDM women [152–154]. Similarly, in this study, afamin and IGFALS, and TTR were up-regulated in the T-EXO. The insulin secretion-related protein RYR2 play a key role in regulating insulin secretion and glucose homeostasis [155]. Here, it was down-regulated in the T-EXO.

In addition, impaired lipid transport and homeostasis are associated with GDM [41]. Lipid metabolism of the placenta is also disturbed in GDM [156]. Our results showed that cholesteryl ester transfer protein (CETP) was down-regulated in the T-EXO, while apolipoprotein was up-regulated in the PLAP-EXO of pregnant women with GDM at 24–28 weeks. In the PLAP-EXO, two other proteins also deserve attention, i.e., up-regulated PKM and down-regulated SHBG. PKM is an important enzyme of glycolysis and any alteration in the enzyme activity will severely affect the glucose utilization [157], which controls signal strength in the insulin secretory pathway [158]. Its expression is increased in the pre-eclampsia placenta at delivery [159]. SHBG, meanwhile, plays an important role in regulating and transferring sex hormones. Its production is controlled by insulin and inversely related to insulin resistance [160]. The studies have shown that the level of SHBG is significantly lower in GDM pregnant women than that in healthy women [160].

Furthermore, we noticed changes in the expression of several pregnancy-related proteins, including PZP, PSG2, PSG5, PSG3, and PSG9. Altered levels of PZP and PSG have been reported in the blood of patients with GDM [52, 57, 161, 162]. As PSGs are intricately involved in immune regulation [163], our results suggest that they may play an important role in maintaining healthy pregnancy.

Collectively, to the best of our knowledge, this study is the first to apply a proteomic approach to study plasma and placental exosomal proteins in patients with GDM in early and mid-pregnancy. The results are beneficial for exploring disease mechanisms and screening biomarkers. The mechanisms involved in DEPs of T-EXO and PLAP-EXO are not exactly the same in pregnant women with GDM in early and mid-gestation and are summarized in Fig. 9. The protein dysregulation found in early gestation suggests that they are involved in the pathogenesis of GDM rather than being the result of subsequent metabolic changes [156]. GDM is a progressive process and mechanisms that emerge early promote

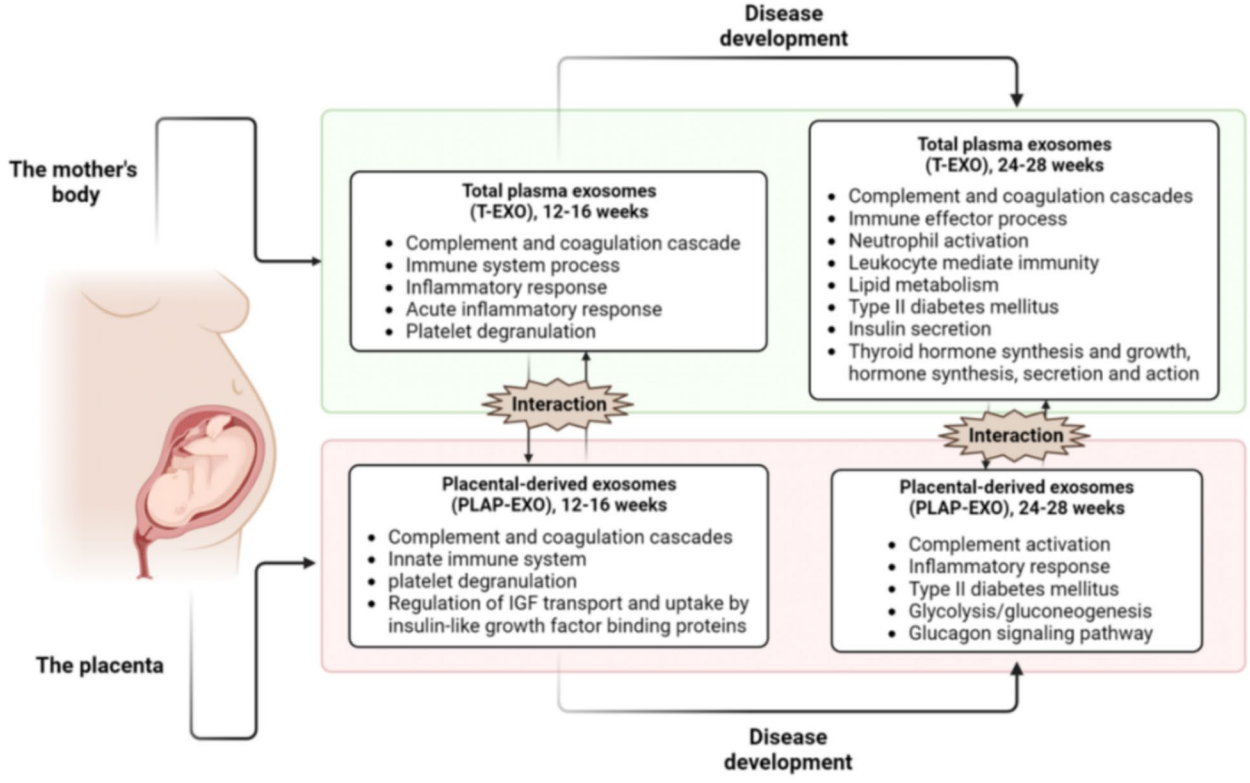

Fig. 9 The mechanism of GDM disease associated with DEPs in plasma total exosomes and placental derived exosomes identified in this study. The results suggest that the mechanisms involved in the early and middle stages are not exactly the same, and that there may be an interaction between maternal and placental factors. Complement and coagulation cascades, immunity and inflammation were common mechanisms in the early and middle stages, whereas in the middle stage, changes in metabolism-related protein expression were detected

disease progression, which is consistent with previous studies [164]. In addition, the mechanisms associated with T-EXO and PLAP-EXO suggest that maternal factors contribute to disease onset or affect the placenta. Maternal metabolic and immune status may alter early placental metabolism and function. The inflammatory environment regulates maternal glucose metabolism, and maternal inflammation is associated with a high susceptibility to GDM [2]. On the other hand, in agreement with previous studies [40], the placenta plays a key role in the pathophysiology of GDM. PLAP-EXO may lead to a pro-inflammatory state associated with GDM [41], while the placenta itself is in an inflammatory state. Moreover, as the mechanisms involved in these DEPs are consistent with the disease mechanisms of GDM and most of them are blood or exosomal proteins reported in previous studies to be associated with GDM, they have the potential to be used as predictive or diagnostic markers, and especially hub protein. They might also be proposed as markers to evaluate the effect of early intervention in GDM. Furthermore, the number of DEPs identified in the two periods was also different, and more were identified in the early stage, suggesting that the search for biomarkers from an exosomal perspective is not only feasible, but also early prediction is significant for GDM prevention and intervention. However, this study has some limitations, such as a small sample size and the use of mixed samples in proteomic analysis, and lack of validation of the DEPs. Therefore, further large sample size studies and validation are still needed [29].

