

Original Article

Placenta inflammation is closely associated with gestational diabetes mellitus

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Abstract: Objective: To investigate the potential role of placenta inflammation in gestational diabetes mellitus (GDM) and construct a model for the diagnosis of GDM. Methods: In this study, transcriptome-wide profiling datasets, GSE70493 and GSE128381 were downloaded from Gene Expression Omnibus (GEO) database. Significant immune-related genes were identified separately to be the biomarkers for the diagnosis of GDM by using random forest model (RF), support vector machine model (SVM), and generalized linear model (GLM). Results: RF was the best model and was used to select the four key immune-related genes (FABP4, DKK1, CXCL10, and IL1RL1) to diagnose GDM. A nomogram model was constructed to predict GDM based on the four key immune-related genes by using “rms” package. The relative proportion of 22 immune cell types were calculated by using CIBERSORT algorithm. Higher M1 macrophage ratio and lower M2 macrophage ratio in GDM placenta compared to normal patients were observed. Conclusions: This study provides clues that inflammation was correlated with GDM and suggests inflammation may be the cause and also the potential targets of GDM.

Keywords: GDM, placenta, inflammation, nomogram, consensus clustering, immune-related genes

Introduction

As the most universal metabolic disturbance of pregnancy, gestational diabetes mellitus (GDM), is defined as “diabetes diagnosed in the second or third trimester of pregnancy which was not clearly overt diabetes prior to gestation” [1]. The morbidity of GDM differs largely among different countries even among states inside the same country due to the using of discrepant criterion or other causes, such as ethnicity and the level of economy [2]. The prevalence of GDM is in the scope of 3.0 to 21.2% in the countries of Asian [3]. In America, the prevalence of GDM exceeds 9% [3] and is increasing with a speed of almost 6.3% [4]. Once diagnosed with GDM, both gravidae and fetus are exposed to many kinds of risks due to the state dysglycemia. For the maternal, the complications include caesarean section, polyhydramnios, pre-eclampsia, shoulder dystocia, and gestational hypertension [2, 5-8]. For the offspring, the common complications contain neonatal hypoglycemia, birth injury, macrosomia, neonatal unit admission, preterm birth, and

respiratory distress [2, 5-9]. What’s more, some studies indicate than women with GDM are more likely to be diagnosed with cardiovascular disease and type 2 diabetes mellitus after conception and their creations are more likely to develop type 2 diabetes mellitus in their early life compared to control [10-12].

At present, the specific pathogenesis of GDM has not been confirmed. Insulin resistance (IR) was the basic pathogenesis of GDM, which has been confirmed as the initiating factor of GDM [13]. Although the concrete mechanism is still unclear, many researches showed that inflammation play a pivotal role in insulin resistance and pancreatic beta cells failure [14-17]. Once pregnant, the body gradually enters into a situation of low-grade systemic inflammation [18]. A number of studies have shown that inflammatory factors are the initiating factors in the development of IR. Placental tissue has a strong endocrine function, and can synthesize and secrete a variety of inflammatory cytokines, which aggravate the chronic inflammatory reaction and the degree of maternal IR. Inflam-

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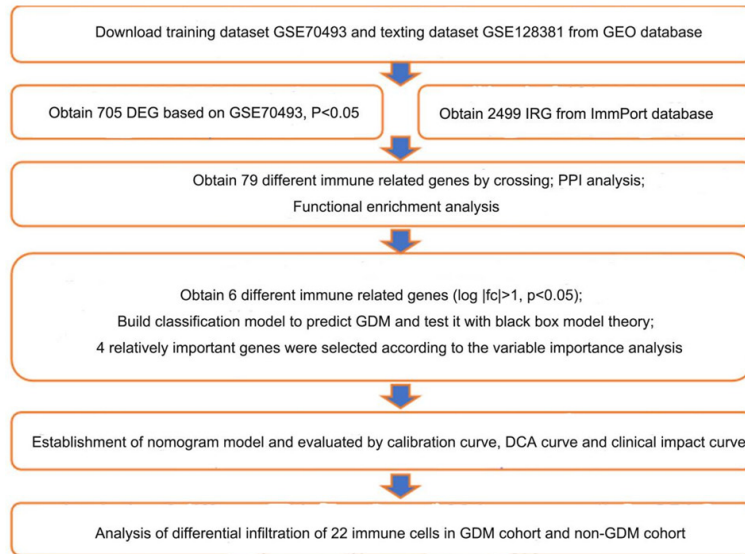


Figure 1. Workflow of the research. Abbreviations are defined as follows, Gene Expression Omnibus database (GEO), differentially expressed gene (DEG), immune-related gene (IRG), protein-protein interaction (PPI), gestational diabetes mellitus (GDM), decision curve analysis (DCA).

matory factors secreted by placental tissue include NK κ B, IL-6, IL-8, IL-1 β and TNF- α . As a pro-inflammatory cytokine, IL-1 β can induce apoptosis of islet β cells and promote the release of IL-6, IL-8 and other inflammatory factors through NK- κ B pathway. IL-6 and IL-8 can stimulate a variety of lymphoid and inflammatory cells and aggravate the inflammatory response. TNF- α is considered as an independent risk factor of GDM because of its function of blocking insulin signal transmission and glucose transport [19-23]. Ines Mrizak et al. report that some immune cells, such as CD68+ or CD14+ cells, are found increased in GDM placentas and the expression of some pro-inflammatory like IL-6, TLR, and TGF- β are also increased in GDM placenta [23]. Zhiyan Yu et al. report that IL-38 is increased both in GDM placentas and serum and may contribute to the development of GDM [24]. This indicates that immune cells and the cytokines they secreted may play a vital role in GDM. However, the concrete role of placenta inflammation in GDM is still unclear.

As part of the microenvironment of placenta, immune cells have been explored preliminary in recent years [23]. However, more work should be done as it is still sealed of the exact relationship between immune cells and placenta inflammation and its role in the occurrence and

process of GDM. Thanks to the rapid development of machine learning, we can further investigate the complicated mechanisms of many diseases from a different angle.

This study aimed to explore the role of placenta inflammation in GDM through comparing the integrated gene expression profiling data downloaded from the Gene expression omnibus (GEO) database. And further try to identify immune related genes as diagnostic biomarkers for GDM patients, which may be helpful for the diagnosis and treatment of GDM. In addition, we also investigated the potential relationship between immune cells and placenta inflammation.

