Identification of monocyte-related transcriptomic signature of peripheral blood mononuclear cells in type 1 diabetes

Min Yin[1], Yan Zhang[1], Juan Huang[1,2], Xinyu Li[1], Haibo Yu[1,3], Xia Li[1]

[1]National Clinical Research Center for Metabolic Diseases, Key Laboratory of Diabetes Immunology, Ministry of Education, and Department of Metabolism and Endocrinology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China;
[2]Department of Internal Medicine, Section of Endocrinology, School of Medicine, Yale University, New Haven, CT, USA;
[3]Hunan Engineering Research Center of Cell Therapy for Diabetes, Changsha, Hunan 410011, China.

To the Editor: Type 1 diabetes (T1D) is caused by autoimmunity-mediated destruction of pancreatic islet beta cells, with islet autoimmunity being the essential component in the pathogenesis of T1D.[1] However, studies on human islets are limited due to sampling difficulties. Therefore, immunological changes in peripheral blood mononuclear cells (PBMCs) should be emphasized. In one way, islet-infiltrating immune cells are presumably in equilibrium with circulating pools and changes in PBMCs might partly reflect islet inflammation. On the other, PBMCs represent a useful tool to detect biomarkers for disease.[2] Traditionally, studies on PBMCs in T1D are characterized by the use of limited numbers of cell-surface markers and secreted cytokines measured using flow detection and other antibody-based assays. Recently, high-throughput sequencing technologies such as gene expression microarray have allowed the identification of PBMC transcriptomic signatures at the population level. Most of these studies focused on the differentially expressed genes (DEGs) in PBMCs, but few have verified the DEGs and investigated their cell types.

To identify the DEGs responsible for T1D, microarray data were obtained from GSE72376,[3] which comprised data of PBMC samples from 15 T1D patients and 15 normal controls (NCs). Only the unstimulated samples were used for analysis [Supplementary Table 1, http://links.lww.com/CM9/B46]. The workflow is presented in Supplementary Figure 1, http://links.lww.com/CM9/B46. For DEG identification, $|\log_2\text{FoldChange}| \geq 0.5$ and P values < 0.05 were considered statistically significant. In total, 318 DEGs were identified, including 98 upregulated and 220 downregulated DEGs [Supplementary Figure 2A, http://links.lww.com/CM9/B46]. The top 10 upregulated and downregulated genes in T1D are presented in Supplementary Table 2, http://links.lww.com/CM9/B46. To explore the functional annotation of DEGs, functional enrichment analysis was performed using the online tool DAVID (https://david.ncifcrf.gov/) [Supplementary Figure 2B, http://links.lww.com/CM9/B46]. Major enrichment in the biological process involved the inflammatory response, positive regulation of tumor necrosis factor biosynthetic process, immune response, and metabolic process. Primary enrichment in the cellular component consisted of clathrin-coated endocytic vesicles, dystrophin-associated glycoprotein complexes, growth cones, and lysosomal lumens. In the molecular function category, DEGs were mainly enriched in chitin-binding [Figure 1A; Supplementary Table 3, http://links.lww.com/CM9/B46].

To identify the most significant hub genes among DEGs, a protein–protein interaction (PPI) network involving 318 DEGs was constructed using STRING (version 10.5; https://string-db.org/) with a PPI score >0.4 and visualized with Cytoscape (https://cytoscape.org/) [Supplementary Figure 2C, http://links.lww.com/CM9/B46]. A total of 10 hub genes were harbored by the PPI network, with *CD86*, *TLR4*, *TLR1*, *VEGFA*, *TREM2*, and *CD33* among the upregulated DEGs and *PRF1*, *CX3CR1*, *FCGR3A*, and *TNFRSF9* among the downregulated DEGs [Figure 1B; Supplementary Table 4, http://links.lww.com/CM9/B46]. Major biological process enrichment of the 10 hub genes involved immune response, cellular response to lipopolysaccharide (LPS), detection of LPS, positive regulation of the ERK1 and ERK2 cascades, and regulation of immune response. The remarkably enriched Kyoto Encyclopedia of Genes and Genome pathways included not only autoimmune diseases (T1D, rheumatoid arthritis, graft-*vs*.-host disease, allograft rejection) but also the TLR signaling