Conclusion

In this study, we used quantitative proteomics techniques to identify DEPs in plasma exosomes and placental-derived exosomes from GDM patients and normoglycemic women in early and mid-pregnancy. These DEPs were able to distinguish GDM patients from controls in early or mid-pregnancy. Functional analysis showed that they were associated with complement, inflammatory, immune, coagulation and metabolism pathways. These mechanisms have been previously reported. The results suggest that GDM is a progressive process and that there is an association between placental and peripheral changes, which may play a key role in the pathogenesis of GDM. Although further research and validation is needed, these proteins especially the hub protein may be potential early biomarkers of GDM in pre-symptomatic women and diagnostic biomarkers for pregnant women with GDM in mid-gestation. The results provide new insights into the pathogenesis and progression of GDM and highlight the potential utility of total plasma proteins and placental-derived exosomal proteins.

Abbreviations

ADIPOQ	Adiponectin
BMI	Body mass index
BP	Biological process
BSA	Bovine serum albumin
CC	Cellular component
CETP	Cholesteryl ester transfer protein
CETP	Cholesteryl ester transfer protein
DEP	Differentially expressed protein
DEPs	Differentially expressed proteins
FC	Fold change
GDM	Gestational diabetes mellitus
GO	Gene ontology
IADPSG	International Association of Diabetes and Pregnancy Study Groups
IGF	Insulin-like growth factor
IGFALS	Insulin-like growth factor-binding protein complex acid labile subunit
IGFBPs	Insulin-like growth factor binding proteins
IR	Insulin resistance
iTRAQ	Isobaric labeling for relative and absolute quantification
MF	Molecular function
MPV	Mean platelet volume
NTA	Nanoparticle tracking analysis
OGTT	Oral glucose tolerance test
PBS	Phosphate-buffered saline
PCA	Principal component analysis
PLAP	Placental alkaline phosphatase
PLAP-EXO	Placental-derived exosomes
PLS-DA	Partial least squares discriminant analysis
PPI	Protein–protein interaction
RYR2	Ryanodine receptor 2
T2DM	Type 2 diabetes mellitus
TEM	Transmission electron microscopy
T-EXO	Total plasma exosomes
TTR	Transthyretin

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-024-06919-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Acknowledgements

We are grateful to the BioRender website and PowerPoint for providing the image materials. We also grateful to the instrument analysis center of Shenzhen University for their assistance.

Author contributions

Liming Shen, Jing Lin, Danqing Zhao and Yi Liang designed the research and performed the experimental procedures; Liming Shen, Zhiyuan Liang, Mingxian Wang, Xiaoxiao Tang, Hongbin Zhuang, Hanghang Wang, Xiaopin Yin, Yuhan Huang, and Li Yin were all involved in the analysis of data and in drafting the manuscript. All authors edited the final manuscript. All authors read and approved the final manuscript.

Funding

This study was financially supported by the National Natural Science Foundation of China (Grant Nos. 81960284, 82160616,31870825), the Shenzhen Bureau of Science, Technology and Information (Nos.

JCYJ20170412110026229), and the Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, 2023SHIBS0003.

Data availability
The data that supports the findings of this study are available in the supplementary material of this article. All raw data and corresponding parameter have been deposited as online resource to the Figshare database with the name: "Proteomic analysis of plasma total exosomes and placenta-derived exosomes in patients with gestational diabetes mellitus in the first and second trimesters". (10.6084/m9.figshare.24986862).

Declarations

Ethics approval and consent to participate
The study was approved by the Human Research Ethics Committee of the Affiliated Hospital of Guizhou Medical University. Informed consent for this study was obtained from all of the participants. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
No applicable.

Competing interests
The authors declare no competing interests.

Received: 20 August 2024 / Accepted: 22 October 2024
Published online: 30 October 2024

