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ORIGINAL ARTICLE

Identification of new key genes for type 1 diabetes through construction and analysis of protein–protein interaction networks based on blood and pancreatic islet transcriptomes

Highlights
- This is the first study describing the concept of a dialogue between pancreatic islets and the immune system in type 1 diabetes (T1D) from a systems biology point of view.
- Interactome–transcriptome analysis revealed high centrality genes in the protein–protein interaction networks that are differentially expressed in peripheral blood mononuclear cells and pancreatic β-cells in T1D.
- This study delineates potential underlying mechanisms of T1D and identifies key markers for further experimental validation using network-based biological analysis in two tissues involved in T1D pathogenesis.

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Abstract

Background: Type 1 diabetes (T1D) is an autoimmune disease in which pancreatic β-cells are destroyed by infiltrating immune cells. Bilateral cooperation of pancreatic β-cells and immune cells has been proposed in the progression of T1D, but as yet no systems study has investigated this possibility. The aims of the study were to elucidate the underlying molecular mechanisms and identify key genes associated with T1D risk using a network biology approach.

Methods: Interactome (protein–protein interaction [PPI]) and transcriptome data were integrated to construct networks of differentially expressed genes in peripheral blood mononuclear cells (PBMCs) and pancreatic β-cells. Centrality, modularity, and clique analyses of networks were used to get more meaningful biological information.

Results: Analysis of genes expression profiles revealed several cytokines and chemokines in β-cells and their receptors in PBMCs, which is supports the dialogue between these two tissues in terms of PPIs. Functional modules and complexes analysis unraveled most significant biological pathways such as immune response, apoptosis, spliceosome, proteasome, and pathways of protein synthesis in the tissues. Finally, Y-box binding protein 1 (*YBX1*), SRSF protein kinase 1 (*SRPK1*), proteasome subunit alpha1/ 3, (*PSMA1/3*), X-ray repair cross complementing 6 (*XRCC6*), Cbl proto-oncogene (*CBL*), SRC proto-oncogene, non-receptor tyrosine kinase (*SRC*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), phospholipase C gamma 1 (*PLCG1*), SHC adaptor protein1 (*SHC1*) and ubiquitin conjugating enzyme E2 N (*UBE2N*) were identified as key markers that were hub-bottleneck genes involved in functional modules and complexes.

Conclusions: This study provide new insights into network biomarkers that may be considered potential therapeutic targets.

Keywords: protein–protein interaction network, topology, transcriptome, type 1 diabetes.

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Introduction

Type 1 diabetes (T1D) is an autoimmune disease with a strong genetic component, during which pancreatic β-cells in the islet of Langerhans are selectively destroyed via activation of cellular immunity against self-antigens on these cells. This may efficiently hamper endogenous insulin production.[1,2]

Data from genome-wide association studies (GWAS) implicate the involvement of classical immunoregulatory pathways, such as modulation of the interleukin (IL)-2 pathway, cytokine signaling, and changes in subsets of T cells in T1D.[3,4] However, it becomes evident from earlier studies that this immunodysregulation results in T1D development, provided that initial β-cells damage has occurred.[5] In this regard, recent studies have shown that pancreatic β-cells themselves express and release many cytokines and chemokines and that early T1D may be influenced by these factors.[6,7] Therefore, it seems that an understanding of changes in gene expression in both pancreatic β-cells and immune effector cells may be needed in order to elucidate the molecular mechanisms underlying this disease. It is not easy to prepare pancreatic samples from patients with new-onset T1D because the death rate with appropriate management is extremely low.[8] To address this issue, there is evidence that the full transcriptome of β-cells exposed to proinflammatory cytokines, such as IL-1β, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, could provide a snapshot of the responses of these cells under conditions that may dominate in early T1D.[9] This procedure is considered as a well-established *in vitro* model of T1D pathogenesis.[10] Further, it has been supposed that islet-infiltrating immune effectors are in equilibrium with circulating pools and may be sampled in peripheral blood mononuclear cells (PBMCs).[11,12] Earlier studies demonstrated that transcriptional profiling of PBMCs is helpful for identifying gene expression signatures of autoimmune diseases.[13,14]

Some biomarkers of human diseases have been successfully identified through genome-wide analysis of gene expression profiles.[15,16] However, this method has failed to introduce reproducible individual gene markers in some studies.[17,18] Furthermore, gene expression measurements for sorting genes into classical pathways or functional categories were not so effective to reveal disease markers because these methods may be limited to prior knowledge. To at least partially address this shortage, network biology and systematic bioinformatics data, such as protein–protein interactions (PPI) and related pathways, were introduced.[19] The aim of PPI studies is to characterize known associations among proteins in the context of biochemistry, signal transduction, and biomolecular networks.[20] In the recent years, integrated analysis of large-scale gene expression data with PPI networks and topological analysis of subnetworks are being considered as a promising approach to obtain a meaningful biological context in terms of functional association for differentially expressed genes.[21–23]

In the present study, query–query PPI (QQPPI) networks were constructed for T1D using genes that have different expression levels in PBMCs and pancreatic β-cells. Bilateral cooperation between two tissues was explored from the viewpoint of systems biology. Topological analyses were performed, and functional modules and complexes were characterized in each network; several biological processes and pathways were identified by these analyses. According to association in functional module and complexes and the degree of centrality measures, some new key markers were identified. The present study is the first in which significant markers have been introduced for T1D by topological and functional analysis of differentially expressed genes in both immune cells and pancreatic β-cells. The strategy of our work is shown in Fig. 1.

Methods

Sources of gene expression data

In the case of the PBMCs gene expression profile, raw data (CEL file) of microarray series data GSE9006 were downloaded from the Gene Expression Omnibus (GEO). The GSE9006 data were published in 2007;[14] blood samples were obtained from 43 newly diagnosed T1D patients and 24 healthy subjects (mean age 9.5 vs 10.9 years, respectively) and a female : male ratio in the two groups of 25 : 18 and 14 : 10, respectively. To obtain a pancreatic β-cell gene expression profile, we used RNA-seq analyzed data of human pancreatic islet transcriptomes (GSE35296) that were prepared in 2012.[7] Human islets were obtained from donors who were beating-heart organ donors with no medical history of diabetes or metabolic disorders. Five islet samples were isolated and cultured under control conditions and in the presence of cytokines (interleukin (IL)-1B and interferon (IFN)-γ). On average, preparations contained 58% β-cells, which is similar to the reported percentages of 54% in isolated human islets and 55% in human pancreas.[7]

Selection of differentially expressed genes

In the case of PBMCs, RNA samples were analyzed by Affymetrix Gene Chips A and B (HG_U133A and