Methods

Data collection

The two transcriptome-wide profiling datasets, GSE70493 [25] and GSE128381 [26] were downloaded from Gene Expression Omnibus database (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). 31 non-GDM and 32 GDM placental tissue specimen data are contained in GSE0493. GSE128381 contains 6 GDM and 177 non-GDM placental tissues. GSE70493 is used as training dataset due to its relative balanced data distribution, and GSE128381 is utilized as testing dataset for analysis. Data of immune-related genes (IRGs) was downloaded from ImmPort database (<https://www.immport.org/shared/>) and finally 2499 immune-related genes are obtained. The schematic of the research is shown in **Figure 1**.

Differentially expression analysis and protein-protein interaction analysis

The differentially expressed genes (DEGs) between GDM and non-GDM of dataset GSE70493 under the criteria of $P < 0.05$ were obtained by using “limma” [27] package in R. We further intersected 705 DEGs and 2499 immune-related genes (IRGs) to obtain different immune-related genes (DIRGs). Functional interactions

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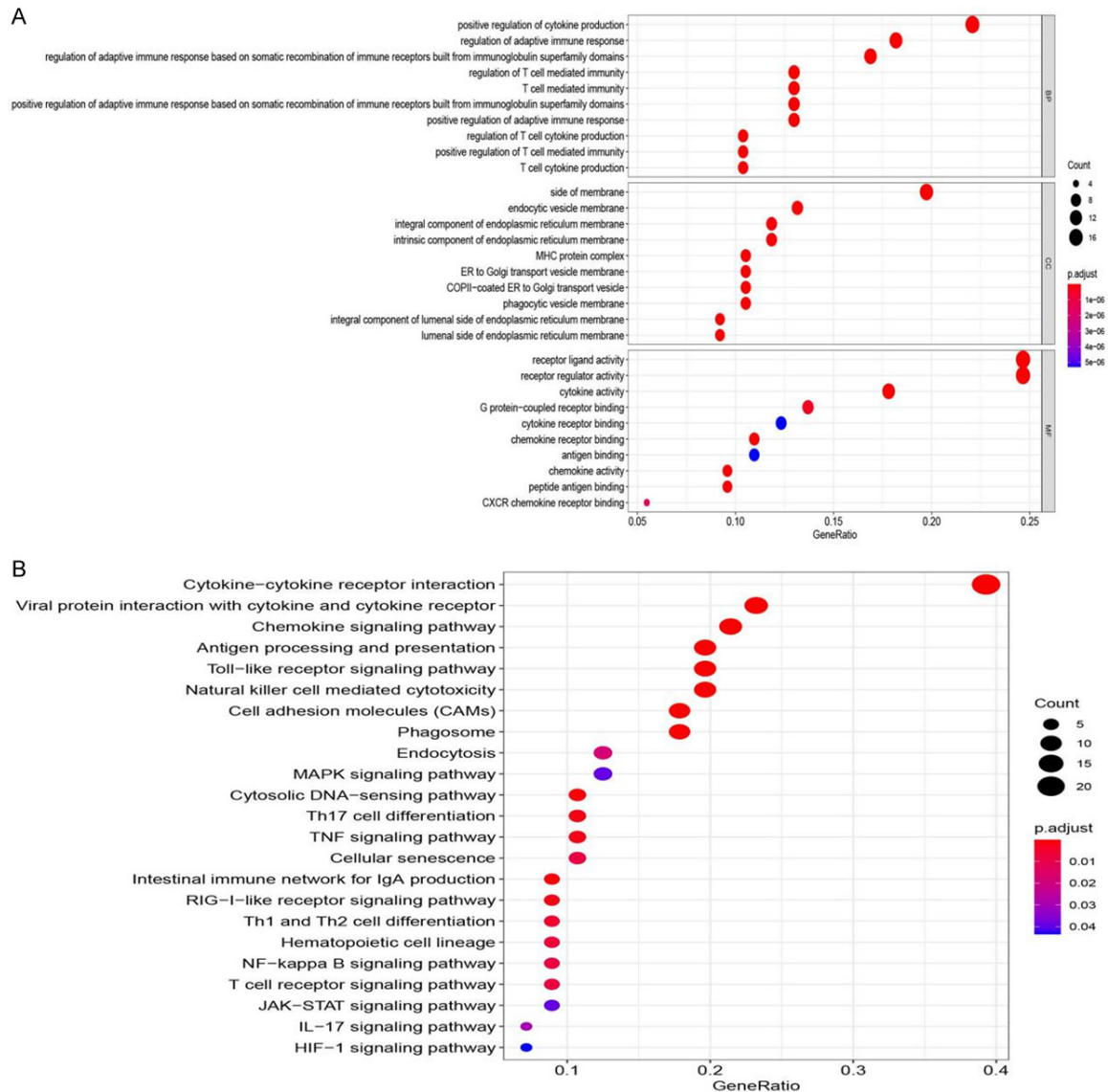


Figure 3. Gene Ontology and KEGG pathway enrichment analysis. A. Biological process (BP, up), cellular component (CC, middle), and molecular function (MF, low) analysis results of 76 DIRGs. B. Result of KEGG pathway enrichment analysis of the 76 DIRGs. Proportion of DIRGs are exhibited in the X-axis and different categories are shown in the Y-axis. The number of genes enriched in particular category are manifested by the size of the circle. The color of the circle denotes different properties.

DEGs and 2499 IRGs and got 79 DIRGs (**Figure 2A**). Then functional interactions of the 79 were investigated by using STRING 11.0. And then the protein-protein interaction (PPI) network was constructed via Cytoscape 3.8.0 as is shown in **Figure 2B**.