References

1. Plows J, Stanley J, Baker P, Reynolds C, Vickers M. The pathophysiology of gestational diabetes Mellitus. Int J Mol Sci. 2018;19(11):3342.
2. Association AD. 2. Classification and diagnosis of diabetes: standards of Medical Care in Diabetes—2020. Diabetes Care. 2019;43(Supplement1):S14–31.
3. Zhang X, Gregg EW, Williamson DF, Barker LE, Thomas W, Bullard KM, Imperatore G, Williams DE, Albright AL. A1C level and future risk of diabetes: a systematic review. Diabetes Care. 2010;33(7):1665–73.
4. Federation ID. IDF diabetes atlas. 8th ed. International Diabetes Federation; 2017. pp. 905–911.
5. Deputy NP, Kim SY, Conrey EJ, Bullard KM. Prevalence and changes in Preexisting Diabetes and Gestational Diabetes among women who had a live birth — United States, 2012–2016. MMWR Morbidity Mortal Wkly Rep. 2018;67(43):1201–7.
6. Garmendia ML, Mondschein S, Montiel B, Kusanovic JP. Trends and predictors of gestational diabetes mellitus in Chile. Int J Gynaecol Obstet. 2020;148(2):210–8.
7. Vince K, Perković P, Matijević R. What is known and what remains unresolved regarding gestational diabetes mellitus (GDM). J Perinat Med. 2020;48(8):757–63.
8. Najafi F, Hasani J, Izadi N, Hashemi-Nazari S-S, Namvar Z, Mohammadi S, Sadeghi M. The effect of prepregnancy body mass index on the risk of gestational diabetes mellitus: a systematic review and dose-response meta-analysis. Obes Rev. 2019;20(3):472–86.
9. Abell SK, De Courten B, Boyle JA, Teede HJ. Inflammatory and other biomarkers: Role in Pathophysiology and Prediction of Gestational Diabetes Mellitus. Int J Mol Sci. 2015;16(6):13442–73.
10. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational diabetes. Lancet Diabetes Endocrinol. 2014;2(6):488–99.
11. Correa PJ, Vargas JF, Sen S, Illanes SE. Prediction of gestational diabetes early in pregnancy: targeting the long-term complications. Gynecol Obstet Invest. 2014;77(3):145–9.
12. Metzger BE, Coustan DR, Trimble ER. Hyperglycemia and adverse pregnancy outcomes. Clin Chem. 2019;65(7):937–8.
13. Vince K, Poljičanin T, Kejla P, Rodin U, Matijević R. Prevalence, risk factors and pregnancy outcomes of women with gestational diabetes in Croatia–a national study. Gynaecologia et perinatologia: J Gynecol Perinatol Reproductive Med Ultrasonic Diagnostics. 2017;26(3–4):124–8.
14. Xiong X, Saunders LD, Wang FL, Demianczuk NN. Gestational diabetes mellitus: prevalence, risk factors, maternal and infant outcomes. Int J Gynaecol Obstet. 2001;75(3):221–8.
15. Hod M, Kapur A, Sacks DA, Hadar E, Agarwal M, Di Renzo GC, Cabero Roura L, McIntyre HD, Morris JL, Divakar H. The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: a pragmatic guide for diagnosis, management, and care. Int J Gynaecol Obstet. 2015;131(Suppl 3):S173–211.
16. Hakanen T, Saha MT, Salo MK, Nummi T, Harjunmaa U, Lipiäinen L, Vuorela N. Mothers with gestational diabetes are more likely to give birth to children who experience early weight problems. Acta Paediatr. 2016;105(10):1166–72.
17. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet. 2009;373(9677):1773–9.
18. Daly B, Toulis KA, Thomas N, Gokhale K, Martin J, Webber J, Keerthy D, Jolly K, Saravanan P, Nirantharakumar K. Increased risk of ischemic heart disease, hypertension, and type 2 diabetes in women with previous gestational diabetes mellitus, a target group in general practice for preventive interventions: a population-based cohort study. PLoS Med. 2018;15(1):e1002488.
19. Griffith RJ, Alsweiler J, Moore AE, Brown S, Middleton P, Shepherd E, Crowther CA. Interventions to prevent women from developing gestational diabetes mellitus: an overview of Cochrane Reviews. Cochrane Database Syst Rev. 2020;6(6):Cd012394.
20. Ratner RE, Christophi CA, Metzger BE, Dabelea D, Bennett PH, Pi-Sunyer X, Fowler S, Kahn SE. Prevention of diabetes in women with a history of gestational diabetes: effects of metformin and lifestyle interventions. J Clin Endocrinol Metab. 2008;93(12):4774–9.
21. Aroda VR, Christophi CA, Edelstein SL, Zhang P, Herman WH, Barrett-Connor E, Delahanty LM, Montez MG, Ackermann RT, Zhuo X, et al. The effect of lifestyle intervention and metformin on preventing or delaying diabetes among women with and without gestational diabetes: the Diabetes Prevention Program outcomes study 10-year follow-up. J Clin Endocrinol Metab. 2015;100(4):1646–53.
22. Werner EF, Pettker CM, Zuckerwise L, Reel M, Funai EF, Henderson J, Thung SF. Screening for gestational diabetes mellitus: are the criteria proposed by the international association of the diabetes and pregnancy study groups cost-effective? Diabetes Care. 2012;35(3):529–35.
23. He C, Zheng S, Luo Y, Wang B. Exosome theranostics: biology and translational medicine. Theranostics. 2018;8(1):237–55.
24. Ludwig AK, Giebel B. Exosomes: small vesicles participating in intercellular communication. Int J Biochem Cell Biol. 2012;44(1):11–5.
25. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol. 2014;14(3):195–208.
26. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–83.
27. Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV, Govorun VM. Isolation of exosomes by differential centrifugation: theoretical analysis of a commonly used protocol. Sci Rep. 2015;5:17319.
28. Machtinger R, Laurent LC, Baccarelli AA. Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation. Hum Reprod Update. 2016;22(2):182–93.
29. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):1535750.
30. Salomon C, Scholz-Romero K, Sarker S, Sweeney E, Kobayashi M, Correa P, Longo S, Duncombe G, Mitchell MD, Rice GE, et al. Gestational diabetes mellitus is associated with changes in the concentration and bioactivity of placenta-derived exosomes in maternal circulation across gestation. Diabetes. 2016;65(3):598–609.
31. Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, Illanes SE, Mitchell MD, Rice GE. A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. PLoS ONE. 2014;9(6):e98667.
32. Lai A, Elfeky O, Rice GE, Salomon C. Optimized specific isolation of placenta-derived exosomes from maternal circulation. Methods Mol Biol. 2018;1710:131–8.
33. Jayabalan N, Nair S, Nuzhat Z, Rice GE, Zuñiga FA, Sobrevia L, Leiva A, Sanhueza C, Gutiérrez JA, Lappas M, et al. Cross talk between adipose tissue and Placenta in obese and gestational diabetes Mellitus pregnancies via Exosomes. Front Endocrinol (Lausanne). 2017;8:239.

