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Original Article

Transcriptome and co-expression network analysis reveal the association between immune system and insulin resistance of type 2 diabetes

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Abstract: Type 2 diabetes (T2D) is a worldwide chronic disease as insulin resistance, malfunction of insulin secretion or a defect of sensitivity to insulin. Although, many researches have demonstrated the association of genetic factor and T2D, the pathogenesis of T2D still remains unknown. Here, we analyzed a global gene expression dataset of T2D with insulin resistance and healthy control individuals with/without T2D family history. Differentially expression analysis revealed that TNFRSF4, PGLYRP1, IL32, HBA1, HBM, MYC, HYAL3 genes involved in immune system are differentially expressed in T2D. Furthermore, coexpression analysis of immune related genes was performed to identify genes that were associated with immune pathway and diabetes. In addition, the coexpression genes were used to construct a protein-protein interaction network to demonstrate the gene interactions occurring in T2D. Overall, our results revealed the association of immune system and insulin resistance T2D and demonstrate the potential genetic identifications involved in T2D.

Keywords: Type 2 diabetes, immune pathway, coexpression network

Introduction

Type 2 diabetes (T2D), a chronic disorder is normally characterized as insulin resistance, malfunction of insulin secretion or a defect of sensitivity to insulin. The liver continues to generate blood sugar and its accumulation is mainly due to the compromise of adipocytes and muscle cells during their glucose intake, causing long term nutrient excess. Research also suggests T2D are linked with inflammation and alteration of immune response [1]. Current opinions regarding inflammation driven T2D are hypoxia, amyloid and lipid deposition, glucotoxicity and oxidative stress [+].

The correlation between T2D and insulin resistance has been widely accepted for decades [2]. It has been identified that skeletal muscle is the major site of insulin-stimulated glucose metabolism and insulin resistance is the central pathogenesis in skeletal muscle that may impact whole body glucose homeostasis [3], while other major metabolic disorders such as cardiovascular, endocrine, hepatic disorders, infections and even cancer might happen afterwards.

Current opinion shows that insulin resistance is determined by the level of insulin receptor including receptor down-regulation and impaired affinity to insulin in T2D. As downstream of hyperglycaemia and other metabolic disturbances, tyrosine kinase activity of insulin receptor is also disturbed by inflammation cytokines such as TNFα [4] in T2D.

Yet studies also identified alterations and dysfunctions of Serine phosphorylation of insulin receptor substrate proteins 1 (IRS1) and IRS2 play important roles in the insulin resistance via their activation kinetics [5] and cellular compartmentalization [6].

Recent researches suggest that inflammation factors and immune responses that are modified during insulin resistance and involved in the innate immune system are worthy of further

Table 1. GO annotation for differentially expressed genes

	GO.ID	Term	Significant	*P* value
T2D vs FH pos	GO:0006955	Immune response	42	4.50E-05
	GO:0044707	Single-multicellular organism process	147	1.00E-04
	GO:0045321	Leukocyte activation	27	1.20E-04
	GO:0032501	Multicellular organismal process	151	1.30E-04
	GO:0050865	Regulation of cell activation	20	1.70E-04
	GO:0002682	Regulation of immune system process	35	3.00E-04
	GO:0045072	Regulation of interferon-gamma biosynthetic process	4	3.10E-04
	GO:0006954	Inflammatory response	23	3.50E-04
	GO:0009617	Response to bacterium	17	3.50E-04
	GO:0046649	Lymphocyte activation	23	3.70E-04
T2D vs FH nag	GO:0002694	Regulation of leukocyte activation	20	3.60E-05
	GO:0002544	Chronic inflammatory response	5	3.70E-05
	GO:0015669	Gas transport	4	6.00E-05
	GO:0051249	Regulation of lymphocyte activation	18	6.30E-05
	GO:0050865	Regulation of cell activation	20	1.00E-04
	GO:0044707	Single-multicellular organism process	142	1.20E-04
	GO:0032501	Multicellular organismal process	146	1.40E-04
	GO:0015671	Oxygen transport	3	2.60E-04
	GO:0072049	Comma-shaped body morphogenesis	3	2.60E-04
	GO:0050670	Regulation of lymphocyte proliferation	11	2.70E-04

discovery in the pathogenesis of T2D [8]. T cells were shown to be involved in the maintenance of self-tolerance and suppression of autoreactive T cells [10], among which CD8+ effector T cells were recruited and promoted a pro-inflammatory cascade together with insulin resistance [13].