Gene ontology and KEGG pathway enrichment analysis

To further explore the enriched pathways and functions of the 79 DIRGs, the genes were

imported in DAVID 6.8, and further visualized the enriched result by using “ggplot2” package in R. These genes are mainly positive regulation of cytokine productions, located in side of membrane, and mainly exhibit receptor ligand activity and receptor regulator activity (**Figure 3A**). And the KEGG enrichment analysis indicated that the 79 DIRGs are mainly take part in cytokine-cytokine receptor interaction, viral protein interaction with cytokine and cytokine receptor (**Figure 3B**). These results indicated that GDM is closely related with inflammation

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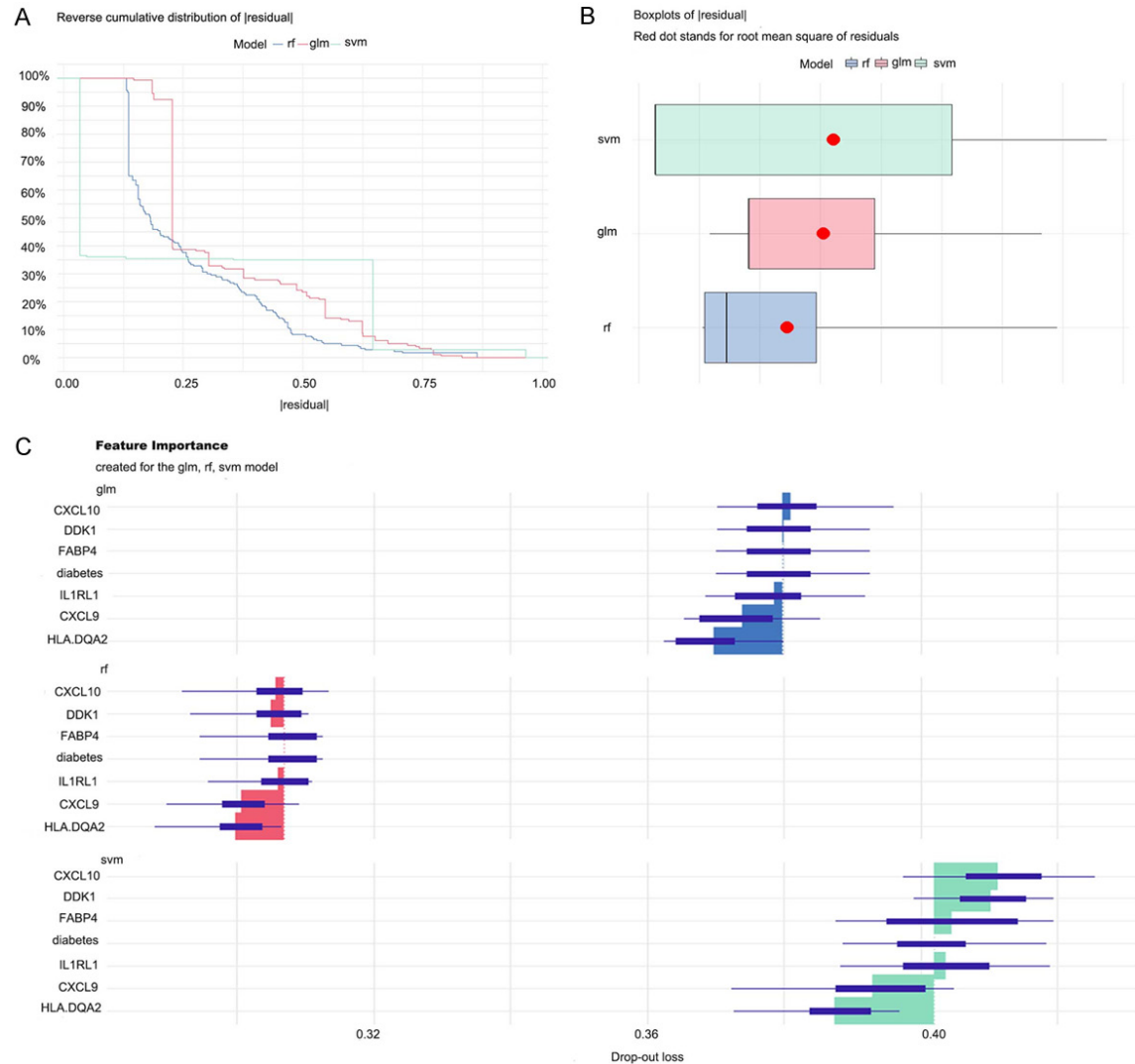


Figure 4. Construction and assessment of RF, GLM and SVM model. A. Cumulative residual distribution map of the sample. B. Boxplots of the residuals of the sample. Red dot stands for root mean square of residuals. C. The importance of the variables in RF, GLM and SVM model.

and placenta inflammation may play a vital role in GDM.

Construction and assessment of RF, GLM and SVM model

To further narrow down the range of key immune-related genes, three models were established. 6 genes (DDK1, ILRL1, CXCL9, HLA-DQA2, CXCL10 and FABP4), whose $\log|FC| > 0.1$, from 76 DIRGs are selected as key genes for the construction of the three models. Random forest model (RF), support vector machine model (SVM), and generalized linear model (GLM) were created independently on the ground of training GSE70493 dataset. Then the

explanatory feature of “DALEX” package in R was utilized to analyze the aforementioned 3 models and residual distribution was plotted to get the best model on the ground of the test set. As shown in **Figure 4A** and **4B**, RF model was identified as the most suitable model as it owns the least sample residual. Then, four explanatory variables (DDK1, ILRL1, FABP4 and CXCL10) were selected from RF model for further analysis (**Figure 4C**).

Further analysis of four important IRGs

Then, four most vital explanatory variables (DDK1, ILRL1, FABP4 and CXCL10) in RF model were selected for further analysis. **Figure**