34. Floriano JF, Willis G, Catapano F, Lima PR, Reis F, Barbosa AMP, Rudge MVC, Emanueli C. Exosomes could offer new options to combat the long-term complications inflicted by gestational diabetes mellitus. Cells. 2020;9(3).
35. Nair S, Jayabalan N, Guanzon D, Palma C, Scholz-Romero K, Elfeky O, Zuñiga F, Ormazabal V, Diaz E, Rice GE, et al. Human placental exosomes in gestational diabetes mellitus carry a specific set of miRNAs associated with skeletal muscle insulin sensitivity. Clin Sci (Lond). 2018;132(22):2451–67.
36. Thamotharan S, Ghosh S, James-Allan L, Lei MYY, Janzen C, Devaskar SU. Circulating extracellular vesicles exhibit a differential miRNA profile in gestational diabetes mellitus pregnancies. PLoS ONE. 2022;17(5):e0267564.
37. Bernea EG, Suica VI, Uyy E, Cerveanu-Hogas A, Boteanu RM, Ivan L, Ceausu I, Mihai DA, Ionescu-Tîrgovişte C, Antohe F. Exosome Proteomics reveals the deregulation of coagulation, complement and lipid metabolism proteins in gestational diabetes mellitus. Molecules. 2022;27(17).
38. Chahrour O, Cobice D, Malone J. Stable isotope labelling methods in mass spectrometry-based quantitative proteomics. J Pharm Biomed Anal. 2015;113:2–20.
39. Shen L, Zhang K, Feng C, Chen Y, Li S, Iqbal J, Liao L, Zhao Y, Zhai J. iTRAQ-Based proteomic analysis reveals Protein Profile in plasma from children with autism. Proteom Clin Appl. 2018;12(3):e1700085.
40. Shen L, Zhao D, Chen Y, Zhang K, Chen X, Lin J, Li C, Iqbal J, Zhao Y, Liang Y, et al. Comparative Proteomics Analysis of Serum Proteins in gestational diabetes during early and middle stages of pregnancy. Proteom Clin Appl. 2019;13(5):e1800060.
41. Zhao D, Shen L, Wei Y, Xie J, Chen S, Liang Y, Chen Y, Wu H. Identification of candidate biomarkers for the prediction of gestational diabetes mellitus in the early stages of pregnancy using iTRAQ quantitative proteomics. Proteom Clin Appl. 2017;11:7–8.
42. Zhang H, Zhao Y, Zhao D, Chen X, Khan NU, Liu X, Zheng Q, Liang Y, Zhu Y, Iqbal J, et al. Potential biomarkers identified in plasma of patients with gestational diabetes mellitus. Metabolomics. 2021;17(11):99.
43. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva A, Hod M, Kitzmiler JL, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010;33(3):676–82.
44. Van Deun J, Mestdagh P, Sormunen R, Cocquyt V, Vermaelen K, Vandesompele J, Bracke M, De Wever O, Hendrix A. The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. J Extracell Vesicles. 2014;3.
45. Subedi P, Schneider M, Philipp J, Azimzadeh O, Metzger F, Moertl S, Atkinson MJ, Tapio S. Comparison of methods to isolate proteins from extracellular vesicles for mass spectrometry-based proteomic analyses. Anal Biochem. 2019;584:113390.
46. Shen L, Yang A, Chen X, Xiao S, Liu X, Lin J, Zhao Y, Zhang K, Li C, Ke J, et al. Proteomic profiling of Cerebrum Mitochondria, myelin sheath, and Synaptosome Revealed Mitochondrial Damage and Synaptic Impairments in Association with 3 × Tg-AD mice Model. Cell Mol Neurobiol. 2022;42(6):1745–63.
47. Du X, Shi Q, Zhao Y, Xie Y, Li X, Liu Q, Iqbal J, Zhang H, Liu X, Shen L. Se-Methylselenocysteine (SMC) improves cognitive deficits by attenuating synaptic and Metabolic Abnormalities in Alzheimer's mice Model: a proteomic study. ACS Chem Neurosci. 2021;12(7):1112–32.
48. Shen L, Feng C, Zhang K, Chen Y, Gao Y, Ke J, Chen X, Lin J, Li C, Iqbal J, et al. Proteomics Study of Peripheral Blood mononuclear cells (PBMCs) in autistic children. Front Cell Neurosci. 2019;13:105.
49. Shen L, Zhang H, Lin J, Gao Y, Chen M, Khan NU, Tang X, Hong Q, Feng C, Zhao Y, et al. A combined proteomics and Metabolomics profiling to investigate the genetic heterogeneity of autistic children. Mol Neurobiol. 2022;59(6):3529–45.
50. Yan X, Zhang X, Li H, Zou Y, Lu W, Zhan M, Liang Z, Zhuang H, Ran X, Ma G, et al. Application of Proteomics and Machine Learning methods to study the Pathogenesis of Diabetic Nephropathy and screen urinary biomarkers. J Proteome Res. 2024;23(8):3612–25.
51. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res. 2012;40(Database issue):D1241–1244.
52. Shen L, Zhao D, Chen Y, Zhang K, Chen X, Lin J, Li C, Iqbal J, Zhao Y, Liang Y. Comparative proteomics analysis of serum proteins in gestational diabetes during early and middle stages of pregnancy. PROTEOMICS–Clinical Appl. 2019;13(5):1800060.
53. Liu X, Sun J, Wen X, Duan J, Xue D, Pan Y, Sun J, Zhang W, Cheng X, Wang C. Proteome profiling of gestational diabetes mellitus at 16-18 weeks revealed by LC-MS/MS. J Clin Lab Anal. 2020;34(9):e23424.
54. Ravnsborg T, Svaneklink S, Andersen LLT, Larsen MR, Jensen DM, Overgaard M. First-trimester proteomic profiling identifies novel predictors of gestational diabetes mellitus. PLoS ONE. 2019;14(3):e0214457.
