Presence of PD-1 similarity genes in monocytes may promote the development of type 1 diabetes mellitus and poor prognosis of pancreatic cancer

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ABSTRACT

Introduction To identify proteins and corresponding genes that share sequential and structural similarity with programmed cell death protein-1 (PD-1) in patients with type 1 diabetes mellitus (T1DM) via bioinformatics analysis.

Research design and methods All proteins with immunoglobulin V-set domain were screened in the human protein sequence database, and the corresponding genes were obtained in the gene sequence database. GSE154609 was downloaded from the GEO database, which contained peripheral blood CD14+ monocyte samples from patients with T1DM and healthy controls. The difference result and the similar genes were intersected. Analysis of gene ontology and Kyoto encyclopedia of genes and genomes pathways was used to predict potential functions using the R package ‘cluster profi le’. The expression differences of intersected genes were analyzed in The Cancer Genome Atlas pancreatic cancer dataset and GTEx database using t-test. The correlation between the overall survival and disease-free progression of patients with pancreatic cancer was analyzed using Kaplan-Meier survival analysis.

Results 2068 proteins with immunoglobulin V-set domain similar to PD-1 and 307 corresponding genes were found. 1705 upregulated differentially expressed genes (DEGs) and 1335 downregulated DEGs in patients with T1DM compared with healthy controls were identified. A total of 21 genes were overlapped with the 307 PD-1 similarity genes, including 7 upregulated and 14 downregulated. Of these, mRNA levels of 13 genes were significantly increased in patients with pancreatic cancer. High expression of MYOM3 and HHLA2 was significantly correlated with shorter overall survival of patients with pancreatic cancer, while high expression of FGFR1, CD274, and SPEG was significantly correlated with shorter disease-free survival of patients with pancreatic cancer.

Conclusions Genes encoding immunoglobulin V-set domain similar to PD-1 may contribute to the occurrence of T1DM. Of these genes, MYOM3 and SPEG may serve as potential biomarkers for the prognosis of pancreatic cancer.

INTRODUCTION

Programmed cell death protein-1 (PD-1) belongs to the CD28/CTLA-4 co-receptor family containing a single extracellular immunoglobulin variable (V) domain. PD-L1 is a ligand for PD-1 and is a member of the CD28/B7 family. PD-1 is a critical immune checkpoint protein in human immune cells, which plays an important role in regulating immune responses and maintaining immune homeostasis by interacting with PD-L1. PD-1 deficiency may lead to various autoimmune diseases. Multiple studies have shown that the incidence of type 1 diabetes mellitus (T1DM) is significantly increased in the presence of PD-1 deficiency in pre-diabetic NOD mice. In addition, PD-1/PD-L1 signaling pathway
is also involved in malignant tumorigenesis and immune escape. It has been reported that the occurrence and immune escape of pancreatic cancer are related to the PD-1/PD-L1 pathway. Previous studies have reported upregulation of PD-L1 in human pancreatic cancer samples. It has also been shown that PD-L1 blockers can effectively inhibit pancreatic cancer in mouse models. These results suggest that the PD-1/PD-L1 pathway plays an important role in pancreatic cancer.

Monocytes, the largest type of white blood cells and part of the innate immune system’s phagocytic cells, have been shown to play a crucial role in the progression of cancer and autoimmune disorders. They are capable of inducing tumor cell killing via cytokines or phagocytosis induction and involved in the progression and regression of inflammation. Notably, PD-1 expression was found to be elevated in the monocytes of patients with hepatocellular carcinoma and contributed to the suppression of CD8 T cell. Individuals diagnosed with T1DM have been shown to exhibit elevated levels of pro-inflammatory monocytes and circulating inflammatory mediators. The aforementioned evidence indicates that monocytes and PD-1/PD-L1 pathway have become crucial regulators in the progression of cancer and autoimmune diseases.

Given the significance of PD-1 pathway in T1DM and pancreatic cancer and the crucial role of monocytes in the progression of cancer and autoimmune disorders, this study aims to use bioinformatics analysis to identify proteins and their corresponding genes that share structural and sequential similarities with PD-1 in patients with T1DM. These identified proteins and genes could serve as potential targets for further investigation in future studies.