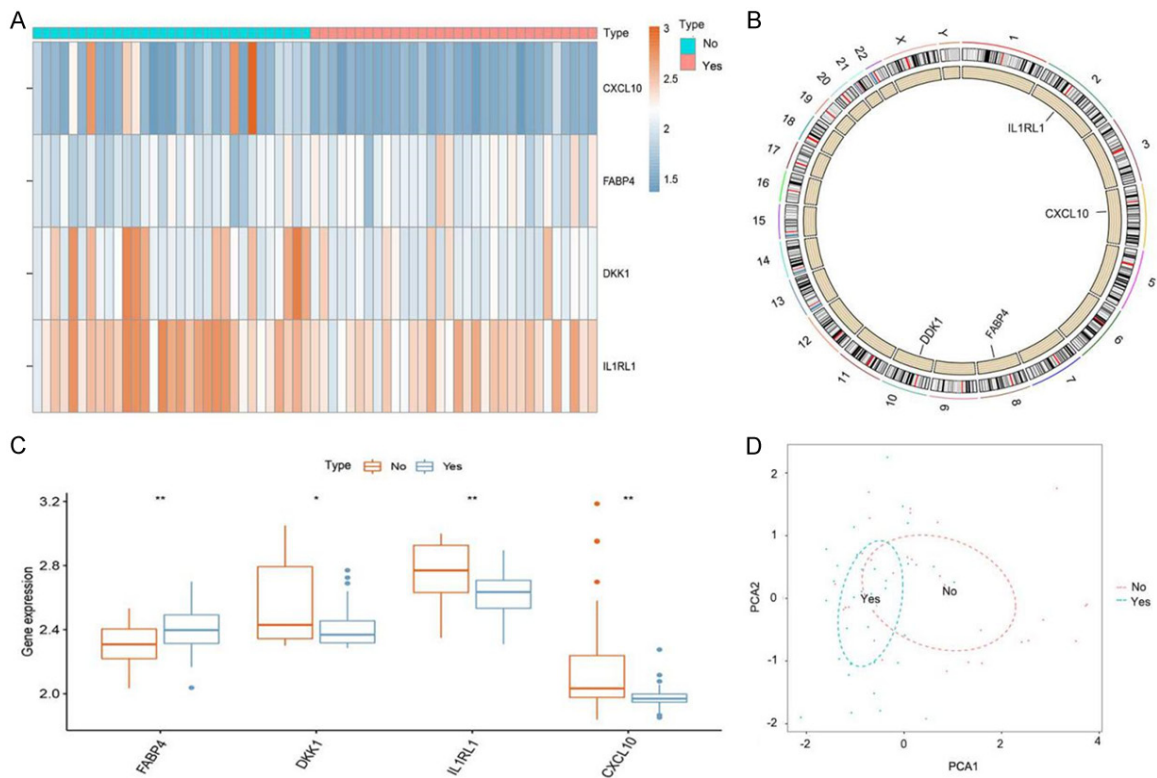


Figure 5. Relative expression level of CXCL10, FABP2, DKK1, IL1RL1. A. Heat map of the expression pattern of CXCL10, FABP4, DKK1 and IL1RL1. B. The chromosomal locations of DKK1, ILRL1, FABP4 and CXCL10. C. The relative expression level of CXCL10, FABP4, DKK1 and IL1RL1 between GDM and non-GDM from GSE70493 dataset. D. Principal component analysis shows that the four genes aforementioned can clearly distinguished GDM and non-GDM.

5B showed the chromosomal locations of DKK1, ILRL1, FABP4 and CXCL10. The expression of DKK1, CXCL10 and IL1RL1 in GDM placenta were less than them in non-GDM placenta. However, FABP4 expression in GDM placenta was higher than it in non-GDM placenta (Figure 5A, 5C). As the principal component analysis result in Figure 5D, the four genes aforementioned can clearly distinguished GDM and non-GDM, which indicated that they may play key roles in the diagnosis of GDM. The correlations of the genes were also analyzed as shown in Figure 6. We can found that HLA.DQA2 and CXCL9 own high correlation coefficients with other four genes (DKK1, ILRL1, FABP4 and CXCL10), which indicated that these two gene have high function similarities with the other four genes. Thus, HLA.DQA2 and CXCL9 can be excluded when select diagnostic biomarkers for GDM, which can reduce unnecessary resource waste.

Construction and assessment of a nomogram model for GDM diagnosis

“Rms” package was used to establish a nomogram model for GDM diagnosis based on the four DIRGs (DKK1, ILRL1, FABP4 and CXCL10) (Figure 7A). Then, calibration curve was used to evaluate the predictive power of the nomogram model. The calibration curve indicated that the error between the actual GDM risk and the predicted risk is very small, suggesting the nomogram model owns high accuracy to predict GDM (Figure 7B). Decision curve analysis (DCA) indicated that the “nomogram” curve was higher than the gray line, “DKK1” curve, “ILRL1” curve, “FABP4” curve, and “CXCL10” curve, suggested that the patients could benefit from the nomogram model at high risk threshold from 0 to 1 and the clinical benefit of the nomogram model was higher than the “DKK1” “ILRL1” “FABP4”, “CXCL10” curve (Figure 7C). To evaluate the clinical effects of the nomogram model more

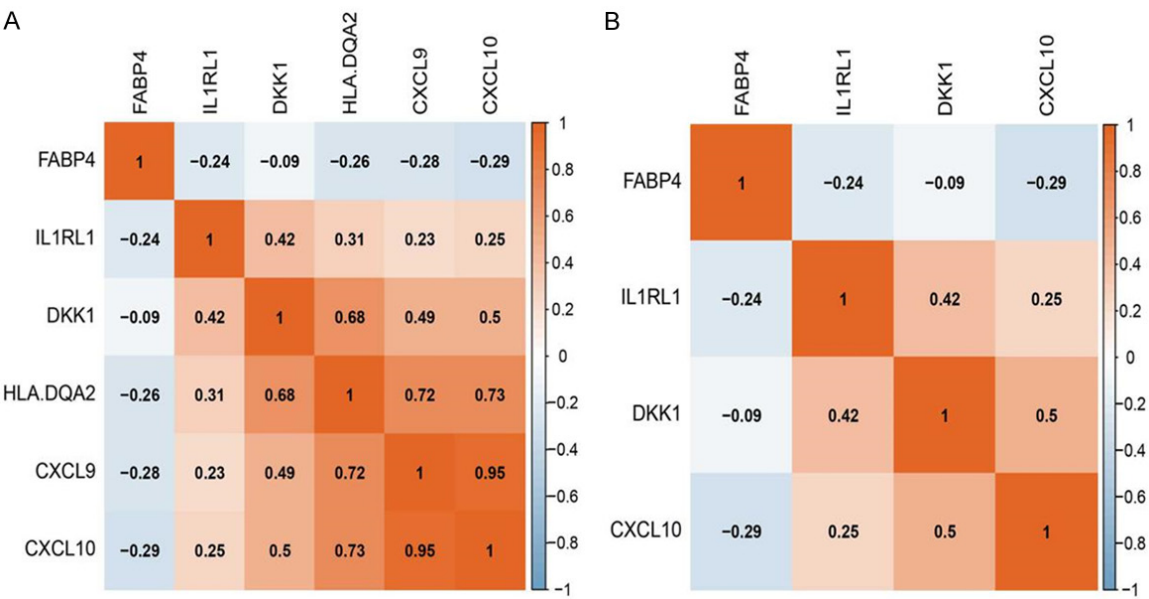


Figure 6. Correlation among selected immune-related genes. A. The correlation among DKK1, ILRL1, CXCL9, HLA-DQA2, CXCL10 and FABP4. B. The correlation among CXCL10, FABP4, DKK1 and IL1RL1.