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Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002142

Correspondence to: Xia Li, National Clinical Research Center for Metabolic Diseases, Key Laboratory of Diabetes Immunology, Ministry of Education, and Department of Metabolism and Endocrinology, The Second Xiangya Hospital, Central South University, No. 139, Renmin Middle Road, Furong District, Changsha, Hunan 410011, China
E-Mail: lixia@csu.edu.cn

Chinese Medical Journal 2022;135(21)
Received: 16-11-2021; **Online:** 28-10-2022 **Edited by:** Lishao Guo

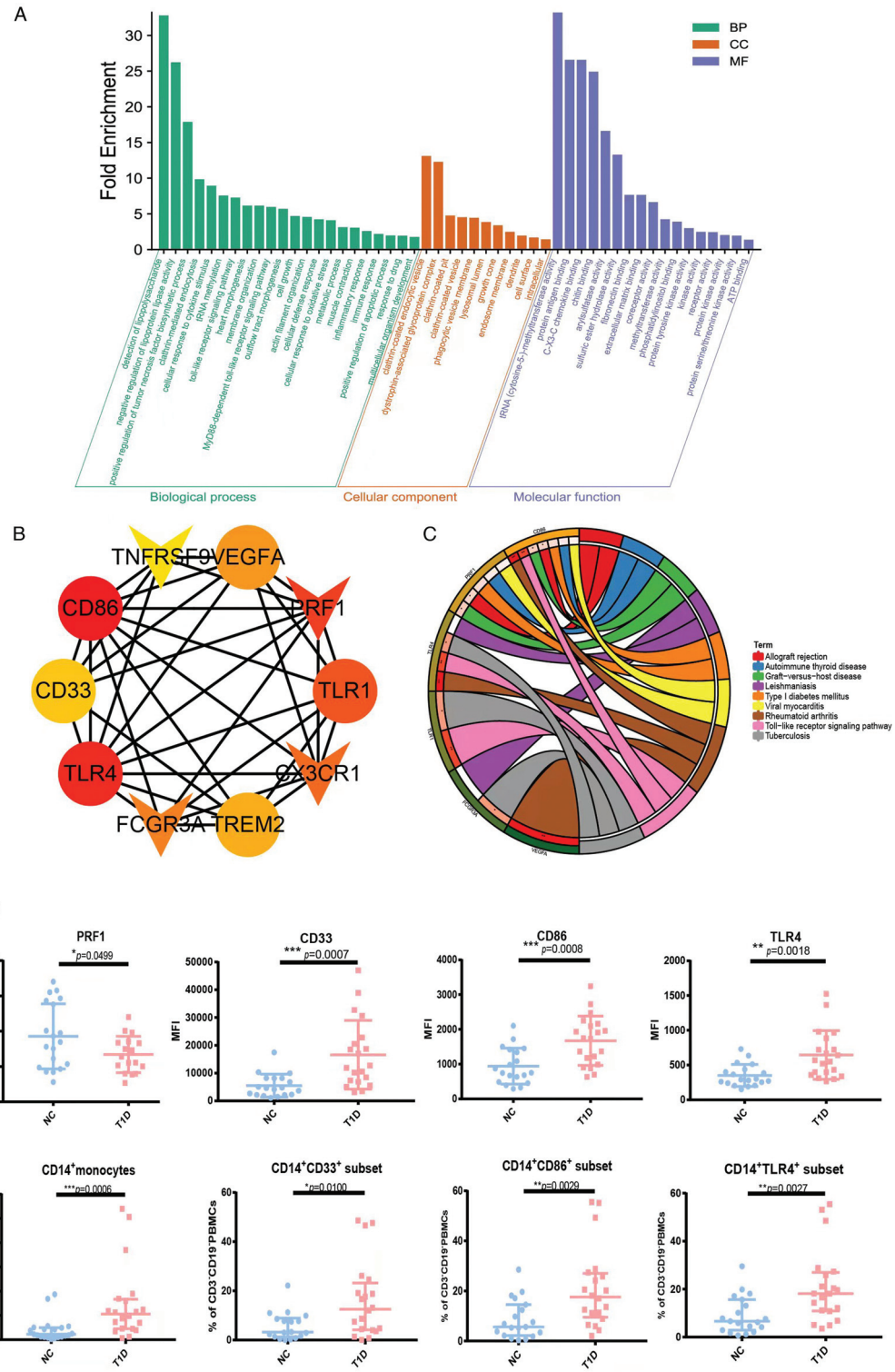

Figure 1: (A) Gene ontology analysis of DEGs in GSE72376 clustered by biological process, cellular component, and molecular function. (B) PPI network of the 10 hub genes. Circles and arrows indicate upregulated and downregulated proteins, respectively. The darker the color, the stronger is the correlation. (C) KEGG pathway enrichment of the hub genes in T1D. (D) MFI of the hub genes in flow cytometry detection. (E) Frequencies of $CD14^+$,$CD14^+CD86^+$,$CD14^+CD33^+$, and $CD14^+TLR4^+$cells in T1D patients and NCs in flow cytometry detection. $^*P$ value < 0.05, $^{**}P$ value < 0.01, $^{***}P$ value < 0.001. DEGs: Differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; MFI: Mean fluorescent intensity; NCs: Normal controls; PPI: Protein-protein interaction; T1D: Type 1 diabetes.

pathway and tuberculosis. In particular, the hub genes *PRF1* and *CD86* were found to be enriched in the T1D pathway [Figure 1C]. To evaluate the diagnostic ability of identified hub genes, a receiver operating characteristic curve analysis was performed, and the respective area under the curve of the hub genes was found to be