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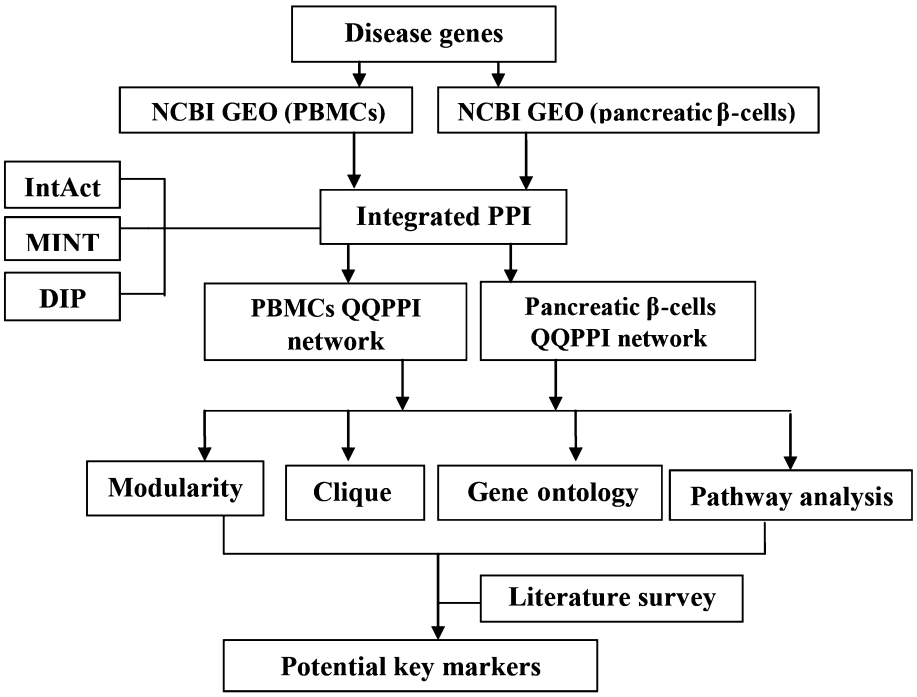

Figure 1 Study workflow. NCBI, National Center for Biotechnology Information; GEO, Gene Expression Omnibus; PBMCs, peripheral blood mononuclear cells; PPI, protein–protein interaction; QQPPI, query–query PPI; IntAct, the IntAct molecular interaction database; MINT, molecular interaction database; DIP, database of interacting proteins.

HG_U133B, respectively) (Affymetrix, Santa Clara, California, USA). Each chip (i.e. HG_U133A and HG_U133B) was normalized separately by the "affy" package of the Bioconductor project.[24] After robust multi-array average normalization, where a gene had more than one probe on the microarray, the average expression of all related probes was used to estimate the expression level of the gene. Differentially expressed genes were computed based on analysis of variance (ANOVA), considering additional variables such as ethnicity, race, age, and gender effects between diabetic and control samples. The genes of corresponding probes with $P < 0.05$ were determined to be abnormally expressed. For each gene, the minimal *P*-value (between HG_U133A and HG_U133B) was chosen. To estimate fold-changes in one condition versus another, linear regression was performed.[25] The fold-changes in the expression of each gene were determined by the chip which gave the more significant *P*-value. In the case of pancreatic β-cell gene expression, over- and underexpressed genes were extracted by Fisher's exact test and *P*-values were corrected by the Benjamin–Hochberg procedure ($P < 0.05$).[7]

Construction and topological analyses of the QQPPI networks

Abnormally expressed genes in PBMCs and pancreatic β-cells were separately located on the human PPI network integrated from three major the International Molecular Exchange (IMEx) consortium [26] public databases, namely the IntAct molecular interaction database (IntAct),[27] the molecular interaction database (MINT),[28] and the database of interacting proteins (DIP),[29] to construct QQPPI networks (i.e. networks consisting of the genes in question as the nodes and direct interactions among them). In a recent study, we showed that IMEx databases (especially IntAct and DIP) have a greater number of significant correlations for their proteins' topological features than all other paired comparisons between the biomolecular interaction network database (BIND), Human Protein Reference Database (HPRD), MINT, IntAct, and DIP databases.[30] The subnetworks of QQPPI were constructed using Cytoscape software 3.2.0 (National Institute of General Medical Sciences (NIGMS), Bethesda, Montgomery, Maryland, USA).[31] Topological properties of QQPPI networks were analyzed using this software.

Topologically significant nodes were extracted from the networks in two steps. First, in the networks, nodes with a degree greater than or equal to the sum of mean and twice the standard deviation (i.e. mean + 2SD) of the degree distribution were considered hubs.[32] Second, we defined bottlenecks as proteins that were in the top 5% in terms of betweenness centrality.

Identification and annotation of functional modules and complexes

The clustering with overlap neighborhood expansion (ClusterONE) algorithm was used to identify the

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connected regions within the network with possible overlap.[33] The modules were identified to have a minimum density of >0.05 and a degree of >5. A cluster with $P < 0.05$ was determined to be a module. The functional meaning for identified modules was further explored, and they were considered candidate functional modules if their genes were significantly enriched in biological process of Gene Ontology (GO) annotation or Kyoto Encyclopedia of Genes and Genomes (KEGG)/Reactome pathways. For this analysis, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) tool,[34] with two cut-off criteria: (i) Benjamin–Hochberg corrected $P < 0.05$; and (ii) two or more genes with specific GO terms.

Using the clique percolation method provided by CFinder software (Department of Biological Physics of Eötvös University, Budapest, Hungary), the complexes involved were extracted from the PPI networks.[35] The complexes in the PPI networks were identified with the help of the CORUM database.[36] To find related complexes, we used each clique forming protein as a query in the CORUM database. Then, all proteins associated with a specific complex were determined using an in-house algorithm.[37]

Results

Determination of differentially expressed genes

For PBMCs, 2466 genes were found be differentially expressed using ANOVA ($P < 0.05$), of which 1024 were upregulated and 1442 were downregulated. The genes and corresponding *P*-values are listed in Table S1, available as Supplementary Material to this paper. In the case of pancreatic β-cells, we used the data of Eizirik *et al.*,[7] who found 3068 genes that were significantly modified by exposure to the proinflammatory cytokines IL-1B plus IFN-γ. Of these genes, 1416 were upregulated and 1652 were downregulated.

Exploration of bilateral cooperation between immune cells and pancreatic β-cells in T1D via gene expression profiles

It has been suggested that β-cells have an active role in inflammation because they can express chemokines and cytokines to attract effector immune cells during inflammation.[7] Given the concept of a "dialogue" between pancreatic β-cells and invading macrophages and T cells during the course of insulitis[6,7] and the fact that no study has attempted to delineate it using a systems approach, we hypothesized that there may be bilateral relationships between chemokine and cytokine genes expression between two PBMCs and β-cells. Indeed, differentially expressed chemokines and cytokines, and their receptors, were found in β-cells and PBMCs, respectively. Table 1 summarizes the results, and Fig. 2 illustrates this cooperation in the context of the PPI networks.