On the other hand, experiment showed that transfer of B cells or serum IgG from mice model into B-cell-deficient recipients also lead to the transfer of insulin resistance [11]. Moreover, NKT cells were shown to play an important role in the visceral adipose tissue in association with tissue specific macrophages during the development of High Fat Diet induced insulin resistance [12]. Glucose intolerance and adipose tissue inflammation was observed to be exacerbated after NKT cells were triggered with α-GalCer [13]. Therefore current studies hold evidences for existence of immune and inflammation alteration in T2D and this may explain the biological mechanism between insulin resistance and T2D.

To further explore the association between immune system and insulin resistance and T2D, we compared the whole-transcriptome expression profiles of insulin resistance T2D with healthy control individuals in this study. The function annotation of Gene Ontology, pathway enrichment was performed to reveal the function alteration of immune system. Furthermore, protein-protein interaction network analysis was constructed to explore the potential molecular interactions involved in T2D.

Results

Gene expression analysis of insulin resistance of type 2 diabetes

To investigate the gene expression alteration in insulin resistance T2D, we download microarray dataset from GEO repository. The GSE25462 data include 10 patient of T2D diabetes with insulin resistance, 15 individuals with 1 or both parents with T2D (FH+) but normoglycemia, and 25 patient with normoglycemia and no family history of diabetes (FH-). The raw data were normalized with limma algorithm. To obtain genes related to insulin resistance of T2D, fold change of gene expression and corresponding t test *P* value were calculated between T2D patients and normoglycemia individuals. Totally, 405 genes meet the criteria of fold change > 1.5 and t test p value < 0.05