55. Li Q, Li C, Jin J, Shen Y, Wang M. Clinical significance of neuregulin 4, afamin, and SERPINB1 in gestational diabetes mellitus and their relationship with insulin resistance. Evidence Based Complementary Alternative Med. 2022;2022.
56. Scholl P, Cole R, Ruczinski I, Gucek M, Diez R, Rennie A, Nathasingh C, Schulze K, Christian P, Yager J. Maternal serum proteome changes between the first and third trimester of pregnancy in rural southern Nepal. Placenta. 2012;33(5):424–32.
57. Zhao D, Shen L, Wei Y, Xie J, Chen S, Liang Y, Chen Y, Wu H. Identification of candidate biomarkers for the prediction of gestational diabetes mellitus in the early stages of pregnancy using iTRAQ quantitative proteomics. PROTEOMICS–Clinical Appl. 2017;11(7–8):1600152.
58. Liao Y, Xu G-F, Jiang Y, Zhu H, Sun L-J, Peng R, Luo Q. Comparative proteomic analysis of maternal peripheral plasma and umbilical venous plasma from normal and gestational diabetes mellitus pregnancies. Medicine. 2018;97(36).
59. Sreckovic I, Birner-Gruenberger R, Besenboeck C, Miljkovic M, Stojakovic T, Scharnagl H, Marsche G, Lang U, Kotur-Stevuljevic J, Jelic-Ivanovic Z. Gestational diabetes mellitus modulates neonatal high-density lipoprotein composition and its functional heterogeneity. Biochim et Biophys Acta (BBA)-Molecular Cell Biology Lipids. 2014;1841(11):1619–27.
60. Roverso M, Brioschi M, Banfi C, Visentin S, Burlina S, Seraglia R, Traldi P, Lapolla A. A preliminary study on human placental tissue impaired by gestational diabetes: a comparison of gel-based versus gel-free proteomics approaches. Eur J Mass Spectrom. 2016;22(2):71–82.
61. Alur V, Raju V, Vastrad B, Tengli A, Vastrad C, Kottureshetti S. Integrated bioinformatics analysis reveals novel key biomarkers and potential candidate small molecule drugs in gestational diabetes mellitus. Biosci Rep. 2021;41(5):BSR20210617.
62. Ai T, Chen F, Zhou S, Zhang J, Zheng H, Zhou Y, Hu W, Liu X, Li L, Lin J. Magnetic bead-based serum peptidome profiling in patients with gestational diabetes mellitus. BioMed Res Int. 2015;2015.
63. Eckmann M, Sheng Q, Baldwin S, Lister RL. Maternal hyperglycemia induces changes in gene expression and morphology in mouse placentas. Gynecol Reproductive Health. 2021;5(1).
64. Jayabalan N, Lai A, Nair S, Guanzon D, Scholz-Romero K, Palma C, McIntyre HD, Lappas M, Salomon C. Quantitative proteomics by SWATH-MS suggest an association between circulating exosomes and maternal metabolic changes in gestational diabetes mellitus. Proteomics. 2019;19(1–2):1800164.
65. Bernea EG, Suica VI, Uyy E, Cerveanu-Hogas A, Boteanu RM, Ivan L, Ceausu I, Mihai DA, Ionescu-Tîrgovişte C, Antohe F. Exosome Proteomics reveals the Deregulation of Coagulation, complement and lipid metabolism proteins in gestational diabetes Mellitus. Molecules. 2022;27(17):5502.
66. Billacura MP, Hanna K, Boocock DJ, Coveney C, Miles AK, Foulds GA, Murphy A, Tan A, Jackisch L, Sayers SR. Carnosine protects stimulus-secretion coupling through prevention of protein carbonyl adduction events in cells under metabolic stress. Free Radic Biol Med. 2021;175:65–79.
67. Zhang L, Zhang J, Han B, Chen C, Liu J, Sun Z, Liu M, Zhou P. Gestational diabetes mellitus-induced changes in proteomes and glycated/glycosylated proteomes of human colostrum. J Agric Food Chem. 2021;69(36):10749–59.
68. Qian Y, Sun H, Xiao H, Ma M, Xiao X, Qu Q. Microarray analysis of differentially expressed genes and their functions in omental visceral adipose tissues of pregnant women with vs. without gestational diabetes mellitus. Biomedical Rep. 2017;6(5):503–12.
69. Ma Y, Gao J, Yin J, Gu L, Liu X, Chen S, Huang Q, Lu H, Yang Y, Zhou H. Identification of a novel function of adipocyte plasma membrane-associated protein (APMAP) in gestational diabetes mellitus by proteomic analysis of omental adipose tissue. J Proteome Res. 2016;15(2):628–37.
70. Coussa A, Hasan HA, Barber TM. Early predictors of gestational diabetes mellitus in IVF-conceived pregnancies. Endocr Pract. 2021;27(6):579–85.
71. Ramachandrarao SP, Hamlin AA, Awdishu L, Overcash R, Zhou M, Proudfoot J, Ishaya M, Aghania E, Madrigal A, Kokoy-Mondragon C. Proteomic analyses of urine exosomes reveal new biomarkers of diabetes in pregnancy. Madridge J Diabetes. 2016;1(1):11.
72. Pillar N, Yoffe L, Hod M, Shomron N. The possible involvement of microRNAs in preeclampsia and gestational diabetes mellitus. Best Pract Res Clin Obstet Gynecol. 2015;29(2):176–82.
73. Mavreli D, Evangelinakis N, Papantoniou N, Kolialexi A. Quantitative comparative proteomics reveals candidate biomarkers for the early prediction of gestational diabetes mellitus: a preliminary study. vivo. 2020;34(2):517–25.