Table 1 Differentially expressed ligand–receptor genes in pancreatic β-cells and peripheral blood mononuclear cells (PBMCs)

PBMC (receptors)	Pancreatic β-cells (ligands)	Major functions
CXCR2	*CXCl1, CXCL2, CXCl3, CXCL5*	Neutrophil recruitment
CXCR3	*CXCL9, CXCl10, CXCl11*	Effector T cell recruitment
CCR1	*CCL3, CCL5*	Mixed leukocyte recruitment
CCR4	*CCL22*	T cell and basophil recruitment
IL6R	*IL-6*	Inflammation and B cell maturation
IL1R2, IL1RAP	*IL-1A*	Inflammatory processes and hematopoiesis
IL1R2, IL1RAP	*IL-1B*	Mediator inflammatory responses

For a definition of all gene symbols, refer to Table S4.

Following are brief descriptions for each pair of chemokines and cytokines, and their receptors, discovered. To start, C-X-C motif chemokine receptor (CXCR) 2 is a chemokine receptor for the chemokines C-X-C motif chemokine ligand (CXCL) 1, CXCL2, CXCL3, and CXCL5. Interaction of this receptor with its chemokine ligands mediates neutrophil migration to sites of inflammation.[38] It has been shown that macrophages and β-cells produce CXCL1 and CXCL2, which recruit CXCR2-expressing neutrophils from the blood to the pancreatic islets during autoimmune diabetes in non-obese diabetic (NOD) mice.[39] The chemokine receptor CXCR3 is activated by three IFN-inducible ligands, namely CXCL9, CXCL10, and CXCL11. Interactions between this receptor and its chemokine ligands induces the recruitment of T cells to inflammatory sites.[38] Antonelli *et al.*[40] indicated that the CXCL10/CXCR3 system had a crucial role in the autoimmune process and destruction of virus-infected β-cells in T1D. The chemokine receptor C-C motif chemokine receptor (CCR) 1, with its ligands C-C motif chemokine ligand (CCL) 3 and CCL5, mediates signal transduction that is critical for the recruitment of effector immune cells to site of inflammations.[38] It has been shown in several mouse models that CCL3 and CCL5 are expressed in pancreatic islets and are implicated in T1D development.[41] The chemokine receptor CCR4 and its ligand CCL22 play a role in the trafficking of activated T lymphocytes to inflammatory sites.[38] A pathogenic role for CCL22 was hypothesized based on reduced insulitis and

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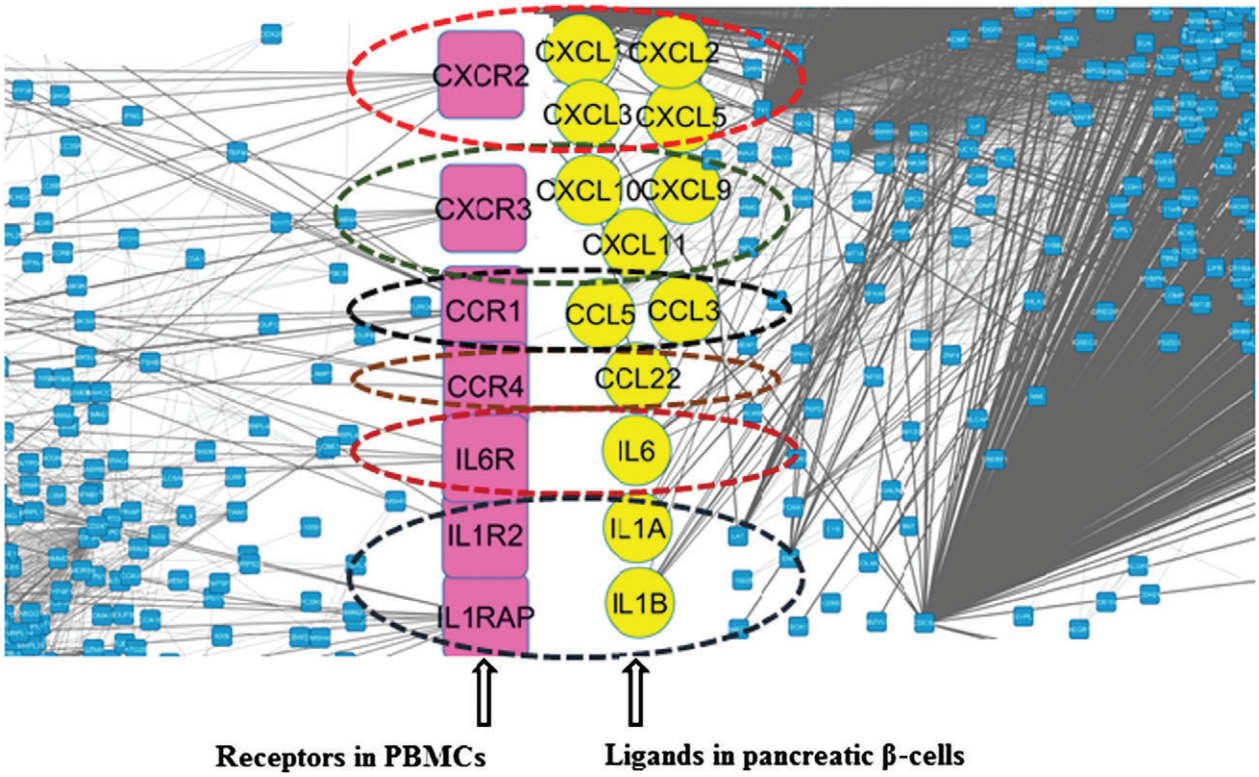

Figure 2 Bilateral cooperation between immune cells and pancreatic β-cells in early type 1 diabetes. For a definition of all gene symbols, refer to Table S4. PBMCs, peripheral blood mononuclear cells.

diabetes frequencies in NOD mice treated with a neutralizing CCL22 antibody.[42] In addition, some interleukins, such as interleukin (IL)-6, IL-1A and IL-1B, in β-cells and their receptors (IL6R, IL1R2 and IL-1 receptor accessory protein [IL1RAP]) in PBMCs appear to have a major role in the immune response, especially inflammation.[38] There is evidence that IL-1, together with tumor necrosis factor (TNF) and IFN-γ, induces apoptosis of pancreatic β-cells and clinical trials with IL-1 antagonists have been initiated in patients with T1D.[43] In the case of IL-6, Ryba-Stanislawowska *et al.*[44] proposed an important regulatory role for IL-6 in the progression of diabetes and its complications. Finally, both IFN-γ and IFN-β1 were found in PBMCs and their receptors (IFNγR2 and IFNαR2) were found in β-cells.