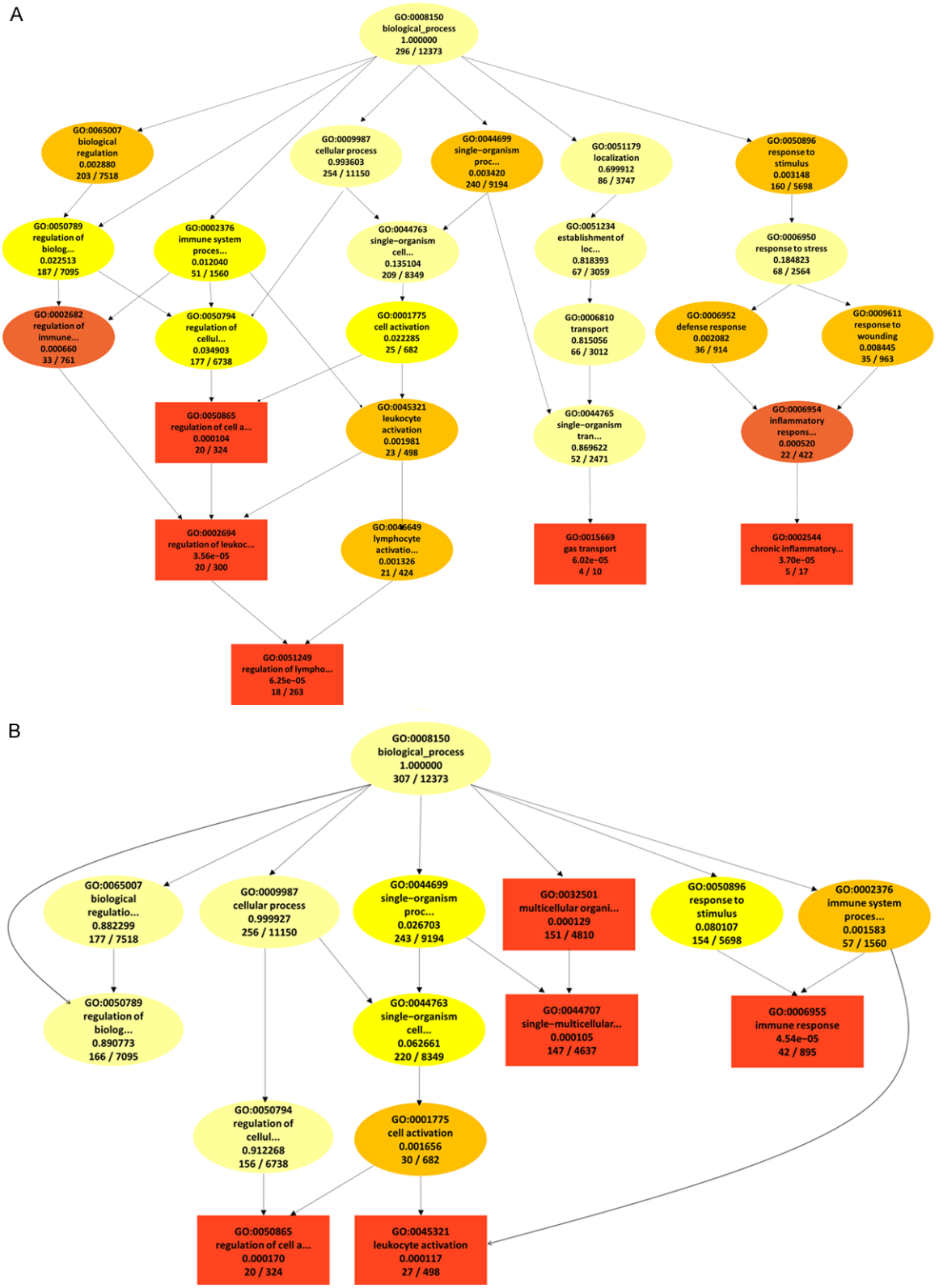

Figure 1. The subgraph induced by the top 5 GO terms identifed by the classic algorithm for scoring GO terms for enrichment. Boxes indicate the 5 most significant terms. Box color represents the relative signifcance, ranging from dark red (most significant) to light yellow (least significant). Black arrows indicate is a relationships and red arrows part-of relationships.

Table 2. Disease association analysis

Term	Count	%	*P* Value
Diabetes, type 1	27	2.824268	2.75E-04
Biliary atresia	5	0.523013	0.00399
Diabetes, type 2; diabetes, type 1	6	0.627615	0.006078
Carbamazepine hypersensitivity	5	0.523013	0.007798

Table 3. Pathway enrichment analysis for 1253 coexpression genes

Term	Count	%	*P* Value
Graft-versus-host disease	11	1.150628	7.70E-06
Type I diabetes mellitus	9	0.941423	5.89E-04
Allograft rejection	8	0.83682	0.001144
Antigen processing and presentation	11	1.150628	0.004856
Autoimmune thyroid disease	8	0.83682	0.00876
Natural killer cell mediated cytotoxicity	13	1.359833	0.020477
Viral myocarditis	8	0.83682	0.046103

were identified as differentially expressed genes by comparing T2D with FH+, and 395 DEGs were identified by comparing T2D with FH-. GO function annotation were performed with these DEGs. The significantly GO annotation results of biological process (BP) terms were listed in **Table 1**. The top enriched GO term is associated with immune system, which includes immune response, leukocyte activation, inflammatory response, and chronic inflammatory response. By comparing with FH- groups, we observed that the "regulation of leukocyte activation", "chronic inflammatory response", "regulation of lymphocyte activation", "regulation of lymphocyte proliferation" pathways were significantly enriched, which were in relation to immune response as well. We next using a topGO method to display the results of gene set enrichment analysis, **Figure 1** shows the relationship between top 5 GO terms. The GO analysis indicates that gene expression alteration in T2D patients are related to immune system. Therefore, we totally select 29 DEGs involved in immune biological process based on the significantly enriched GO term for following analysis.