74. Boyle KE, Hwang H, Janssen RC, DeVente JM, Barbour LA, Hernandez TL, Mandarino LJ, Lappas M, Friedman JE. Gestational diabetes is characterized by reduced mitochondrial protein expression and altered calcium signaling proteins in skeletal muscle. PLoS ONE. 2014;9(9):e106872.
75. Liu M, Chen Y, Chen D. Association between transthyretin concentrations and gestational diabetes mellitus in Chinese women. Arch Gynecol Obstet. 2020;302:329–35.
76. Cai B, Du J. Role of bone morphogenic protein–4 in gestational diabetes mellitus–related hypertension. Experimental Therapeutic Med. 2021;22(1):1–8.
77. Zhang Q, Wu S, Sun G, Zhang R, Li X, Zhang Y, Huang F, Yuan D. Hyperglycemia aggravates monocyte-endothelial adhesion in human umbilical vein endothelial cells from women with gestational diabetes mellitus by inducing Cx43 overexpression. Ann Transl Med. 2021;9(3).
78. Sun X, Qu T, He X, Yang X, Guo N, Mao Y, Xu X, Sun X, Zhang X, Wang W. Screening of differentially expressed proteins from syncytiotrophoblast for severe early-onset preeclampsia in women with gestational diabetes mellitus using tandem mass tag quantitative proteomics. BMC Pregnancy Childbirth. 2018;18(1):1–11.
79. Teliga-Czajkowska J, Sienko J, Zareba-Szczudlik J, Malinowska-Polubiec A, Romejko-Wolniewicz E, Czajkowski K. Influence of glycemic control on coagulation and lipid metabolism in pregnancies complicated by pregestational and gestational diabetes mellitus. Adv Biomed. 2019:81–8.
80. Chen J, Yang X, Huang L, Zhang Z, Yao J, Liang H, Zhou W. Insulin resistance biomarkers in small-for-gestational-age infants born to mothers with gestational diabetes mellitus. J Maternal-Fetal Neonatal Med. 2022;35(25):9061–5.
81. Ramanjaneya M, Butler AE, Bashir M, Bettahi I, Moin ASM, Ahmed L, Elrayess MA, Hunt SC, Atkin SL, Abou-Samra AB. apoA2 correlates to gestational age with decreased apolipoproteins A2, C1, C3 and E in gestational diabetes. BMJ Open Diabetes Res Care. 2021;9(1):e001925.
82. Fuller H, Iles M, Moore JB, Zulyniak MA. Unique metabolic profiles associate with gestational diabetes and ethnicity in low-and high-risk women living in the UK. J Nutr. 2022;152(10):2186–97.
83. Singh A, Subramani E, Ray CD, Rapole S, Chaudhury K. Proteomic-driven biomarker discovery in gestational diabetes mellitus: a review. J Proteom. 2015;127:44–9.
84. Ramanjaneya M, Butler AE, Alkasem M, Bashir M, Jerobin J, Godwin A, Moin ASM, Ahmed L, Elrayess MA, Hunt SC. Association of complement-related proteins in subjects with and without second trimester gestational diabetes. Front Endocrinol. 2021;12:641361.
85. Ge L, Huang P, Miao H, Yu H, Wu D, Chen F, Lin Y, Lin Y, Li W, Hua J. The new landscape of differentially expression proteins in placenta tissues of gestational diabetes based on iTRAQ proteomics. Placenta. 2023;131:36–48.
86. Rayis DA, Ahmed AB, Sharif ME, ElSouli A, Adam I. Reliability of glycosylated hemoglobin in the diagnosis of gestational diabetes mellitus. J Clin Lab Anal. 2020;34(10):e23435.
87. Hu Z, Zhang M. Establishment of clinical diagnostic models using glucose, lipid, and urinary polypeptides in gestational diabetes mellitus. J Clin Lab Anal. 2021;35(7):e23833.
88. Hu Z, Tian Y, Li J, Hu M, Zhang M. Urinary peptides associated closely with gestational diabetes mellitus. Dis Markers. 2020;2020.
89. Miao Z, Wang J, Wang F, Liu L, Ding H, Shi Z. Comparative proteomics of umbilical vein blood plasma from normal and gestational diabetes mellitus patients reveals differentially expressed proteins associated with childhood obesity. Proteomics–Clinical Appl. 2016;10(11):1122–31.
90. Hu Z, Hou J, Zhang M. Levels of inter-alpha-trypsin inhibitor heavy chain H4 urinary polypeptide in gestational diabetes mellitus. Syst Biology Reproductive Med. 2021;67(6):428–37.
91. Chen L, Wang C-T, Forsyth NR, Wu P. Transcriptional profiling reveals altered biological characteristics of chorionic stem cells from women with gestational diabetes. Stem Cell Res Ther. 2020;11(1):1–15.
92. Shore N, Khurshid R, Saleem M. Alpha-1 microglobulin: a marker for early detection of tubular disorders in diabetic nephropathy. J Ayub Med Coll Abbottabad. 2010;22(4):53–5.
93. Siddiqui K, George TP, Joy SS, Nawaz SS. Association of sex hormone binding globulin with gestational age and parity in gestational diabetes mellitus. J Maternal-Fetal Neonatal Med. 2022;35(8):1433–8.
94. Sriboonvorakul N, Hu J, Boriboonhirunsarn D, Ng LL, Tan BK. Proteomics studies in gestational diabetes mellitus: a systematic review and meta-analysis. J Clin Med. 2022;11(10).
95. Roverso M, Dogra R, Visentin S, Pettenuzzo S, Cappellin L, Pastore P, Bogialli S. Mass spectrometry-based omics technologies for the study of gestational diabetes and the discovery of new biomarkers. Mass Spectrom Rev. 2023;42(4):1424–61.
96. Zhou T, Huang L, Wang M, Chen D, Chen Z, Jiang SW. A critical review of proteomic studies in gestational diabetes mellitus. J Diabetes Res. 2020;2020:6450352.
97. Bogdanet D, O'Shea PM, Halperin J, Dunne F. Plasma glycated CD59 (gCD59), a novel biomarker for the diagnosis, management and follow up of women with gestational diabetes (GDM) - protocol for prospective cohort study. BMC Pregnancy Childbirth. 2020;20(1):412.
98. Hertle E, van Greevenbroek MM, Stehouwer CD. Complement C3: an emerging risk factor in cardiometabolic disease. Diabetologia. 2012;55(4):881–4.
99. Ramanjaneya M, Butler AE, Alkasem M, Bashir M, Jerobin J, Godwin A, Moin ASM, Ahmed L, Elrayess MA, Hunt SC, et al. Association of Complement-Related Proteins in subjects with and without second trimester gestational diabetes. Front Endocrinol (Lausanne). 2021;12:641361.
100. Kennelly MA, Killeen SL, Phillips CM, Alberdi G, Lindsay KL, Mehegan J, Cronin M, McAuliffe FM. Maternal C3 complement and C-reactive protein and pregnancy and fetal outcomes: a secondary analysis of the PEARS RCT-An mHealth-supported, lifestyle intervention among pregnant women with overweight and obesity. Cytokine. 2022;149:155748.
101. Engström G, Hedblad B, Eriksson KF, Janzon L, Lindgärde F. Complement C3 is a risk factor for the development of diabetes: a population-based cohort study. Diabetes. 2005;54(2):570–5.
102. Peake PW, Kriketos AD, Campbell LV, Charlesworth JA. Response of the alternative complement pathway to an oral fat load in first-degree relatives of subjects with type II diabetes. Int J Obes (Lond). 2005;29(4):429–35.
103. Kopylov AT, Papysheva O, Gribova I, Kotaysch G, Kharitonova L, Mayatskaya T, Sokerina E, Kaysheva AL, Morozov SG. Molecular pathophysiology of diabetes mellitus during pregnancy with antenatal complications. Sci Rep. 2020;10(1):19641.
104. Moreno-Navarrete JM, Martínez-Barricarte R, Catalán V, Sabater M, Gómez-Ambrosi J, Ortega FJ, Ricart W, Blüher M, Frühbeck G, de Rodríguez S, et al. Complement factor H is expressed in adipose tissue in association with insulin resistance. Diabetes. 2010;59(1):200–9.
105. Hebecker M, Okemefuna AI, Perkins SJ, Mihlan M, Huber-Lang M, Józsi M. Molecular basis of C-reactive protein binding and modulation of complement activation by factor H-related protein 4. Mol Immunol. 2010;47(6):1347–55.
106. Skerka C, Zipfel PF. Complement factor H related proteins in immune diseases. Vaccine. 2008;26(Suppl 8):I9–14.
107. Ai T, Chen F, Zhou S, Zhang J, Zheng H, Zhou Y, Hu W, Liu X, Li L, Lin J. Magnetic bead-based serum peptidome profiling in patients with gestational diabetes mellitus. Biomed Res Int. 2015;2015:586309.
108. Girardi G, Lingo JJ, Fleming SD, Regal JF. Essential role of complement in pregnancy: from implantation to Parturition and Beyond. Front Immunol. 2020;11:1681.
109. Tschopp J, Chonn A, Hertig S, French LE. Clusterin, the human apolipoprotein and complement inhibitor, binds to complement C7, C8 beta, and the b domain of C9. J Immunol. 1993;151(4):2159–65.
110. Lepedda AJ, Lobina O, Rocchiccioli S, Nieddu G, Ucciferri N, De Muro P, Idini M, Nguyen HQ, Guarino A, Spirito R, et al. Identification of differentially expressed plasma proteins in atherosclerotic patients with type 2 diabetes. J Diabetes Complications. 2016;30(5):880–6.
111. Abdulwahab RA, Alaiya A, Shinwari Z, Allaith AAA, Giha HA. LC–MS/MS proteomic analysis revealed novel associations of 37 proteins with T2DM and notable upregulation of immunoglobulins. Int J Mol Med. 2019;43(5):2118–32.
112. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. Placenta. 2015;36(7):709–15.
113. Ozgu-Erdinc AS, Yilmaz S, Yeral MI, Seckin KD, Erkaya S, Danisman AN. Prediction of gestational diabetes mellitus in the first trimester: comparison of C-reactive protein, fasting plasma glucose, insulin and insulin sensitivity indices. J Matern Fetal Neonatal Med. 2015;28(16):1957–62.
114. Volpe L, Di Cianni G, Lencioni C, Cuccuru I, Benzi L, Del Prato S. Gestational diabetes, inflammation, and late vascular disease. J Endocrinol Invest. 2007;30(10):873–9.
115. Liu W, Huang Z, Tang S, Zhang Z, Yu Q, He J. Changes of serum sex hormone-binding globulin, Homocysteine, and hypersensitive CRP levels during pregnancy and their relationship with gestational diabetes Mellitus. Gynecol Obstet Invest. 2021;86(1–2):193–9.

116. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342(12):836–43.
117. Maguire PJ, Power KA, O'Higgins AC, Jackson S, Harley R, le Roux CW, Turner MJ. Maternal C-reactive protein in early pregnancy. Eur J Obstet Gynecol Reprod Biol. 2015;193:79–82.
118. Winzer C, Wagner O, Festa A, Schneider B, Roden M, Bancher-Todesca D, Pacini G, Funahashi T, Kautzky-Willer A. Plasma adiponectin, insulin sensitivity, and subclinical inflammation in women with prior gestational diabetes mellitus. Diabetes Care. 2004;27(7):1721–7.
119. Di Benedetto A, Russo GT, Corrado F, Di Cesare E, Alessi E, Nicocia G, D'Anna R, Cucinotta D. Inflammatory markers in women with a recent history of gestational diabetes mellitus. J Endocrinol Invest. 2005;28(1):34–8.
120. Ling Y, Su J, Lin J, Wang S. Screening of serum biomarkers of preeclampsia by proteomics combination with bioinformatics. Hypertens Pregnancy. 2019;38(3):184–92.
121. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turning offensive. Nat Rev Nephrol. 2016;12(7):383–401.
122. Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. Placenta. 2015;36(2):101–14.
123. Jiang J, Wang H, Liu K, He S, Li Z, Yuan Y, Yu K, Long P, Wang J, Diao T, et al. Association of complement C3 with Incident Type 2 diabetes and the Mediating role of BMI: a 10-Year Follow-Up study. J Clin Endocrinol Metab. 2023;108(3):736–44.
124. Stoikou M, Grimolizzi F, Giaglis S, Schäfer G, van Breda SV, Hoesli IM, Lapaire O, Huhn EA, Hasler P, Rossi SW, et al. Gestational diabetes Mellitus is Associated with altered neutrophil activity. Front Immunol. 2017;8:702.
125. De Luccia TPB, Pendeloski KPT, Ono E, Mattar R, Pares DBS, Yazaki Sun S, Daher S. Unveiling the pathophysiology of gestational diabetes: studies on local and peripheral immune cells. Scand J Immunol. 2020;91(4):e12860.
126. Omazić J, Viljetić B, Ivić V, Kadivnik M, Zibar L, Müller A, Wagner J. Early markers of gestational diabetes mellitus: what we know and which way forward? Biochem Med (Zagreb). 2021;31(3):030502.
127. Siennicka A, Kłysz M, Chełstowski K, Tabaczniuk A, Marcinowska Z, Tarnowska P, Kulesza J, Torbe A, Jastrzębska M. Reference values of D-Dimers and fibrinogen in the course of physiological pregnancy: the potential impact of selected risk factors-a pilot study. Biomed Res Int. 2020;2020:3192350.
128. Dong C, Gu X, Chen F, Long Y, Zhu D, Yang X, Qiu X, Gao G, Qi W. The variation degree of coagulation function is not responsible for extra risk of hemorrhage in gestational diabetes mellitus. J Clin Lab Anal. 2020;34(4):e23129.
129. Abdel Gader AG, Khashoggi TY, Habib F, Awadallah SB. Haemostatic and cytokine changes in gestational diabetes mellitus. Gynecol Endocrinol. 2011;27(5):356–60.
130. Gorar S, Alioglu B, Ademoglu E, Uyar S, Bekdemir H, Candan Z, Saglam B, Koc G, Culha C, Aral Y. Is there a tendency for thrombosis in gestational diabetes Mellitus? J Lab Physicians. 2016;8(2):101–5.
131. Kopylov AT, Kaysheva AL, Papysheva O, Gribova I, Kotaysch G, Kharitonova L, Mayatskaya T, Krasheninnikova A, Morozov SG. Association of proteins modulating immune response and insulin clearance during gestation with antenatal complications in patients with gestational or type 2 diabetes mellitus. Cells. 2020;9(4).
132. Ding L, Fan L, Xu X, Fu J, Xue Y. Identification of core genes and pathways in type 2 diabetes mellitus by bioinformatics analysis. Mol Med Rep. 2019;20(3):2597–608.
133. Chu SG, Becker RC, Berger PB, Bhatt DL, Eikelboom JW, Konkle B, Mohler ER, Reilly MP, Berger JS. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. J Thromb Haemost. 2010;8(1):148–56.
134. Domingueti CP, Dusse LM, Carvalho M, de Sousa LP, Gomes KB, Fernandes AP. Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. J Diabetes Complications. 2016;30(4):738–45.
135. Weisel JW. Fibrinogen and fibrin. Adv Protein Chem. 2005;70:247–99.
136. Chapman J, Dogan A. Fibrinogen alpha amyloidosis: insights from proteomics. Expert Rev Proteom. 2019;16(9):783–93.
137. Ozder A, Eker HH. Investigation of mean platelet volume in patients with type 2 diabetes mellitus and in subjects with impaired fasting glucose: a cost-effective tool in primary health care? Int J Clin Exp Med. 2014;7(8):2292–7.
138. Santilli F, Simeone P, Liani R, Davì G. Platelets and diabetes mellitus. Prostaglandins Other Lipid Mediat. 2015;120:28–39.
139. Politou M, Mougiou V, Kollia M, Sokou R, Kafalidis G, Iliodromiti Z, Valsami S, Boutsikou T, Iacovidou N. High-risk pregnancies and their impact on neonatal primary hemostasis. Semin Thromb Hemost. 2020;46(4):435–45.
140. Iyidir OT, Degertekin CK, Yilmaz BA, Toruner FB, Akturk M, Arslan M. Elevated mean platelet volume is associated with gestational diabetes mellitus. Gynecol Endocrinol. 2014;30(9):640–3.
141. Kebapcilar L, Kebapcilar AG, Ilhan TT, Ipekci SH, Baldane S, Pekin A, Kulaksizoglu M, Celik C. Is the Mean platelet volume a predictive marker of a low apgar score and Insulin Resistance in gestational diabetes Mellitus? A retrospective case-control study. J Clin Diagn Res. 2016;10(10):Oc06–10.