Topological analyses of QQPPI networks

The QQPPI networks investigated herein were undirected and unweighted PPI networks based on the differential expression of genes in both PBMCs and pancreatic β-cells in T1D. After removal of all orphan nodes, the QQPPI networks included 949 proteins and 1776 interactions in PBMCs and 1358 proteins and 3505 interactions in β-cells that were used for further analysis. The QQPPI networks were studied by several topological parameters, by which more information has been provided about the interaction networks. Some global properties of the networks are listed in Table 2. The power law of node degree distribution is one of the most important criteria of biological networks. The degree values approximately followed power law distributions ($P(k) = k^{-\lambda}$, where $P(k)$ is a distribution of node degree, k is a degree and λ is a degree exponent), with λ= 2.13 and λ=1.95 for the PBMC and β-cell networks, respectively (Fig. 3), which indicates that the QQPPI networks were scale free. There were 29 and 48 hubs and bottlenecks, respectively, in PBMC networks and 40 and 68, respectively, in pancreatic β-cell networks. The hub–bottleneck nodes in PBMC and β-cell networks are illustrated in Fig. 4. A list of all nodes, hubs and bottlenecks in PBMCs and β-cells, along with their topological parameters as obtained from Cytoscape software, are available in Tables S2 and S3.

Identification of functional modules

To better understand the biological processes or molecular functions of differentially expressed genes in T1D,

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Table 2 Global properties of the networks in pancreatic β-cells and peripheral blood mononuclear cells (PBMCs)

	No. nodes	No. edges	Average degree	Average betweenness	Average CC	Average ASP	Average CF
PBMCs	949	1776	3.75	0.011	0.268	4.404	0.048
β-Cells	1358	3508	5.16	0.005	0.276	4.037	0.060

CC, closeness centrality; ASP, average shortest path length; CF, clustering coefficient.

in the present study the PPI networks were decomposed into 11 and 12 functional modules in PBMCs and pancreatic β-cells, respectively, using the ClusterONE algorithm in Cytoscape. In the case of PBMCs, the biological processes of inflammatory response, chemotaxis, defense response, immune response, and related pathways (e.g. chemokine signaling pathway, cytokine–cytokine receptor interaction and signaling in immune system) demonstrated immune cell intermediation in disease pathogenesis. Responses to DNA damage, viral infection, and non-homologous end-joining (NHEJ) pathways were the other significant biological processes and pathways. Finally, mRNA metabolic process, RNA splicing, ATP metabolic process, modification-dependent macromolecule catabolic process, proteolysis, translational initiation, and proteasomal ubiquitin-dependent protein catabolic process were the remaining biological processes. The relevant significant pathways included spliceosome, oxidative phosphorylation, metabolism proteins, and proteasomes (Table 3).

In the case of β-cells, most biological processes and pathways were related to immune responses and apoptosis (Table 4). The more significant biological processes were chemotaxis, regulation of the IκB kinase/nuclear factor (NF)-κB cascade, and antigen processing and presentation of peptide or polysaccharide antigen via major histocompatibility complex (MHC) Class I. In this sense, natural killer cell-mediated cytotoxicity, NOD-like receptor signaling, chemokine signaling, B cell receptor signaling, T cell receptor signaling, cytokine–cytokine receptor and Toll-like receptor signaling pathways were the remarkable immune pathways. Apoptosis and p53 signaling were the apoptotic-enriched pathways. In addition, there were some major biological processes, such as regulation of protein modification process, positive regulation of DNA metabolic process, proteolysis, ubiquitin-dependent protein catabolic process, spliceosomes, small nuclear ribonucleo protein (snRNP) biogenesis, and regulation of RNA metabolic process. Janus tyrosine kinases (Jak)/signal transducers and activators of transcription (STAT) signaling and insulin signaling pathways were two noticeable pathways related to the genetic information processing class. Positive regulation of cell proliferation, cell migration and cell communication were the last significant biological processes. Regulation of the actin cytoskeleton and focal adhesion were two significant pathways for the cellular processes class. The last remarkable pathway was the neurotrophin signaling pathway, which has been established to be involved in T1D pathogenesis.[45]

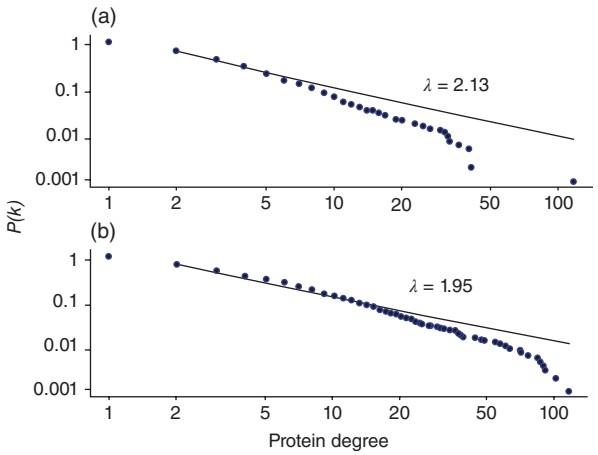

Figure 3 Power law distribution of node degrees. Degree distribution of the query–query protein–protein interaction network in (a) peripheral blood mononuclear cells (PBMCs) and (b) pancreatic β-cells. $P(k) = k^{-\lambda}$, where $P(k)$ is a distribution of node degree, k is a degree, and λ is a degree exponent.

Identification of complexes

Several cliques with three and four nodes (three-clique and four-clique, respectively) were identified in the QQPPI networks using CFinder software. The corresponding complexes were retrieved from the CORUM database and are listed in Tables 5 and 6. For PBMCs, the complexes identified were involved in many biological processes, like protein processing, proteasomal degradation, stress response, protein binding, protease activator (ID: 38, 39, 181, 191, 192, 193, 194), protein biosynthesis (ID: 306), RNA processing and RNA binding (ID: 351, 1181, 1332), translation initiation (ID: 742, 1097), phosphate metabolism, transcription activation and protein modification (ID: 2601), transcription repression and DNA binding (ID: 2918), ribosome biogenesis (3055), cell cycle and RNA synthesis (ID: 5593), and apoptosis (ID: 5623).