**MATERIALS AND METHODS**

**Screening for proteins with similar structures to PD-1 protein**


**Protein subcellular localization analysis**

The subcellular distribution and functions of the obtained similar proteins were predicted using the Hum-mPLoc 3.0 database (http://www.csbio.sjtu.edu.cn/bioinf/hum-mPLoc3/).

**Identification of differentially expressed genes in patients with T1DM**

The GSE154609 dataset containing 12 T1DM samples and 12 healthy controls was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154609) to identify differentially expressed genes (DEGs) in patients with T1DM using wilcox-test (R language, wilcox.test; R V.4.1.2 (https://www.r-project.org/)). Data were visualized using box plots. During comparison, filter according to the upper and lower limit of box plot, that is, the upper limit of each group of data was Q3+1.5 IQR, the lower limit was Q1-1.5 IQR (IQR=Q3-Q1). After the difference result (p<0.05) was obtained, the similar genes obtained in the first step were intersected. P value less than 0.05 was considered statistically significant.

**Gene ontology and Kyoto encyclopedia of genes and genomes**

DEGs were subjected to gene ontology (GO) functional annotation (http://www.geneontology.org/) and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis (http://www.kegg.jp/). Statistical analysis of GO and KEGG enrichment data was carried out using the R package ‘clusterProfiler’. A p value less than 0.05 was considered statistically significant.

**Prognosis and differential expression analysis in pancreatic cancer**

To compare the mRNA levels of the genes related to both T1DM and PD-1 similarity, 179 pancreatic cancer samples and 4 normal pancreatic samples were downloaded from The Cancer Genome Atlas (TCGA) database. To increase the normal control sample size, 167 normal pancreatic samples were downloaded from the GTEx database. Expression differences of intersected genes were analyzed by t-test. Kaplan-Meier survival analysis was performed to assess the correlations of the genes related to both T1DM and PD-1 similarly with the prognosis (overall survival and disease-free progression) of patients with pancreatic cancer. A p value less than 0.05 was considered statistically significant.

**RESULTS**

**Identification of proteins sharing sequential and structural similarity with PD-1**

According to the structural similarity, the proteins with immunoglobulin V-set domain similar to PD-1 and their corresponding genes were screened in the Pfam.
of pancreatic cancer. Furthermore, high expression levels of SIGLEC10, SPEG, BTN3A1, CD300LF, CEACAM1, compared with those in normal controls, the mRNA was significantly correlated with shorter overall survival of patients with pancreatic cancer. As shown in figure 1A and online supplemental table 4).

Identification of DEGs in patients with T1DM and enrichment analysis
To identify the genes that might contribute to T1DM occurrence due to analogous pathway of PD-1 in T cells, we sought to find out the genes in the intersection between T1DM-related genes and the 307 genes encoding potential analogs (immunoglobulin V-set domain) of PD-1. In the GSE154609 dataset containing 12 peripheral blood CD14+ monocyte samples of patients with T1DM and 12 healthy controls, we identified 1705 upregulated DEGs and 1335 downregulated DEGs in patients with T1DM compared with healthy controls (online supplemental table 5). Among these DEGs, a total of 21 genes (figure 1B) were overlapped with the 307 PD-1 homologs, including 7 upregulated (figure 1C) and 14 downregulated (figure 1D) genes (online supplemental tables 6 and 7). The 1705 upregulated and 1335 downregulated DEGs were further subjected to GO annotation and KEGG pathway analysis. These genes were mainly annotated as receptor complex, external side of plasma membrane, and M band (figure 2A and online supplemental table 8). KEGG pathway analysis revealed that these genes were mostly enriched in the prostate cancer, central carbon metabolism in cancer, and EGFR tyrosine kinase inhibitor resistance pathways (figure 2B and online supplemental table 9).

