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Original Article

Key regulatory genes and signaling pathways involved in islet culture: a bioinformatic analysis

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Abstract: Type 1 diabetes (T1D) is characterized by non-ideal mass and low survival rate of islets. Therefore, it is necessary to find intrinsic factors that prolong the survival of islets. This study aimed to track out hub genes and pathways in the process of islet culture by bioinformatic analysis. We downloaded the gene expression microarray of GSE42591 from the Gene Expression Omnibus (GEO). Aberrant Differentially methylated genes (DMGs) were obtained using the GEO2R tool. Gene ontology (GO) analysis and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment analyses were performed on selected genes by using the Database for Annotation Visualization and Integrated Discovery (DAVID). A protein-protein interaction (PPI) network was constructed with the Retrieval of Interacting Genes (STRING) and visualized in Cytoscape 3.7.2. A total of 434 genes were overexpressed and 114 genes underexpressed in fresh to cultured 4 h tissue. KEGG pathway enrichment analyses revealed the TGF-beta signaling pathway, MAPK signaling pathway, or VEGF signaling pathway. The genes FN1, MKI67, IGF1, MAPK14, COL1A1 might be involved in islet culture. In general, this work scrutinized islet culture-relevant knowledge and provided insight into the regulation and mediation of islet survival.

Keywords: Type 1 diabetes, islet culture, bioinformatics analysis, KEGG pathway

Introduction

Type 1 diabetes (T1D) is the result of autoimmune damage to insulin-producing beta cells in the pancreas and destruction of the heart, eye, kidney, nervous system and other organs. There is an urgent need to identify new therapy for T1D [1]. Islet transplantation is a possible treatment for type 1 diabetes but is restricted by non-ideal transplantable islet mass and low survival of islets after transplantation [2]. Even with available donor organs, the current method of islet isolation and culture also results in islet cell loss by cell death in a short period of time [3]. Understanding the biologic processes of islets during culture could provide ideas for ways to prolong the survival time of islets in the future.

Bioinformatic analysis can screen out candidate genes that may affect the survival status of islets through differential expression and annotation of genes in biologic processes, which provides a strong basic support for the further study of islet survival. Such as the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins) can predict information for primary-secondary target interaction *in silico* [4], and KEGG pathway enrichment analysis can investigate functions of all protein targets (including direct protein targets and indirect protein targets) [5]. DAVID (Database for Annotation, Visualization and Integrated Discovery) can identify functional biologic categories of all genes [6]. In general, the results of this bioinformatics analysis could help provide information on the key genes for islet survival.

Therefore, our goal was to find the key genes that related to islet survival time by analyzing biologic changes in the process of islet culture with various bioinformatic methods, in order to provide a basis for further exploration of prolonging islet survival time.

Material and methods

Chip data source

The gene expression microarray of GSE42591 was obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/gds/) of the National Center for Biotechnology Information (NCBI). GSE 42951 was the expression profile of C57BL6 mice at different culture times. In this study, we screened 6 sample data numbers of GSM10104566, GSM10104567, GSM10104576, GSM10104577, GSM10104578, GSM10104578 of GSE42591, then we divided them into 3 groups by their culture time. The samples of GSM104566, GSM104567 were fresh as group A. The samples of GSM104576, GSM 104577 were cultured 1 h as group B. The samples of GSM104578, GSM104579 were cultured 4 h as group C. The chip platform was GPL1261 [Mouse430_2] Affymetrix Mouse Genome 430 Array, expression profiling by array.

Data processing

GEO2R was used in the analysis of GSE42591 and screened DMGs within different groups (in our study those were group A compared to group B, group A to group C, and group B to group C). Then we normalized the original data. Differentially expressed gene sets (DEGs) were identified with |log FC|>2 and $P<0.05$ and Sanger Box software was used to make volcano plots.

Data Go and KEGG pathway enrichment analysis

In order to understand these DEGs better, we subsequently subjected significantly DEGs to GO and KEGG pathway enrichment analysis by the online analytical database DAVID (http://david.abcc.ncifcrf.gov/) to cluster them according to their biologic functions.

Network visualization and screen key gene

The significantly DEGs were in turn subjected to retrieval in online analytical website STRING database (http://www.string-db.org/) to get the DEGs' protein interaction network and export it into TSV format, then integrated into a PPI network and visualized using Cytoscape software (version 3.7.2). The key genes were the top 5 genes screened by degree in descending order from Cytoscape 3.7.2.