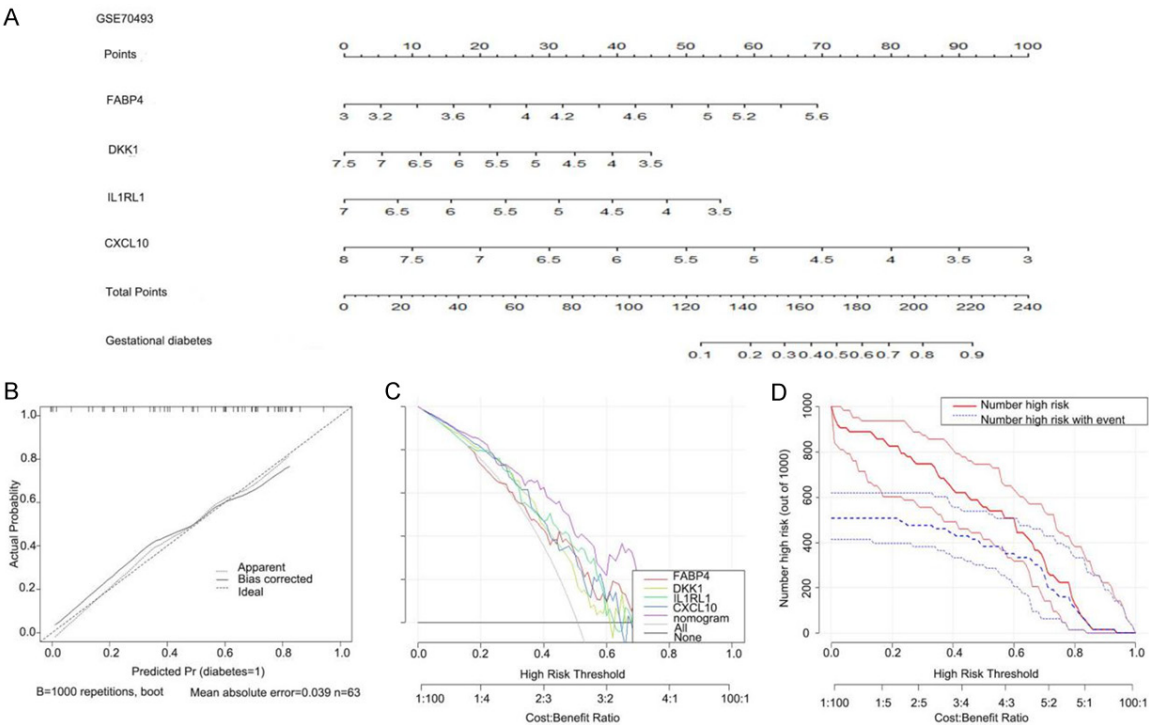


Figure 7. Construction and validation of a nomogram model for GDM diagnosis based on the training dataset GSE70493. A. Nomogram to predict the occurrence of GDM. B. Calibration curve to assess the predictive power of the nomogram model. C. DCA curve to evaluate the clinical value of the nomogram model. D. Clinical impact curve based on the DCA curve to assess the nomogram model.

visually, the clinical impact curve on the ground of DCA curve was drawn. The “Number high risk” curve was closely to the “Number

high risk with event” curve at high risk threshold from 0.3 to 1, which indicated that the nomogram model owns extraordinary predic-

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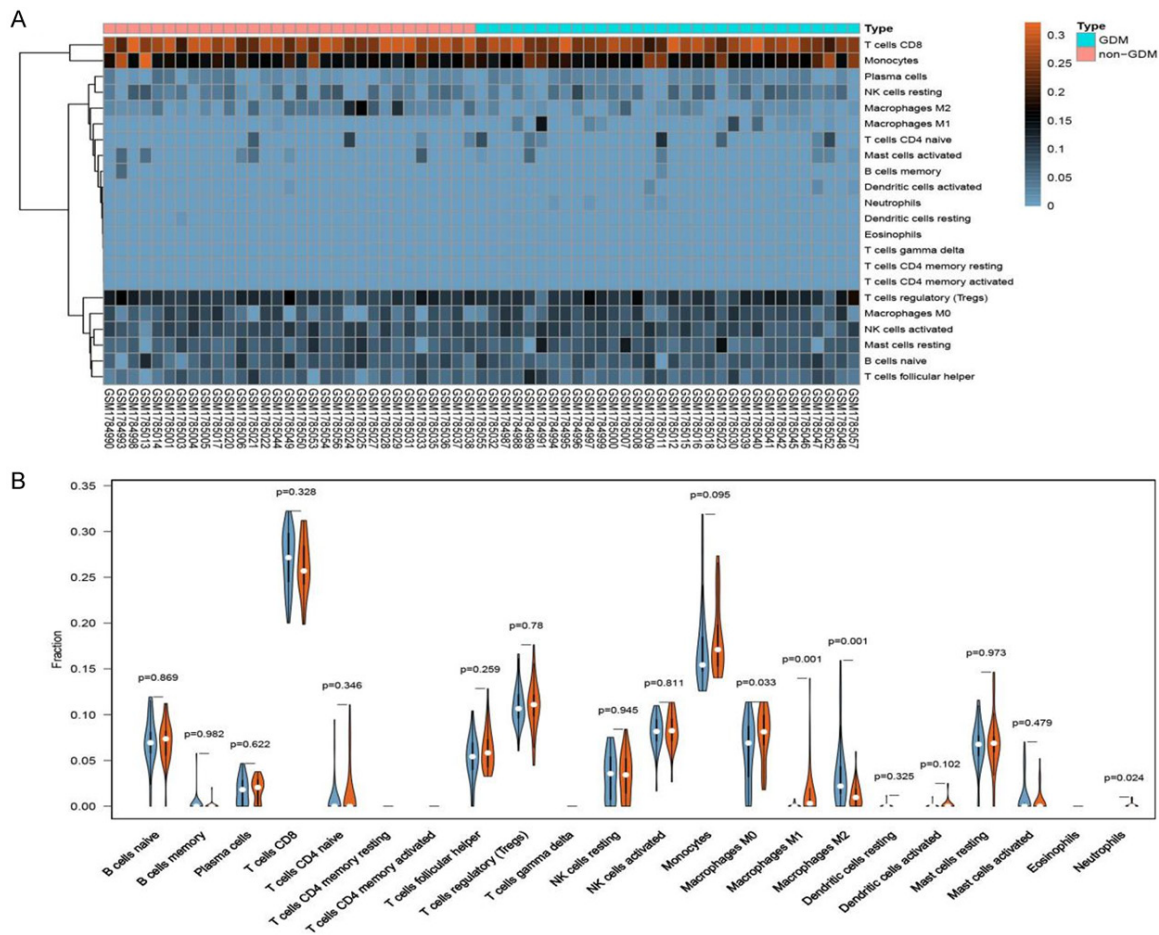