T1DM-related genes are highly expressed in pancreatic cancer and associated with the prognosis of pancreatic cancer
To investigate the clinical significance of the 21 common genes related to both both T1DM and PD-1 similarity, we compared their mRNA levels between pancreatic cancer tissue samples (n=179) from the TCGA database and normal tissue samples from TCGA database (n=4) and GTEx database (n=167). As shown in figure 3A, compared with those in normal controls, the mRNA levels of SIGLEC10, SPEG, BTN3A1, CD300LF, CEACAM1, FCERIA, HHLA2, IL1R2, MCAM, MYOM3, PDGFRB, PIGR, and PILRA were significantly increased in patients with pancreatic cancer. Furthermore, high expression of MYOM3 and HHLA2 was significantly correlated with shorter overall survival of patients with pancreatic cancer (figure 3B) and that high expression of FGFR1L, CD274, and SPEG was significantly correlated with shorter disease-free survival of patients with pancreatic cancer (figure 3C). These results suggest that these genes may serve as risk factors for T1DM onset and prognostic factors in patients with pancreatic cancer.

DISCUSSION
PD-1 is a member of the CD28/CTLA-4 co-receptor family, sharing 25% sequence identity. It is recognized as a type I membrane protein possessing a singular extracellular immunoglobulin V domain. PD-1 and PD-L1 interact using their V domains A‘GFCC’ β-sheets to form a pair of V domains in an Fv-like structure, similar to the V domains of antibodies, T cell receptors, or Fv paired (TCR) and CD8 to function. Due to its significant involvement in the regulation of peripheral tolerance, a lack of PD-1 may contribute to the development of multiple autoimmune diseases. Multiple studies have shown the presence of PD-1 deficiency in pre-diabetic NOD mice significantly increases the incidence of T1DM. Antibodies specific for PD-1 or PD-L1 but not PD-L2 accelerate insulin in NOD mice, and also induce T1DM within 10 days. Not only do T cells act through the PD-1 pathway, but our study found that through gene encoding, a functionally similar protein was also present in peripheral blood mononuclear cells of T1DM.

The study found that islet macrophages (derived from monocytes) are located near blood vessels, and they communicate with cellular and acellular components of the blood through filopodia that extend into the vascular lumen. They also interact with β-cells to capture insulin and deliver it to self-reactive T cells, which are essential for immune responses. This led us to wonder if monocytes could play the same role. On the one hand, multiple studies have shown that diabetes, through high blood glucose levels, alters monocyte/macrophage metabolism, leading to failure of innate immune and inflammatory processes, and dysregulation of macrophage-specific signal transduction. On the other hand, found that macrophages can protect and aggravate T1DM by increasing islet inflammation and affecting β-cell proliferation. However, whether monocytes/macrophages affect T1DM through the PD-1 pathway has not been studied. Therefore, this aspect of research may be a direction in the future. Although the mechanisms of immune checkpoint inhibitor (ICI)-induced T1DM remain unclear, studies have linked the onset of T1DM with autoimmune responses. Because the pathogenesis of ICI-induced T1DM is the same as that of T1DM, the PD-1 pathway may be blocked. In addition, epidemiological studies have suggested a close relationship between diabetes mellitus and pancreatic cancer. Some researchers even suggest that new-onset diabetes may be an early manifestation of pancreatic cancer, which provides important clues for the early diagnosis of pancreatic cancer. Meanwhile, several studies have reported that diabetes mellitus affects the prognosis of patients with pancreatic cancer. However, the underlying molecular mechanism is still unclear. It is worth mentioning that pancreatic cancer evades the immune response by...
Inducing the development of immunosuppressive T cells, thereby blocking the PD-1 pathway. Based on the role of PD-1/PD-L1 pathway in T1DM and pancreatic cancer, in this article, we discuss these two diseases together.

In this study, we aimed to identify proteins and the corresponding genes sharing sequential and structural similarity with PD-1 in T1DM. We found 21 genes associated with both T1DM and PD-1 similarity which were...
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screened from peripheral blood CD14+ monocyte samples of patients with T1DM. We identified 7 upregulated and 14 downregulated genes, out of which 13 genes, including BTN3A1 and CEACAM1, were significantly upregulated in pancreatic cancer. High expression of MYOM3, HHLA2, FGFRL1 and CD274 and low expression of SPEG were significantly associated with poor prognosis of patients with pancreatic cancer. These genes may also be potential risk factors for T1DM and prognostic factors for pancreatic cancer, and may serve as potential biomarkers for PD-1 inhibitor-induced T1DM.