Co-expression of DEGs involved in immune process and disease association analysis

Gene co-expression analysis assumes that genes with similar expression patterns are hypothesized to have similar functions or involved in same biological process. To further characterize the role of immune system in T2D and reveal the mechanism of T2D and insulin resistance, coexpression analysis was performed of the 29 selected genes by calculating the Spearman correlation coefficient for each gene with the 29 selected genes. The threshold of correlation coefficient larger than 0.8 was applied as a criteria of coexpression gene selection, we identified an interaction network which included 1253 genes (R2 > 0.8). The genes coexpressed with 29 immune genes in T2D dataset may indicate the relationship of these genes and T2D. Therefore, we download disease-gene association data form GAD, and Fisher exact test *p* value was calculated for each disease with the 1253 genes. The top enriched diseases were listed in **Table 2**, including type 1 diabetes, biliary atresia, type 2 diabetes and carbamazepine hypersensitivity. In the GAD database, 27 out of 1253 selected genes involved in type 1 diabetes and 6 genes involved in type 2 diabetes.

Pathway enrichment analysis of coexpression genes

To clarify the biological function of these network genes, all the 1253 genes were used to query the KEGG database for pathway enrichment analysis. **Table 3** lists the enriched pathways. Graft-versus-host disease pathway is top enriched and 11 genes involved in this pathway; the Graft-versus-host disease pathway is in relation to immunologic rejection. In addition, the Type I diabetes mellitus and pathways associated with immune system which includes Allograft rejection, Antigen processing and presentation, Autoimmune thyroid disease, Natural killer cell mediated cytotoxicity were all enriched. The activation of NKT cells is associated with insulin resistance. **Figure 2** shows the key elements of the Natural killer cell mediated cytotoxicity pathway, like HLA, CD94, CD48 and several other genes are coexpressed genes in our dataset.