Figure 8. Distribution of the immune cells in placenta. A. Characteristics of infiltrated immune cells. B. Differences of the infiltrate immune cells between the GDM group and non-GDM group.

tive power (Figure 7D). These results, in some respects, also indicated that the four genes may play key role in the process of GDM.

Distribution of the immune cells in placenta

To make a better understand of the relationship between inflammatory and GDM, the relative proportion of 22 immune cell types in each samples were calculated. Then, we compared the 22 immune cells infiltration between non-GDM and GDM samples and the results are visualized using the heat map and the histogram. We found that the infiltration abundance of M0 macrophage cells, M1 macrophage cells and neutrophils cells were higher in GDM samples than in non-GDM samples, while the infiltration abundance of M2 macrophage cells in GDM samples were lower. The infiltration abundance of the other immune cells has no statistical difference (Figure 8A and 8B).

Discussion

Gestational diabetes mellitus is a most universal metabolic disturbance of pregnancy [1]. GDM places both the maternal and the offspring at risk of many kinds of diseases [29]. Although the main causes of GDM, increasing maternal age, adiposity and less of physical exercise, have been defined, more work should be done to promote the diagnosis and treatment of GDM. Recently, inflammation has been found to exert import role during GDM. Many studies enriched in systemic inflammation [30, 31], the inflammation of placenta is less explored. Thus, by using machine learning, the role of placenta inflammation is investigated. The differentially expressed immune-related genes between GDM and non-GDM placenta were got. A model for the diagnosis of GDM is also constructed based on the differentially expressed immune-related genes.

Recent studies show that tumor necrosis factor- α (TNF- α) and IL-6 are closely associated with GDM as their expression are both increased in GDM placenta [32-34]. However, the role of many other inflammatory mediators in GDM are still unclear. In this study, 79 differentially expressed immune-related genes were obtained and made for Gene Ontology and KEGG pathway analysis. Further construction and assessment of RF, GLM and SVM model lead our view to four key genes (DKK1, IL1RL1, FABP4 and CXCL10). DKK1, which can inhibit LRP5/6 interaction with WNT and form a ternary complex with KREMEN then promote LRP5/6 internalization, can antagonize canonical WNT signaling. DKK1 takes part in inflammation in many diseases like periodontitis [35], hepatocellular carcinoma, and hepatitis [36]. Lucia D'Amico et al. [37] reported that DKK1 can exhibit immune suppressive effects via targeting β -catenin. Xueqian Zhuang et al. reported that DKK1 can suppress the recruitment of PTGS2-induced macrophage and neutrophil recruitment in lung metastases [38]. Our results showed that DKK1 is down-regulated in placenta of GDM patients compared to normal control. This may be one of the reasons why macrophage proportion is higher in GDM placenta (see latter). As a Fatty acid-binding protein, FABP4 is mainly derived from macrophage and adipocyte [39] and also resolved in many diseases such as type2 diabetes mellitus [40], acute lung injury [41], and insulin resistance [42]. Research of Jee Young Chung et al. shows that silencing of FABP4 can result in reduction of inflammation and then alleviate insulin resistance [42], which indicates that FABP4 may play as an impeller during inflammation. In fact, our results also found that the relative expression of FABP4 is higher in GDM placenta than the non-GDM dose. IL1RL1 (interleukin-1 receptor-like 1), also known as ST2, is reported to participate in inflammation as well [43]. By binding IL-33, IL1RL1 exerts its role like the promotion of hepatic inflammation [44] and the recruitment of macrophage [45]. We found the relative expression of IL1RL1 in GDM is lower in non-GDM. Fragment of CXCL10, C-X-C motif chemokine 9, has been reported to diminish the recruitment of neutrophil and joint inflammation in antigen-induced arthritis [46]. CXCL10 is one of the markers of M1 macrophage, which can recruit activated T cells by binding its receptor CXCR3 [47]. What we observed is that

CXCL10 expression is relative lower in GDM than non-GDM. This is interesting as it is widely believed CXCL10 is a kind of inflammation markers. We then constructed a nomogram model for GDM diagnosis by using the DKK1, IL1RL1, FABP4 and CXCL10. We found that this model owns extraordinary predictive power and patients can benefit from the nomogram model at high risk threshold from 0 to 1. There are many minimally invasive methods for obtaining placental tissue, such as ultrasound-guided puncture. However, given the high risk and cost, the clinical application of this Nomogram is limited. It is hoped that safer and more economical methods of obtaining placental tissue will be developed in the future.