142. Rusak T, Misztal T, Rusak M, Branska-Januszewska J, Tomasiak M. Involvement of hyperglycemia in the development of platelet procoagulant response: the role of aldose reductase and platelet swelling. Blood Coagul Fibrinolysis. 2017;28(6):443–51.
143. Challier JC, Basu S, Bintein T, Minium J, Hotmire K, Catalano PM, Hauguel-de Mouzon S. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. Placenta. 2008;29(3):274–81.
144. Angelo AGS, Neves CTC, Lobo TF, Godoy RVC, Ono É, Mattar R, Daher S. Monocyte profile in peripheral blood of gestational diabetes mellitus patients. Cytokine. 2018;107:79–84.
145. Retnakaran A, Retnakaran R. Adiponectin in pregnancy: implications for health and disease. Curr Med Chem. 2012;19(32):5444–50.
146. Turer AT, Scherer PE. Adiponectin: mechanistic insights and clinical implications. Diabetologia. 2012;55(9):2319–26.
147. Bozkurt L, Göbl CS, Baumgartner-Parzer S, Luger A, Pacini G, Kautzky-Willer A. Adiponectin and leptin at early pregnancy: association to actual glucose disposal and risk for GDM-A prospective cohort study. Int J Endocrinol. 2018;2018:5463762.
148. Shen L, Zhao Y, Zhang H, Feng C, Gao Y, Zhao D, Xia S, Hong Q, Iqbal J, Liu XK. Advances in biomarker studies in autism spectrum disorders. Rev Biomark Stud Psych Neurodegenerative Dis. 2019:207–33.
149. Eroğlu H, Örgül G, Tonyalı NV, Biriken D, Polat N, Yücel A, Yazihan N, Şahin D. The role of Afamin and other Trace Elements in the prediction of GDM: a Tertiary Center Experience. Biol Trace Elem Res. 2021;199(12):4418–22.
150. Vora N, Kalagiri R, Mallett LH, Oh JH, Wajid U, Munir S, Colon N, Raju VN, Beeram MR, Uddin MN. Proteomics and metabolomics in Pregnancy-An overview. Obstet Gynecol Surv. 2019;74(2):111–25.
151. Zemany L, Bhanot S, Peroni OD, Murray SF, Moraes-Vieira PM, Castoldi A, Manchem P, Guo S, Monia BP, Kahn BB. Transthyretin antisense oligonucleotides lower circulating RBP4 levels and improve insulin sensitivity in obese mice. Diabetes. 2015;64(5):1603–14.
152. Liu M, Chen Y, Chen D. Association between transthyretin concentrations and gestational diabetes mellitus in Chinese women. Arch Gynecol Obstet. 2020;302(2):329–35.
153. Xiong T, Zhong C, Zhou X, Chen R, Xiao M, Wu Y, Hu X, Wang W, Li X, Liu C, et al. Maternal circulating transthyretin level is longitudinally Associated with increased risk of gestational diabetes Mellitus: it is not just an Indicator of Nutritional Status. Diabetes Care. 2017;40(5):e53–4.
154. Choi SH, Kwak SH, Youn BS, Lim S, Park YJ, Lee H, Lee N, Cho YM, Lee HK, Kim YB, et al. High plasma retinol binding protein-4 and low plasma adiponectin concentrations are associated with severity of glucose intolerance in women with previous gestational diabetes mellitus. J Clin Endocrinol Metab. 2008;93(8):3142–8.
155. Santulli G, Pagano G, Sardu C, Xie W, Reiken S, D'Ascia SL, Cannone M, Marziliano N, Trimarco B, Guise TA, et al. Calcium release channel RyR2 regulates insulin release and glucose homeostasis. J Clin Invest. 2015;125(11):4316.
156. Valent AM, Choi H, Kolahi KS, Thornburg KL. Hyperglycemia and gestational diabetes suppress placental glycolysis and mitochondrial function and alter lipid processing. Faseb j. 2021;35(3):e21423.
157. Mohammad S, Taha A, Akhtar K, Bamezai RN, Baquer NZ. In vivo effect of Trigonella foenum graecum on the expression of pyruvate kinase, phosphoenolpyruvate carboxykinase, and distribution of glucose transporter (GLUT4) in alloxan-diabetic rats. Can J Physiol Pharmacol. 2006;84(6):647–54.
158. Lewandowski SL, Cardone RL, Foster HR, Ho T, Potapenko E, Poudel C, VanDeusen HR, Sdao SM, Alves TC, Zhao X, et al. Pyruvate Kinase Controls Signal Strength in the insulin secretory pathway. Cell Metab. 2020;32(5):736–e750735.
159. Bahr BL, Price MD, Merrill D, Mejia C, Call L, Bearss D, Arroyo J. Different expression of placental pyruvate kinase in normal, preeclamptic and intrauterine growth restriction pregnancies. Placenta. 2014;35(11):883–90.
160. Faal S, Abedi P, Jahanfar S, Ndeke JM, Mohaghegh Z, Sharifipour F, Zahedian M. Sex hormone binding globulin for prediction of gestational diabetes

mellitus in pre-conception and pregnancy: a systematic review. Diabetes Res Clin Pract. 2019;152:39–52.
161. Liu X, Sun J, Wen X, Duan J, Xue D, Pan Y, Sun J, Zhang W, Cheng X, Wang C. Proteome profiling of gestational diabetes mellitus at 16–18 weeks revealed by LC-MS/MS. J Clin Lab Anal. 2020;34(9):e23424.
162. Wei Y, He A, Huang Z, Liu J, Li R. Placental and plasma early predictive biomarkers for gestational diabetes mellitus. Proteom Clin Appl. 2022;16(4):e2200001.
163. Temur M, Serpim G, Tuzluoğlu S, Taşgöz FN, Şahin E, Üstünyurt E. Comparison of serum human pregnancy-specific beta-1-glycoprotein 1 levels in pregnant women with or without preeclampsia. J Obstet Gynaecol. 2020;40(8):1074–8.
164. Gunderson EP. The role of lactation in GDM women. Clin Obstet Gynecol. 2013;56(4):844–52.

Publisher's note