Results

DEGs in each group

According to online tool GEO2R analysis, the microarrary data of 6 samples (GSM104566, GSM104567 were group A, GSM104576, GSM104577 were group B, and the GSM104578, GSM104579 were group C) of GSE42591 were screened to obtain the differentially expressed data (A-B is Supplement 1, B-C is Supplement 2, A-C is Supplement 3), then we diagrammed the DEGs in each group by Sanger Box software.

Group A vs Group B: 515 genes were identified as differentially expressed, of which 349 genes were overexpressed and 166 genes underexpressed (**Figure 1A**).

Group B vs Group C: 251 genes were identified as differentially expressed, of which 94 were overexpressed and 157 underexpressed (**Figure 1B**).

Group A vs Group C: 548 genes were identified as differentially expressed, of which 79.2% were overexpressed and 20.8% underexpressed (**Figure 1C**).

Gene set enrichment analysis

To understand the biologic processes of the DEGs between the three groups, we performed Gene Ontology (GO) term enrichment analysis of the DEGs lists which was divided into molecular function (MF), biologic process (BP) and cellular component (CC). We used the DAVID functional annotation tool to carry out the GO and KEGG enrichment analysis of the DEG lists of three groups. The DEGs between group A and B revealed that the MF were mainly concentrated in categories of nucleotide binding and cytokine binding. The BP were focused on cellular response to stress, regulation of cell adhesion and cell communication, and the CC were mainly extracellular region parts, extracellular matrix, and basement membrane (**Figure 2**). The DEGs of groups B to C showed categories of nucleotide binding, cofactor and coenzyme binding in MF, biologic and cell adhesion, locomotory behavior, and regulation of cell adhesion in BP, and plasma membrane, extracellular region, and extracellular region part in CC (**Figure 3**). The DEGs listed in groups A to C most involved: anion transmembrane transporter activity, chloride

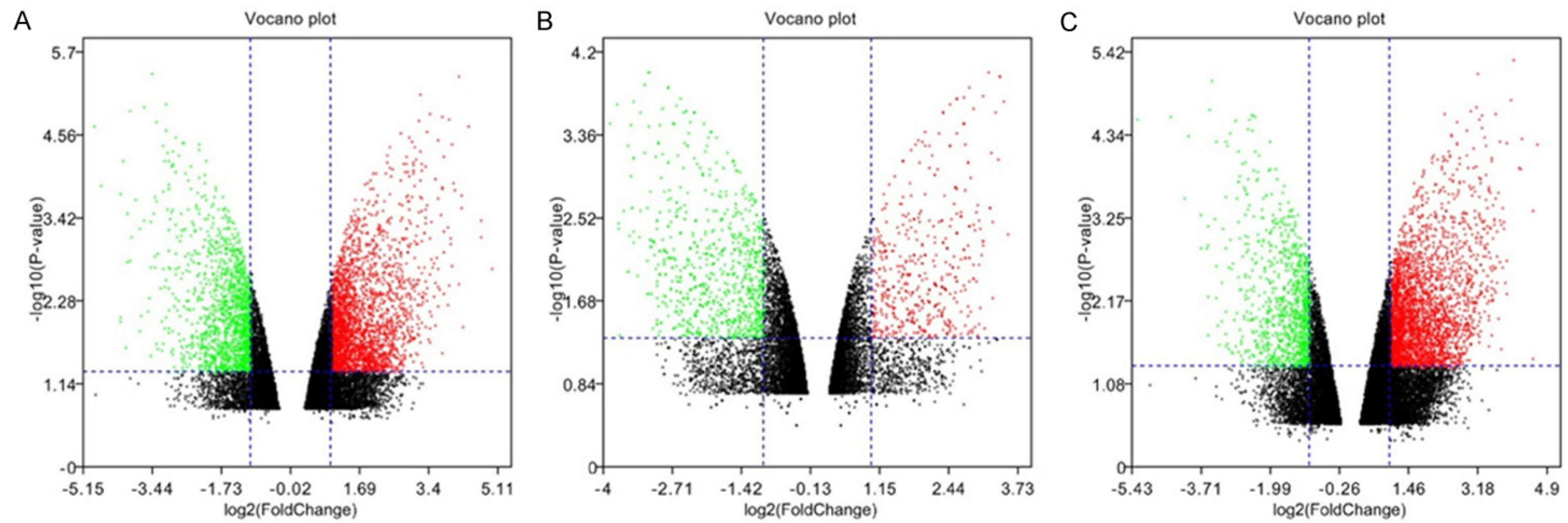

Figure 1. Differentially expressed gene sets in each group. Note: Each data point indicates the differentially expressed genes (DEGs). The red dot on the right indicates the gene expression is upregulated, the green dot on the left showed the gene expression is downregulated and the black spot at the middle is genes without differentially expression. A. Fresh islets compared with islets cultured 1 h, at left. B. Islets cultured 1 h compared with islets cultured 4 h, in middle. C. Fresh islets compared with islets cultured 4 h, at right.