Immune cells infiltration is studied in tumor in depth. However, there are little reports about the immune cells infiltration in GDM placenta. Thus, for further investigation of the distribution of immune cells in placenta, we used CIBERSORT algorithm to explore the thing. We found that the ratio of M1 macrophage in GDM placenta is much higher than non-GDM placenta. And M2 macrophage ratio is less in GDM placenta than it in non-GDM. It is generally believed that, the M1 macrophage (classical macrophage) can produce many kinds of pro-inflammatory cytokines, such as CXCL10, and mediators which result in inflammation. However, M2 macrophage (alternative) has an effect of anti-inflammation. We also found that the relative M1 macrophage ratio is higher in GDM placenta and the M2 macrophage relative ratio is the opposite. Those results in consistent with the overall cognitive of macrophage. Indeed, Ines Mrizak et al. reported that the macrophage infiltration is increased in GDM placenta compared to the normal group [23]. All these results indicate that macrophage is one of the most important contributor of placenta inflammation in GDM. These results may shed light on further research of GDM.

There are also some shortcomings in our study. The results, like the expression level of DKK1, IL1RL1, FABP4 and CXCL10 may need further quantitative polymerase chain reaction and western blot or immunohistochemistry verification. And the nomogram model may need further examining before the clinical application due to the limitation of sample capacity. We did not analyze the prognosis of GDM based on the

nomogram as the associated clinical characteristics is nonexistent in GEO.

In conclusion, we investigated the potential correlation between placenta inflammation and the occurrence of GDM by machine learning and found their tight relationships. Some immune-related genes, like DKK1, ILRL1, FABP4, and CXCL10, are highly expressed in GDM placenta. And M1 macrophage ratio is higher in GDM placenta compared to the control group. Thus placenta inflammation may play a vital role in GDM.

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Disclosure of conflict of interest

None.

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References

- [1] American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. *Diabetes Care* 2018; 41 Suppl 1: S13-S27.
- [2] Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS and Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; 358: 1991-2002.
- [3] Yuen L and Wong VW. Gestational diabetes mellitus: challenges for different ethnic groups. *World J Diabetes* 2015; 6: 1024-1032.
- [4] Carpenter MW and Coustan DR. Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol* 1982; 144: 768-773.
- [5] Fadl HE, Ostlund IK, Magnuson AF and Hanson US. Maternal and neonatal outcomes and time trends of gestational diabetes mellitus in Sweden from 1991 to 2003. *Diabet Med* 2010; 27: 436-441.
- [6] Wendland EM, Torloni MR, Falavigna M, Trujillo J, Dode MA, Campos MA, Duncan BB and Schmidt MI. Gestational diabetes and pregnancy outcomes—a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. *BMC Pregnancy Childbirth* 2012; 12: 23.
- [7] Billionnet C, Mitancher D, Weill A, Nizard J, Alla F, Hartemann A and Jacqueminet S. Gestational diabetes and adverse perinatal outcomes from 716,152 births in France in 2012. *Diabetologia* 2017; 60: 636-644.
- [8] O'Sullivan EP, Avalos G, O'Reilly M, Dennedy MC, Gaffney G and Dunne F. Atlantic Diabetes in Pregnancy (DIP): the prevalence and outcomes of gestational diabetes mellitus using new diagnostic criteria. *Diabetologia* 2011; 54: 1670-1675.
- [9] Mortier I, Blanc J, Tosello B, Gire C, Bretelle F and Carcopino X. Is gestational diabetes an independent risk factor of neonatal severe respiratory distress syndrome after 34 weeks of gestation? A prospective study. *Arch Gynecol Obstet* 2017; 296: 1071-1077.
- [10] Bellamy L, Casas JP, Hingorani AD and Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* 2009; 373: 1773-1779.
- [11] HAPO Study Cooperative Research Group. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study. *Int J Gynaecol Obstet* 2002; 78: 69-77.
- [12] Sullivan SD, Umans JG and Ratner R. Gestational diabetes: implications for cardiovascular health. *Curr Diab Rep* 2012; 12: 43-52.
- [13] Lavery JA, Friedman AM, Keyes KM, Wright JD and Ananth CV. Gestational diabetes in the United States: temporal changes in prevalence rates between 1979 and 2010. *BJOG* 2017; 124: 804-813.
- [14] Glass CK and Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. *Cell Metab* 2012; 15: 635-645.
- [15] Shoelson SE, Lee J and Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006; 116: 1793-1801.
- [16] Ying W, Fu W, Lee YS and Olefsky JM. The role of macrophages in obesity-associated islet inflammation and β -cell abnormalities. *Nat Rev Endocrinol* 2020; 16: 81-90.
- [17] Eizirik DL, Colli ML and Ortis F. The role of inflammation in insulinitis and beta-cell loss in type 1 diabetes. *Nat Rev Endocrinol* 2009; 5: 219-226.
- [18] Mor G, Cardenas I, Abrahams V and Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. *Ann N Y Acad Sci* 2011; 1221: 80-87.
- [19] Hunt JS and Robertson SA. Uterine macrophages and environmental programming for pregnancy success. *J Reprod Immunol* 1996; 32: 1-25.
- [20] Hauguel-de Mouzon S and Guerre-Millo M. The placenta cytokine network and inflammatory signals. *Placenta* 2006; 27: 794-798.