Consistent with our results, previous researches have linked several genes to cancer, diabetes, and immune checkpoint pathways. For instance, SIGLEC10, a member of the Siglec family, is involved in the modulation of immune tolerance and is associated with the pathogenesis of autoimmune diseases, inflammatory reactions, and tumors.30 31 Therapeutic drugs targeting Siglec antibodies...
Genetics/Genomes/Proteomics/Metabolomics

or glycosylation ligands have been developed and used in the treatment of various Siglec-associated diseases. Additionally, butyrophilin (BTN) and butyrophilin-like (BTNL) families have been found to participate in the progression of inflammatory diseases and tumors by modulating antigen-specific responses of αβ T and γδ T cells. This suggests that targeting SIGLEC10 through immunotherapy may hold promise for treating certain
Given its diverse functions, CEACAM1 is a crucial molecule in insulin signaling and metabolism. Additionally, the extracellular domains of BTN and BTN-like molecules share a structural similarity with the B7 family of co-stimulatory ligands, which includes PD-L1, B7-H3, and B7-H4. Consequently, BTN and BTN-like molecules are classified as members of this family. Studies have shown that these families can play a role in the progression of inflammatory diseases and tumors by modulating the antigen-specific responses of CD8T and γδ-T cells. For example, anti-BTN3A antibodies have shown efficacy in acute myeloid leukemia and pancreatic cancer both in vitro and in vivo. The BTN3A1 homolog BTN3A2 is related to gastric cancer and T1DM. Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a glycoprotein belonging to the carcinoembryonic antigen family, plays a crucial role in tumor progression, immune regulation, and inflammation. Additionally, CEACAM1 is a crucial molecule in insulin signaling and metabolism. Given its diverse functions, CEACAM1 has the potential to emerge as a significant therapeutic target for both autoimmune disorders and cancer treatment in the future. HHLA2 is a newly identified immune checkpoint protein that is rarely expressed in normal pancreatic tissue but widely expressed in pancreatic cancer lesions. Several studies have highlighted a strong correlation between HHLA2 expression and poor clinical outcomes in various types of cancer, including pancreatic cancer. Furthermore, the inhibitory effects of HHLA2 on CD4 and CD8 T cells suggest that targeting HHLA2 could be a promising approach for the treatment of cancer and autoimmune disorders. ILIR2, an interleukin-1 receptor family member, acts as a decoy receptor that competitively binds to IL1β, inhibiting its interaction with IL1R1 and thereby impeding IL1β signaling in inflammatory conditions. Overexpression of ILIR2 has been associated with various cancers, as well as conditions such as arthritis, Alzheimer’s disease, and diabetes. CD146, also known as melanoma cell adhesion molecule (MCAM), shares homology with multiple cell adhesion molecules. It is involved in critical biological processes such as signal transduction, cell migration, angiogenesis, and immune response. Several investigations have reported a marked increase in CD146 T cells at sites of inflammation in patients with autoimmune disorders. In addition, our study identified potential new candidate genes, such as MYOM3 and SPEG, that have not been associated with cancer and diabetes, and may serve as new potential candidates for future studies.

However, it is important to note the limitations of our study. First, whether monocytes can play a role in the PD-1 pathway of T cells, there is no relevant research, and further experiments are needed to verify our findings. Second, our samples were obtained from databases and may not fully represent the population characteristics in other regions. Monocytes are known to interact with T cells to perform functions, such as in tuberculosis disease. However, there is limited research on whether monocytes are also involved in the development of T1DM, pancreatic cancer and ICI-induced fulminant T1DM through the PD-1 pathway. Here, we investigated whether monocytes would play a similar role to T cells and sought to identify the genes involved.

CONCLUSIONS

In this study, we find the presence of PD-1 similarity genes in monocytes in patients with T1DM. These 21 similarity genes encode immunoglobulin V-set domain similar to PD-1. Our results suggest that these genes can promote the development of T1DM and contribute to the poor prognosis of pancreatic cancer.

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