approximately 0.7 [Supplementary Figure 3, http://links.lww.com/CM9/B46], suggesting that hub genes may be used as biomarkers of T1D.

To further validate the importance of these hub genes, we used external datasets and validation groups to prove this conclusion. *PRF1, TLR1, and TLR4* were validated in GSE9006, while *PRF1,* TLR4, and *VEGFA* were validated in GSE55100 [Supplementary Figure 4A, http://links.lww.com/CM9/B46]. TLR1 was DEG in GSE9006 and PRF1 was DEG in GSE55100. Next, we performed quantitative polymerase chain reaction and flow cytometry analysis of hub genes *in vitro* [Supplementary Table 5, http://links.lww.com/CM9/B46]. Twenty T1D patients and 19 NCs were enrolled for validation, which were age and sex matched. This study followed the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from all participants. Compared with NCs, the mRNA expression of *CD33,* CD86,and *TLR4* showed an upward trend ($P = 0.0676$, 0.0953, and 0.1586, respectively), whereas the mRNA expression of *PRF1* was significantly decreased in the T1D group ($P = 0.0060$) [Supplementary Figure 4C, http://links.lww.com/CM9/B46]. At the protein level, CD33, CD86, TLR4 were markedly increased ($P = 0.0007$, 0.0008, and 0.0018, respectively), while PRF1 and CX3CR1 decreased significantly in the T1D group ($P = 0.0499$ and 0.0302, respectively) [Figure 1C; Supplementary Figure 4D, http://links.lww.com/CM9/B46].