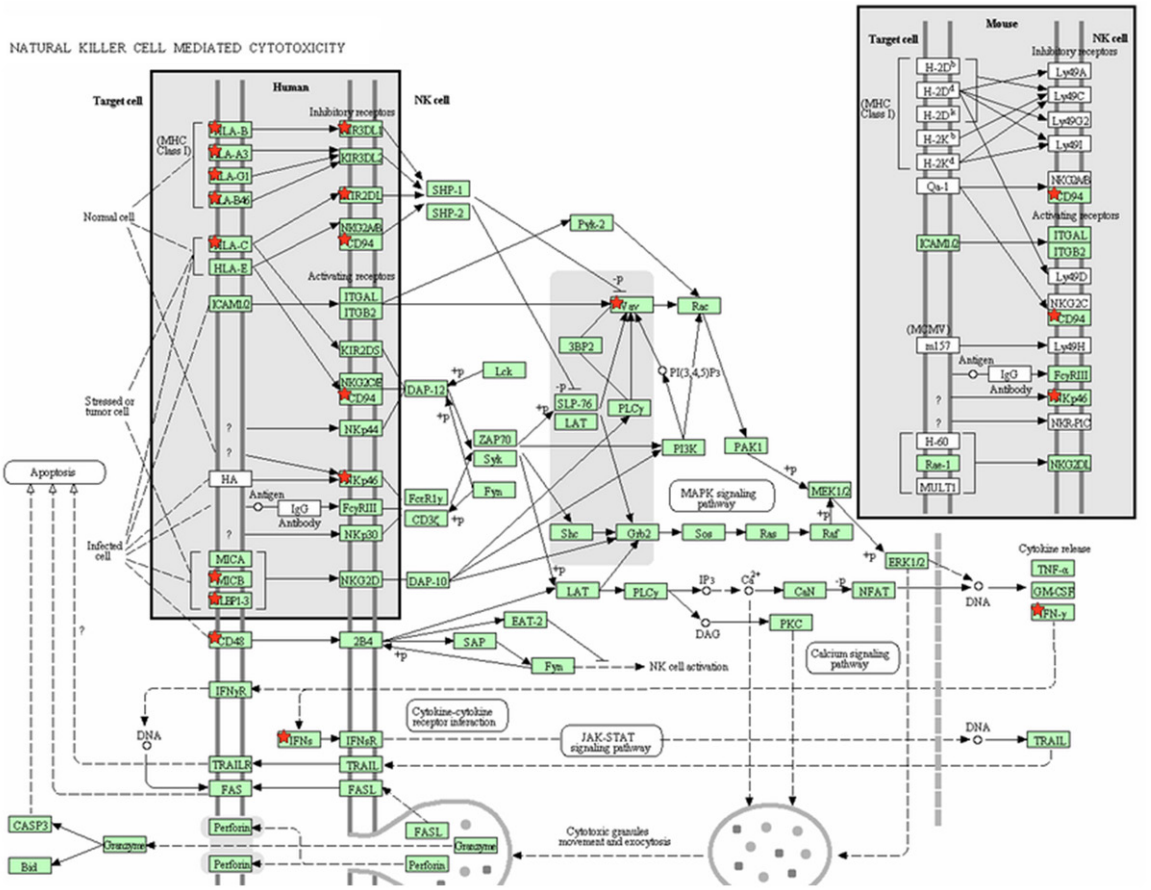

Figure 2. Natural killer cell mediated cytotoxicity signaling pathway. Coexpression genes were marked with a star.

Protein-protein interaction for coexpression genes

To further investigate the relationship of coexpression genes, we performed protein-protein interaction analysis for each gene. The STRING database includes known and predicted protein interactions, and it also provided experimental evidence for part of interactions. We query the database with all the 1253 coexpression genes to find the protein-protein interaction of these genes. In order to identify high confidence interactions, we only take the results with experimental supported into consideration, and apply a threshold confidence score larger than 0.7 (high confidence). The **Figure 3** shows the protein-protein interactions. Totally, 33 proteins have at least one interaction with other proteins. This interaction network includes several immune related genes, such as, Complement Component 3 (c3) and CD28. Several hub genes in the network were identified, like SRC and STAT1.

Discussion

Although, current study have demonstrated various factors that may contribute to diabetes mellitus and insulin resistance, such as, adipocytokines, hormones released [14], glucose transporters [15] and Pancreatic β cells failure [16], however, the pathogenesis of diabetes mellitus and insulin resistance has not been revealed completely. This study presents a comprehensive gene expression profile analysis linking gene expression alteration of immune system with T2D and insulin resistance. The analyses identified a list of DEGs, GO analysis indicates these DEGs are related to immune biological process. Based on the GO annotation results, we pick up 29 genes are involved in immune biological process for the coexpression and protein-protein interaction analysis.

In the 29 genes, TNFRSF4, PGLYRP1, IL32, HBA1, HBM, MYC, HYAL3 involved in immune biological process are differentially expressed.