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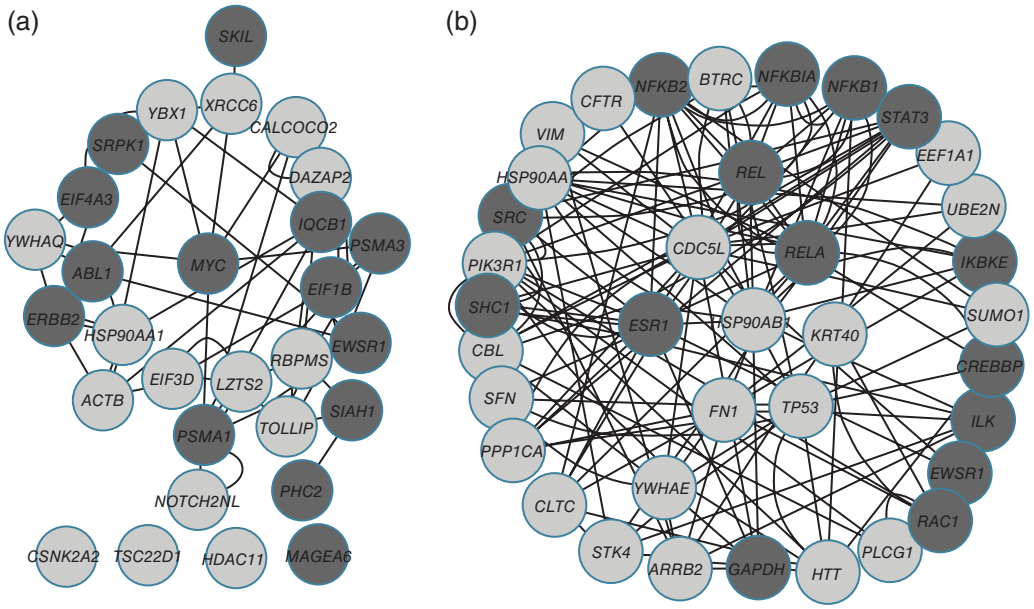

Figure 4 Hub–bottleneck nodes in (a) peripheral blood mononuclear cells (PBMCs) and (b) pancreatic β-cells. Genes with upregulated and downregulated expression are indicated by dark gray and light gray colors, respectively. For a definition of all gene symbols, refer to Table S4.

In the case of pancreatic β-cells, these complexes mediated various biological functions, such as protein processing, proteasomal degradation, stress response, protein binding, protease activator (ID: 38, 39, 181, 191, 192, 193, 194), ribosome biogenesis and protein biosynthesis (ID: 306, 3055), RNA processing and RNA binding (ID: 351, 1189, 1335, 5615), regulation of transcription (ID: 2084, 2086, 1335), assembly of protein complexes (ID: 2242), protein binding and cell adhesion (ID: 2376, 2383, 5342), cell cycle, protein modification and cellular signaling and adaptive cell-mediated response (T cells; ID: 2470, 2529, 2551), apoptosis, (ID: 2684, 4158, 5623), the IκB kinase/NF-κB cascade and cytokine activity (ID: 5193,5196, 5228, 5232, 5233, 5269, 5464, 5492), intracellular signaling cascade (ID: 5220, 5615), G-protein-coupled receptor protein signaling pathway, and cell migration ID: 5342).

Table 3 List of pathways enriched in modules for peripheral blood mononuclear cells

Module ID	Pathway	*P*-value
M1	hsa03040: Spliceosome	1.6×10^{-5}
M2	hsa00190: Oxidative phosphorylation	7.4×10^{-5}
M5	REACT_1762: 3′ UTR-mediated translational regulation	2.7×10^{-9}
	REACT_17015: Metabolism of proteins	9.5×10^{-8}
	REACT_71: Gene expression	1.1×10^{-6}
M6	hsa03050: Proteasome	1.1×10^{-5}
M7	hsa04062: Chemokine signaling pathway	1.8×10^{-4}
	hsa04060: Cytokine–cytokine receptor interaction	3.3×10^{-4}
	hsa05120: Epithelial cell signaling in *Helicobacter pylori* infection	5.9×10^{-3}
M9	hsa03450: Non-homologous end-joining	3.2×10^{-4}
M10	REACT_6900: Signaling in immune system	7.1×10^{-3}

UTR, untranslated region.

Identification of key markers for T1D

To identify key markers in QQPPI networks, we prepared the list of proteins that were hub–bottlenecks in each QQPPI network, and then chose the hub–bottleneck nodes that incorporated in both functional modules and complexes identified in the networks. It has been established that hubs and bottlenecks play pivotal role in networks and they are considered biologically significant proteins.[46,47] The key markers and their functions are listed in Table 7 and their locations in functional modules are shown in Fig. 5.

Discussion

Given the complex genetics of T1D and the notion that ongoing molecular mechanisms of the disease contribute to the processes mediated at the level of both the immune system and the pancreatic β-cell,[7] we combined transcriptome and interactome data in PBMCs and pancreatic β-cells to determine the underlying biological

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Table 4 List of pathways enriched in modules for pancreatic β-cells

Module ID	Pathway	*P*-value
M1	hsa04650: Natural killer cell-mediated cytotoxicity	8.2×10^{-6}
	hsa04810: Regulation of actin cytoskeleton	8.2×10^{-6}
	hsa04630: Jak/STAT signaling pathway	6.7×10^{-6}
	hsa04012: ErbB signaling pathway	5.9×10^{-6}
	hsa04666: FcγR-mediated phagocytosis	3.1×10^{-6}
	hsa04510: Focal adhesion	2.5×10^{-5}
	hsa04062: Chemokine signaling pathway	5.1×10^{-4}
	hsa04664: FcγRI signaling pathway	1.8×10^{-4}
	hsa04662: B cell receptor signaling pathway	1.6×10^{-4}
	hsa04660: T cell receptor signaling pathway	1.4×10^{-4}
	hsa04060: Cytokine-cytokine receptor interaction	4.3×10^{-3}
	hsa04910: Insulin signaling pathway	1.4×10^{-2}
	hsa04722: Neurotrophin signaling pathway	4.8×10^{-2}
M2	REACT_604: Hemostasis	3.8×10^{-2}
M3	hsa04920: Adipocytokine signaling pathway	7.0×10^{-5}
	hsa04621: NOD-like receptor signaling pathway	8.1×10^{-5}
	hsa04622: RIG-I-like receptor signaling pathway	9.6×10^{-4}
	hsa04620: Toll-like receptor signaling pathway	5.2×10^{-3}
	hsa04940: Type I diabetes mellitus	4.4×10^{-8}
	hsa04514: CAMs	2.3×10^{-6}
M6	hsa03050: Proteasome	1.4×10^{-11}
	hsa04612: Antigen processing and presentation	3.8×10^{-3}
M7	REACT_11052: Metabolism of non-coding RNA	1.2×10^{-2}
M8	hsa03440: Homologous recombination	1.4×10^{-3}
M11	hsa04115: p53 signaling pathway	3.4×10^{-2}
M12	hsa04115: p53 signaling pathway	6.3×10^{-3}
	REACT_578: Apoptosis	1.1×10^{-4}

CAM, cell adhesion molecules; Jak, Janus tyrosine kinases; STAT, signal transducers and activators of transcription; NOD, nucleotide-binding oligomerization domain; RIG-I, retinoic acid-inducible gene 1.

pathways and decipher the missing heritability of this complex disease.