To identify the corresponding cell type of these hub genes, CIBERSORTx analysis was used, which further revealed that the hub genes were mainly derived from monocytes [Supplementary Table 6, http://links.lww.com/CM9/B46]. In order to determine the proportions of monocytes in T1D, immune infiltration analysis was performed using ImmuCellAI (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/). Monocytes showed an upregulation trend in T1D ($P = 0.054$) [Supplementary Figure 5, http://links.lww.com/CM9/B46]. Consistent with the immune infiltration analysis, the proportion of $CD14^+$ monocytes was higher in the T1D group according to flow cytometry data ($P = 0.0006$). Moreover, CD33, CD86, and TLR4 were mainly expressed in monocytes, which verified the results obtained in the immune infiltration analysis [Figure 1D]. All these results were consistent with bioinformatic analyses data, suggesting that *CD33, CD86, TLR4,* and *PRF1* expression patterns may effectively distinguish between T1D patients and NCs, establishing their status as potential biomarkers for T1D diagnosis and treatment. Surprisingly, through immune infiltration analysis, the proportion of four types of innate immune cells – namely, natural killer (NK) cells, NK T cells, mucosal-associated invariant T (MAIT) cells, and neutrophils – were significantly different between T1D and NC, with MAIT and neutrophil upregulation, and NK and NKT downregulation in T1D. Unfortunately, flow cytometry did not confirm the above findings, possibly due to the small quantity of NK and NKT cells in PBMCs. In addition, transcription factors (TFs) and miRNAs prediction analysis showed that hub genes in the PPI network were regulated by multiple TFs and miRNAs [Supplementary Figure 6, http://links.lww.com/CM9/B46].

Although T cells are considered dominant in most cases of insulitis brought on by T1D, increasing evidence reveals that other immune cells contribute to this disease.[4] Monocytes showed an upward trend in T1D patients by bioinformatic analysis. In flow cytometry experiments, monocytes increased significantly in the T1D group. Most importantly, hub genes *CD33, CD86,* and *TLR4* were located primarily in monocytes. All these suggest that monocyte-related innate immunity plays an important role in the pathogenesis of T1D.[5] We assert that two hub proteins – CD33 and PRF1 – are likely candidates for new markers in the diagnosis of T1D. Furthermore, CD86 and TLR4 may be valuable targets for the treatment of T1D. The results of the present study further our understanding of the pathophysiology of T1D. However, our findings must be investigated further by conducting more experimental verification.

Acknowledgments

We appreciate the "GEO database" for providing high quality biological data.

Funding

This study was supported by grants from the National Natural Science Foundation of China (No. 82070812), the Fundamental Research Funds for the Central Universities of Central South University (No. 2021zzts0361), and the Hunan Provincial Innovation Foundation for Postgraduate (No. CX20210363).

Conflicts of interest

None.

References

1. Ilonen J, Lempainen J, Veijola R. The heterogeneous pathogenesis of type 1 diabetes mellitus. Nat Rev Endocrinol 2019;15:635–650. doi: 10.1038/s41574-019-0254-y.
2. Kent SC, Chen Y, Clemmings SM, Viglietta V, Kenyon NS, Ricordi C, *et al*. Loss of IL-4 secretion from human type 1A diabetic pancreatic draining lymph node NKT cells. J Immunol 2005;175:4458–4464. doi: 10.4049/jimmunol.175.7.4458.
3. Yamamoto S, Whalen E, Chujo D, Nattamai D, Baldwin N, O'Brien K, *et al*. A systems approach to the characterization and classification of T-cell responses. bioRxiv 2017;175620. doi: 10.1101/175620.
4. Kolb H, von Herrath M. Immunotherapy for type 1 diabetes: why do current protocols not halt the underlying disease process? Cell Metab 2017;25:233–241. doi: 10.1016/j.cmet.2016.10.009.
5. Thiem K, van Dierendonck X, Janssen AWM, Boogaard JP, Riksen NP, Tack CJ, *et al*. A high glycemic burden relates to functional and metabolic alterations of human monocytes in patients with type 1 diabetes. Diabetes 2020;69:2735–2746. doi: 10.2337/db20-0568.

How to cite this article: Yin M, Zhang Y, Huang J, Li X, Yu H, Li X. Identification of monocyte-related transcriptomic signature of peripheral blood mononuclear cells in type 1 diabetes. Chin Med J 2022;135:2608–2610. doi: 10.1097/CM9.0000000000002142