- [21] Pantham P, Aye IL and Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta* 2015; 36: 709-715.
- [22] Radaelli T, Varastehpour A, Catalano P and Hauguel-De Mouzon S. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. *Diabetes* 2003; 52: 2951-2958.
- [23] Mrizak I, Grissa O, Henault B, Fekih M, Bouslema A, Boumaiza I, Zaouali M, Tabka Z and Khan NA. Placental infiltration of inflammatory markers in gestational diabetic women. *Gen Physiol Biophys* 2014; 33: 169-176.
- [24] Yu Z, Liu J, Zhang R, Huang X, Sun T, Wu Y, Hambly BD and Bao S. IL-37 and 38 signalling in gestational diabetes. *J Reprod Immunol* 2017; 124: 8-14.
- [25] Binder AM, LaRocca J, Lesseur C, Marsit CJ and Michels KB. Epigenome-wide and transcriptome-wide analyses reveal gestational diabetes is associated with alterations in the human leukocyte antigen complex. *Clin Epigenetics* 2015; 7: 79.
- [26] Cox B, Tsamou M, Vrijens K, Neven KY, Winckelmans E, de Kok TM, Plusquin M and Nawrot TS. A co-expression analysis of the placental transcriptome in association with maternal pre-pregnancy BMI and newborn birth weight. *Front Genet* 2019; 10: 354.
- [27] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015; 43: e47.
- [28] Gao Y, Chen L, Cai G, Xiong X, Wu Y, Ma D, Li SC and Gao Q. Heterogeneity of immune microenvironment in ovarian cancer and its clinical significance: a retrospective study. *Oncoimmunology* 2020; 9: 1760067.
- [29] Cutchie WA, Cheung NW and Simmons D. Comparison of international and New Zealand guidelines for the care of pregnant women with diabetes. *Diabet Med* 2006; 23: 460-468.
- [30] Skórzyńska-Dziduszko KE, Kimber-Trojanar Ż, Patro-Małyza J, Olszewska A, Zaborowski T and Małecka-Massalska T. An interplay between obesity and inflammation in gestational diabetes mellitus. *Curr Pharm Biotechnol* 2016; 17: 603-613.
- [31] Lekva T, Norwitz ER, Aukrust P and Ueland T. Impact of systemic inflammation on the progression of gestational diabetes mellitus. *Curr Diab Rep* 2016; 16: 26.
- [32] Aye IL, Lager S, Ramirez VI, Gaccioli F, Dudley DJ, Jansson T and Powell TL. Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways. *Biol Reprod* 2014; 90: 129.
- [33] Yu J, Zhou Y, Gui J, Li AZ, Su XL and Feng L. Assessment of the number and function of macrophages in the placenta of gestational diabetes mellitus patients. *J Huazhong Univ Sci Technolog Med Sci* 2013; 33: 725-729.
- [34] Zhang J, Chi H, Xiao H, Tian X, Wang Y, Yun X and Xu Y. Interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α) single nucleotide polymorphisms (SNPs), inflammation and metabolism in gestational diabetes mellitus in inner mongolia. *Med Sci Monit* 2017; 23: 4149-4157.
- [35] Liu J, Ren X, Zhang M, Lei Y, Chen Y and He H. Roles of Wnt3a and Dkk1 in experimental periodontitis. *J Dent Sci* 2017; 12: 220-225.
- [36] Zhang R, Real CI, Liu C, Baba HA, Gerken G, Lu M and Broering R. Hepatic expression of onco-genes Bmi1 and Dkk1 is up-regulated in hepatitis B virus surface antigen-transgenic mice and can be induced by treatment with HBV particles or lipopolysaccharides in vitro. *Int J Cancer* 2017; 141: 354-363.
- [37] D'Amico L, Mahajan S, Capietto AH, Yang Z, Zamani A, Ricci B, Bumpass DB, Meyer M, Su X, Wang-Gillam A, Weilbaecher K, Stewart SA, DeNardo DG and Faccio R. Dickkopf-related protein 1 (Dkk1) regulates the accumulation and function of myeloid derived suppressor cells in cancer. *J Exp Med* 2016; 213: 827-840.
- [38] Zhuang X, Zhang H, Li X, Li X, Cong M, Peng F, Yu J, Zhang X, Yang Q and Hu G. Differential effects on lung and bone metastasis of breast cancer by Wnt signalling inhibitor DKK1. *Nat Cell Biol* 2017; 19: 1274-1285.
- [39] Zhong CQ, Zhang XP, Ma N, Zhang EB, Li JJ, Jiang YB, Gao YZ, Yuan YM, Lan SQ, Xie D and Cheng SQ. FABP4 suppresses proliferation and invasion of hepatocellular carcinoma cells and predicts a poor prognosis for hepatocellular carcinoma. *Cancer Med* 2018; 7: 2629-2640.
- [40] Aleksandrova K, Drogan D, Weikert C, Schulze MB, Fritsche A, Boeing H and Pischon T. Fatty acid-binding protein 4 and risk of type 2 diabetes, myocardial infarction, and stroke: a prospective cohort study. *J Clin Endocrinol Metab* 2019; 104: 5991-6002.
- [41] Gong Y, Yu Z, Gao Y, Deng L, Wang M, Chen Y, Li J and Cheng B. FABP4 inhibitors suppress inflammation and oxidative stress in murine and cell models of acute lung injury. *Biochem Biophys Res Commun* 2018; 496: 1115-1121.
- [42] Chung JY, Ain QU, Song Y, Yong SB and Kim YH. Targeted delivery of CRISPR interference system against Fabp4 to white adipocytes ameliorates obesity, inflammation, hepatic steatosis, and insulin resistance. *Genome Res* 2019; 29: 1442-1452.
- [43] Molofsky AB, Savage AK and Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity* 2015; 42: 1005-1019.

- [44] Strobel HJ and Russell JB. Regulation of beta-glucosidase in *Bacteroides ruminicola* by a different mechanism: growth rate-dependent de-repression. *Appl Environ Microbiol* 1987; 53: 2505-2510.
- [45] Fang M, Li Y, Huang K, Qi S, Zhang J, Zgodzinski W, Majewski M, Wallner G, Gozdz S, Macek P, Kowalik A, Pasiarski M, Grywalska E, Vatan L, Nagarsheth N, Li W, Zhao L, Kryczek I, Wang G, Wang Z, Zou W and Wang L. IL33 promotes colon cancer cell stemness via JNK activation and macrophage recruitment. *Cancer Res* 2017; 77: 2735-2745.
- [46] Boff D, Crijns H, Janssens R, Vanheule V, Menezes GB, Macari S, Silva TA, Amaral FA and Proost P. The chemokine fragment CXCL9 (74-103) diminishes neutrophil recruitment and joint inflammation in antigen-induced arthritis. *J Leukoc Biol* 2018; 104: 413-422.
- [47] Dangaj D, Bruand M, Grimm AJ, Ronet C, Barras D, Duttagupta PA, Lanitis E, Duraiswamy J, Tanyi JL, Benencia F, Conejo-Garcia J, Ramay HR, Montone KT, Powell DJ Jr, Gimotty PA, Facciabene A, Jackson DG, Weber JS, Rodig SJ, Hodi SF, Kandalaft LE, Irving M, Zhang L, Foulkas P, Rusakiewicz S, Delorenzi M and Coukos G. Cooperation between constitutive and inducible chemokines enables T cell engraftment and immune attack in solid tumors. *Cancer Cell* 2019; 35: 885-900, e810.