In the present study we were able to show, for the first time, bilateral relationships between PBMCs and pancreatic β-cells considering differentially expressed chemokines, cytokines, and their receptors in both PBMCs and β-cells using a systems approach. The results verified that β-cells are not passive victims during the pathogenesis of T1D. Pathway enrichment analysis indicated that immune response, apoptosis, spliceosome, and proteasome pathways of protein synthesis were the most significantly enriched pathways in both tissues. However, in PBMCs, the metabolism (oxidative phosphorylation) and phosphate metabolism complex (ID: 2601) classes comprised one of the significant enriched pathways in functional modules and biological processes in complexes. It has been proposed that chronic hyperglycemia itself may directly or indirectly affect the gene expression profile of PBMCs in untreated diabetes. [48] Thus, PBMCs reflect systemic metabolic changes as well as abnormal immune regulation.

We prepared compendium annotations of most relevant key markers, hub–bottleneck nodes involved in both functional modules and complexes, in terms of their up- and downregulation of expression to describe their possible role in T1D pathogenesis. For PBMCs, the first key marker was the Y-box binding protein 1 (YBX1/YB1) in Module 1 and complex (ID: 3055). It was downregulated and incorporated in the enriched spliceosome pathway. It has been reported that the *YBX1* gene plays role in cytokine mRNA stability, which is important in autoimmune diseases.[49] In addition, there is evidence of an important role for YB1 as a regulator of prototypic protein tyrosine phosphatase (PTP1B) expression, which is considered as a critical regulator of insulin- and cytokine-mediated signal transduction.[50] The second and third markers were proteasome subunit alpha 1 and 3 (*PSMA1*, *PSMA3*), genes involved in Module 6 and the proteasome complex; both *PSMA1* and *PSMA3* were upregulated. They are members of the enriched proteasome pathway. The ubiquitin proteasome system has a salient biological role in the antigen processing and immune response because it could potentially be involved in the pathogenesis of many immune-related diseases.[51] The fourth marker, DNA non-homologous end-joining repair gene (*XRCC6*) was downregulated and found in Module 9, in which Non-homologous end-joining (NHEJ) was an enriched pathway, and in complex (ID: 2918). The *XRCC6* gene encodes an enzyme involved in variable division joining (V(D)J) recombination,[52] the process of genetic rearrangement in generating B cell receptor and T cell receptor diversity. The work of Khanna *et al.* showed that proteomic defects in *XRCC6* may cause not only lower double-strand break repair capacity, but are also related to severe combination immune deficiency due to severely impaired V(D)J recombination capacity.[53] The last, namely the SR (serine/arginine) protein kinase (*SRPK1*) gene, in Module 4 and complex (ID: 3055), was upregulated. The *SRPK1* gene is activated early during apoptosis and possible biological roles for SRPKs (1 and 2) are involvement in signaling

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Table 5 Complexes identified in peripheral blood mononuclear cells

Symbol	Complex
PSMA1, PSMA3, PSMA4, PSMB7, PSMB3, PSMC2, PSMD6	Proteasome (ID: 38, 39, 181, 191, 192, 193, 194)
RPS7, RPL35, RPL31,RPS10	Ribosome, cytoplasmic (ID: 306)
EIF4A3, PABPC1, SNRPA, SNRPA1, AQR, RBM22, IGF2BP3	Spliceosome (ID: 351)
EIF3C, EIF3D, EIF3F, EIF3H, EIF3E, EIF3K, EIF3J, EIF3M	eIF3 complex (ID: 742, 1097)
EIF4A3, PABPC1, SNRPA, SNRPA1, AQR, RBM22, HNRNPA2B1, HNRNPM	C complex (ID: 1181)
SRPK1, EWSR1, FUS, HNRNPM	Large Drosha complex (ID: 1332)
CCNT1, CDK9, MED1	P-TEFb–BRD4–TRAP220 complex 1 (ID: 2601)
XRCC5, YY1, XRCC6	Ku antigen–YY1–αMyHC promoter complex (2918)
RPS7, YBX1, SRPK1, RPL35, RPL31, SLC25A5, SRP14, HNRNPM	Nop56p-associated pre-rRNA complex (ID: 3055)
LIN37, LIN54, RBBP4	LINC core complex (ID: 5589, 5593)
HSP90AA1, MME, MAP3K5	Ask1–HSP90–AKT1 complex (ID: 5623)

For a definition of all gene symbols, refer to Table S4.
P-TEFb, positive transcription elongation factor; BRD4, bromodomain containing 4; TRAP220, thyroid hormone receptor-associated protein complex 220 kDa component; αMyHC, α-myosin heavy chain promoter; LINC, linker of nucleoskeleton and cytoskeleton; Ask1, apoptotic signal-regulating kinase 1; HSP90, heat shock protein 90; AKT1, AKT serine/threonine kinase 1.