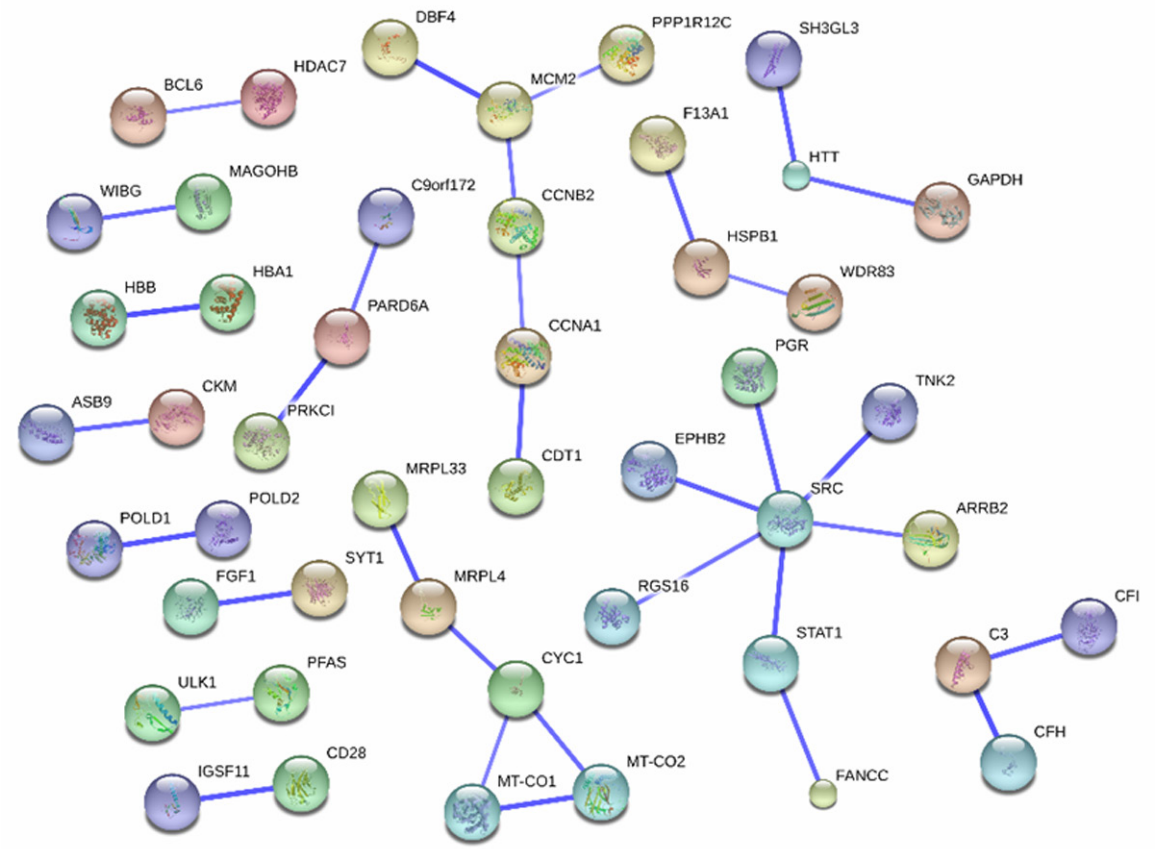

Figure 3. Protein-protein interaction of coexpressed genes by using STRING database.

TNFRSF4 also known as CD134, a member of the TNF-receptor superfamily, the stimulation of Ox40 is known to enhance activation of effector T cells and to inhibit induction and suppressive function of Treg. It is reported that OX40 is a key factor in shaping Treg sensitivity to IL-2 and promoting their proliferation and survival, toward accurate immune regulation [17]. Knockout studies in mice suggested that this receptor promotes the expression of apoptosis inhibitors BCL2 [18]. The Bcl-2 family has a double-edged effect in diabetes. These proteins are crucial controllers of the mitochondrial pathway of β-cell apoptosis induced by pro-inflammatory cytokines or lipotoxicity. In parallel, some Bcl-2 members also regulate glucose metabolism and β-cell function [19]. In the DEGs, PGLYRP1 is also a member of the TNF-receptor superfamily plays a role in innate immunity, and IL32 induces the production of TNF alpha from macrophage cells [19]. It was well known that TNF members are associated with in the pathogenesis of insulin resistance and type 2 diabetes [21, 22].

HBA1 (Hemoglobin Alpha Chain) gene and its paralog gene HBM are both dysregulated in our analysis. Plasma glycated hemoglobin levels, which reflect the mean ambient fasting and postprandial glycaemia over a 2- to 3-month period, are the most widely used marker of long-term glycoregulation [23]. Recent study has examined the relationship between plasma Hemoglobin levels and the incidence of chronic obstructive pulmonary disease (COPD) in Chinese patients with type 2 diabetes [24]. The Hemoglobin level is also widely recommended as a therapeutic guideline for preventing cardiovascular complications in diabetic patients [25]. Moreover, MYC gene plays an important role in beta-cell growth and differentiation, activation of c-Myc in beta-cells leads to proliferation and apoptosis, it also can initial β-Cell hyperplasia hyperplasia with amorphous islet organization, and selective downregulation of insulin gene expression and the development of overt diabetes [26].

The 29 genes involved in immune biological process were coexpressed with 1253 genes in

our analysis. According to the KEGG pathway enrichment analysis, the molecular function of these co-expressed genes is mainly focused in immune system. Notably, 9 genes are involved in Type I diabetes mellitus pathway and 13 genes are involved in Natural killer cell mediated cytotoxicity pathway. The association between Natural killer cell and diabetes and insulin deficiency has been well demonstrated in previous studies [27-29].

In conclusion, our study systematically demonstrated the association between immune system and T2D diabetes in terms of gene expression profiling. Our study indicates that several key factors of immune biological process may involve in the pathogenesis of T2D diabetes and insulin resistance. This study provided clues for further researches on the involvement immune system in type 2 diabetes.

Disclosure of conflict of interest

None.

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