Table 6 Complexes identified in pancreatic β-cells

Symbol	Complex
PSMA3, PSMA6, PSMA7, PSMB1, PSMB7, PSMB8, PSMB9, PSMC4	Proteasome (ID: 38, 39, 181, 191, 192, 193, 194)
RPL11, RPL5, RPS13, RPS16, RPS4X	Ribosome, cytoplasmic (ID: 306)
CDC5L, DDX17, DDX5, EIF4A3, PRPF8, SNRNP200, SNRPE, SNRPG, SRRM1	Spliceosome (ID: 351)
CDC5L, DDX5, EIF4A3, HNRNPC, HNRNPM, PRPF8, SNRNP200, SNRPE, SNRPG, SRRM1	C complex spliceosome (ID: 1189)
CDC5L, DYNC1H1, GCN1L1, PPP1CA, SRRM1	CDC5L complex (ID: 1183)
CDC5L, DDB1, EEF1A1, PFKL, PRPF8, SNRNP200, TUBB	SNW1 complex (ID: 1335)
NFKB1, NFKB2, RELA, RELB	NFKB1-NFKB2-REL-RELA-RELB complex (ID: 2084, 2086)
CALM1, HTT, TGM2	TGM2-HD-CALM1 complex (ID: 2242)
FN1, ITGB3, ITGA5, TGM2	ITGA2B–ITGB3–FN1–TGM2 complex, ITGA5–ITGB1–FN1–TGM2 complex (ID: 2376, 2383)
BCAR1, ESR1, PIK3R1, SRC	p130Cas–ERα–cSrc kinase–PI3K p85 subunit complex (ID: 2470)
CBL, PIK3R1, PLCG1, VAV1	LAT–PLCγ1–p85–GRB2–CBL–VAV–SLP-76 signaling complex (ID: 2529)
PDGFRA, PIK3R1, PLCG1	PDGFRA–PLCγ1–PI3K–SHP-2 complex, PDGF stimulated (ID: 2551)
BCL2L1, TP53	p53–Bcl-xL complex, DNA-damage induced (ID: 2684)
EEF1A1, HNRNPM, RPL11, RPL5, RPS13, RPS16, SLC25A5	Nop56p-associated pre-rRNA complex (ID: 3055)
CALM1, HSP90AA1, HSP90AB1	HSP90–FKBP38–CAM–Ca^{2+} complex (ID: 4158)
NFKB1, NFKB2, NFKBIA, NFKBIB, NFKBIE, RELA, RELB, TNIP2, BTRC, IKBKE, GTF2I, MAP3K8, RPS13, IQGAP2, HSP90AA1, HSP90AB1	TNF-α/NF-κB signaling complex (ID: 5193, 5196, 5233, 5269)
IQGAP2, RELA, TNIP2	CHUK–IQGAP2–AKAP8L–RELA–TNIP2 complex (ID: 5220)
MAP3K8, RELA, TNIP2	REL–MAP3K8–RELA–TNIP2–PAPOLA complex (ID: 5228)
NFKB1, NFKB2, NFKBIA, NFKBIE, RELA, TNIP1, TNIP2	CHUK–NFKB2–REL–IKBKG–SPAG9–NFKB1–NFKBIE–COPB2–TNIP1–NFKBIA–RELA–TNIP2 complex (ID: 5230)
LMO1, RAC1	ELMO1–DOCK1–RAC1 complex (ID: 5342)
NFKB1, NFKBIA, RELA	IκBα–NF-κBp50–NF-κBp65 complex, IKBA–NF-κBp65–NF-κBp50 complex (ID: 5464,5492)
HDGF, NMI, PDCD4, YWHAE	Emerin complex 52 (ID: 5615)
HSP90AA1, HSP90AB1, MAP3K5	Ask1–HSP90–AKT1 complex (ID: 5623)

For a definition of all gene symbols, refer to Table S4.
SNW1, SNW domain containing 1; ITGA2B, integrin subunit alpha 2b; ITGB1, integrin subunit beta 1; PI3K, phosphoinositide-3-kinase; LAT, linker for activation of T cells; GRB2, growth factor receptor bound protein 2; SLP-76, SH2 domain-containing leukocyte protein of 76 kDa; SHP-2/PTPN11, protein tyrosine phosphatase, non-receptor type 11; PDGF, platelet-derived growth factor; NOP56p, nucleolar protein 56; FKBP38, FK506 binding protein 8; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-κB; CHUK, conserved helix–loop–helix ubiquitous kinase; AKAP8L, A-kinase anchoring protein 8 like; PAPOLA, poly(A) polymerase alpha; IKBKG, inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma ; SPAG9, sperm associated antigen 9; COPB2, coatomer protein complex subunit beta 2 ; ELMO1, engulfment and cell motility 1; DOCK1, dedicator of cytokinesis 1; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha.

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pathways governing apoptosis, alternative mRNA splicing, RNA stability, and possibly the generation of autoantibodies directed against splicing factors.[54]

In case of pancreatic β-cells, there were five key markers, namely E3 ubiquitin-protein ligase CBL (*CBL*), non receptor tyrosine kinase (*SRC*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), phospholipase C, gamma-1 (*PLCG1*), and SHC-transforming protein-1 (*SHC1*), which mapped on module 1. The *CBL* gene, which is located in complex (ID: 2529), is inherently involved in the cbl/Cbl-activating protein (cap) pathway, which is parallel to insulin action for uptake glucose via the glucose GLUT4 translocator. Downregulation of the *CBL* gene may be correlated with insulin resistance.[55] The *SRC* gene was upregulated and incorporated in complex (ID: 2470). Inhibition of Src (c-Src) activation by exendin-4 was reported to reduce endogenous reactive oxygen species production and increase ATP production in diabetic Goto-Kakizaki (GK) rat islets.[56] The *PIK3R1* gene was downregulated and involved in complexes (ID: 2470, 2529, and 2551). Mutations in PIK3R1 as a regularity subunit cause primary immunodeficiencies, especially antibody deficiencies (hypogammaglobinemia or agammaglobulinemia).[57] Type 1 diabetes has been reported in X-linked agammaglobulinemia (XLA) patients.[58] The *PLCG1* gene was downregulated and involved in natural killer cell-mediated cytotoxicity, the T cell receptor signaling pathway, the FcγRI signaling pathway and in complexes (ID: 2529, 2551). The *SHC1* gene was upregulated and located in the ErbB signaling pathway, chemokine signaling pathway, and insulin signaling pathway. Upregulation of p46Shc, which induces decreased insulin signaling sensitivity, has been reported

Table 7 Brief description of hub–bottleneck nodes in functional modules and complexes

Complex	Module	Fold-change*	Symbol	Function
PBMCs				
ID: 2918	9	−1	*XRCC6*	ATP-dependent helicase involved in DNA NHEJ
ID: 3055	1	−1	*YBX1*	Mediates pre-mRNA alternative splicing regulation
ID: 3055	4	1	*SRPK1*	Protein kinase involved in regulation of splicing
ID: 38, 39, 191	6	1	*PSMA1*	Proteasome subunit 1 that cleaves peptides in ATP/ubiquitin-dependent process
ID: 191, 192, 193	6	1	*PSMA3*	Proteasome subunit 1 that cleaves peptides in ATP/ubiquitin-dependent process
	5	1	*EIF1B*	Probably involved in translation
ID: 742, 1097	8	−1	*EIF3D*	Participates in the initiation of protein synthesis
β-Cells				
ID: 2529	1	−1	*CBL*	Adapter protein that functions as a negative regulator of many signaling pathways
ID: 2470	1	1	*SRC*	Non-receptor protein tyrosine kinase involved in signal transductions
ID: 2470, 2529, 2551	1	−1	*PIK3R1*	Binds to activated (phosphorylated) protein tyrosine kinases and acts as an adapter
ID: 2529, 2551	1	−1	*PLCG1*	Mediates the production of DAG and IP_3, which have an important roles in signaling cascades
	1	1	*SHC1*	Signaling adapter that couples activated growth factor receptors to signaling pathway
ID: 5220, 5230	3,10	1	*RELA (NFKB3)*	NF-κB is a pleiotropic transcription factor and endpoint of a series of signal transduction events
	3	1	*REL*	Proto-oncogene that regulate genes involved in apoptosis, inflammation, the immune response
ID: 2086, 5230	3	1	*NFKB2*	Functions as a central activator of genes involved in inflammation and immune function
ID: 2084, 5464	3	1	*NFKB1*	NF-κB is a pleiotropic transcription factor and endpoint of a series of signal transduction events
ID: 5230, 5494	3	1	*NFKBIA*	Inhibits NF-κB/REL complexes that are involved in inflammatory responses
	5	−1	*UBE2N*	Member of the E2 ubiquitin-conjugating enzyme family
	9	−1	*SUMO1*	Ubiquitin-like protein that covalently attaches to proteins
ID: 2684	11	−1	*TP53*	Acts as a tumor suppressor in many tumor types

For a definition of all gene symbols, refer to Table S4.

*A positive number indicates an increase, a negative number indicates a decrease.

NHEJ, non-homologous end joining; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; NK-κB, nuclear factor-κB.

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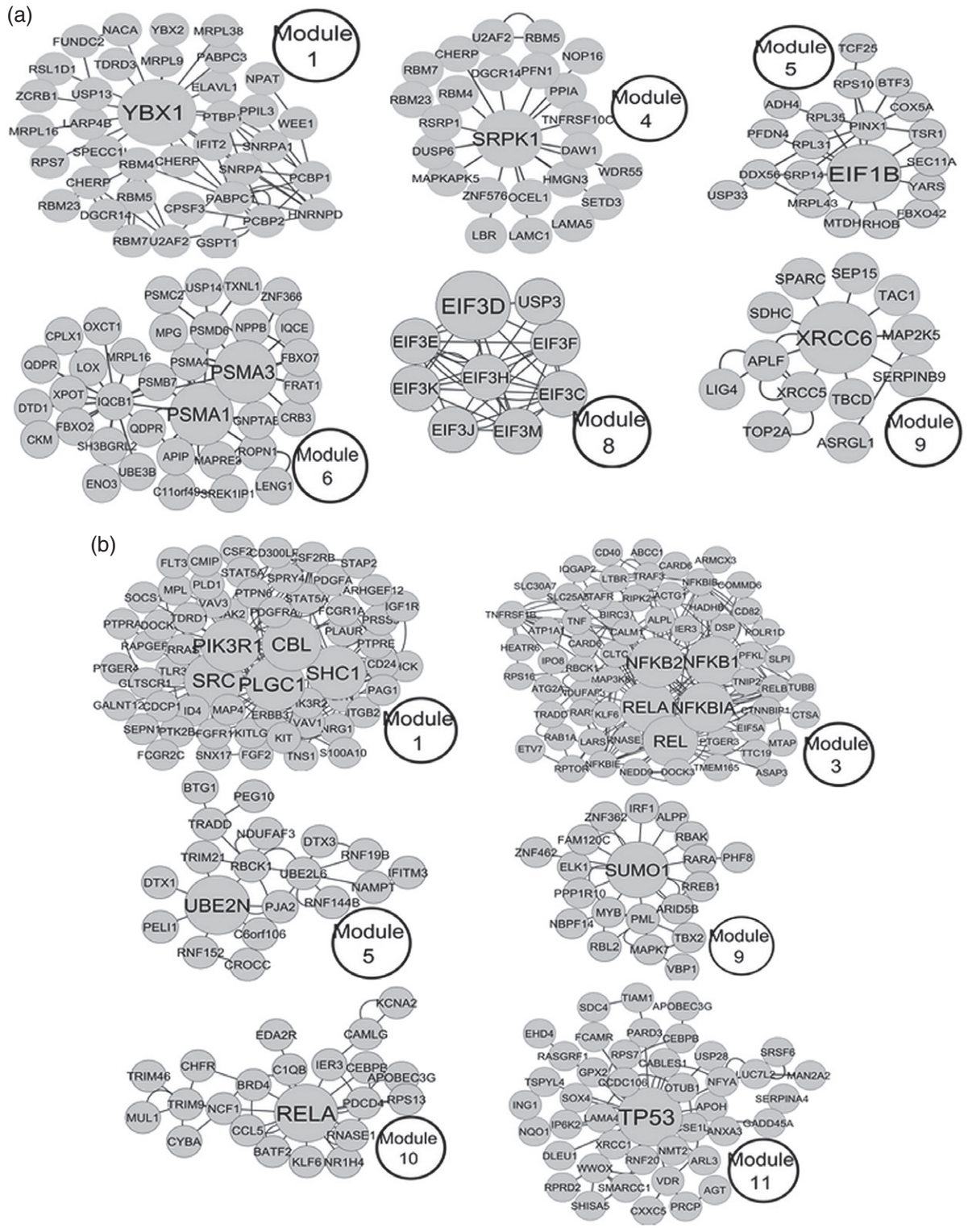

Figure 5 Key markers in functional modules in (a) peripheral blood mononuclear cells (PBMCs) and (b) pancreatic β-cells. Key markers are indicated by the larger font size. In case of PBMCs, modules represent several biological processes/pathways such as splicesome (module 1), RNA processing (module 4), translational regulation (module 5), proteosem (module 6), translation (module 8) and Non-homologous end-joining (module 9). For pancreatic β-cells, modules represent immunological responses (module 1,3,10), proteolysis involved in cellular protein catabolic process (module 5), transcription (module 9) and p53 signaling pathway (module 11). All symbols are for genes. For a definition of all gene symbols, refer to Table S4.

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in a p66Shc-knockout strain of mice (ShcP).[59] All five markers were incorporated in ErbB signaling pathway. Moreover, the *CBL*, *SHC1*, and *PIK3R1* genes were involved in both natural killer cell-mediated cytotoxicity and the neurotrophin signaling pathway, whereas *CBL*, *SHC1*, and *PIK3R1* were incorporated in the insulin signaling pathway. Finally, ubiquitin-conjugating enzyme E2N (*UBE2N*/*UBC13*) in Module 5 was downregulated. It has been reported that *Ubcl13* plays a crucial role in maintenance of the in vivo immunosuppressive function of T regulatory (T_{reg}) cells and in inhibition of the conversion of T_{reg} cells into T helper (Th) 1- and Th17-like effector T cells in a manner dependent on its downstream target IκB kinase (IKK) in mice.[60]

In summary, the present study shows that network biology can be considered as an effective approach to obtain information regarding the underlying etiology of complex diseases, such as T1D, and that data integration is pivotal to such analyses. The analysis of transcriptomes of PBMCs and pancreatic β-cells propagated the concept of dialogue between pancreatic islets and the immune system, mediated by cytokine and chemokine signaling pathways. Centrality, modularity, and clique analyses of the constructed networks resulted in the identification of significant biological pathways and genes. Finally, the present network-based analysis facilitated experimental identification of new diagnostic biomarkers and the development of therapeutic targets.

Disclosure

The authors declare that they have no conflicts of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of differentially expressed genes in peripheral blood mononuclear cells.

Table S2 Topological properties of all nodes in the peripheral blood mononuclear cell network.

Table S3 Topological properties of all nodes in the pancreatic β-cell network.

Table S4 List of full names for all gene symbols.

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