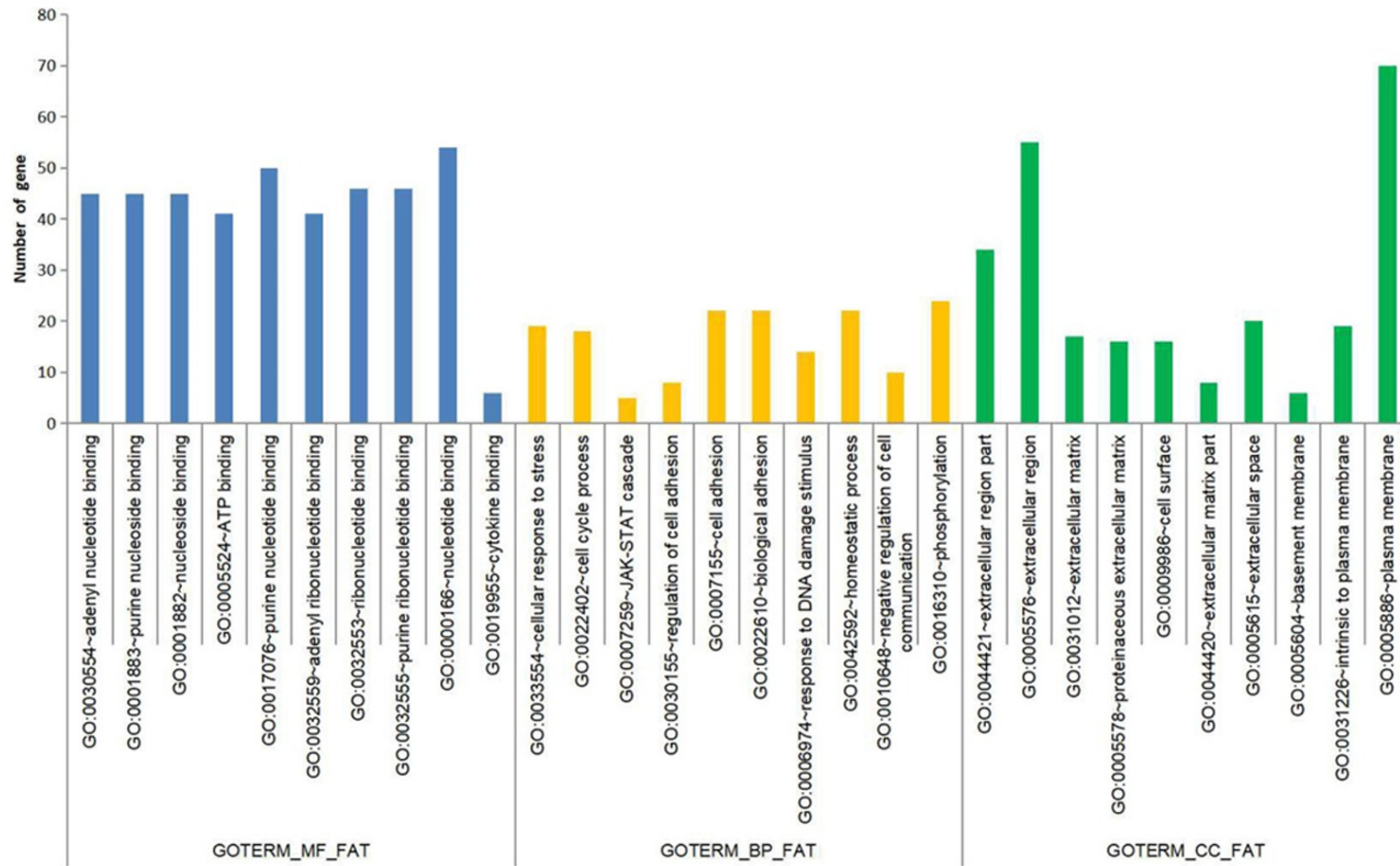

Figure 2. Gene Ontology (GO) term enrichment analysis of the DEGs from fresh islet to islets cultured 1 h.

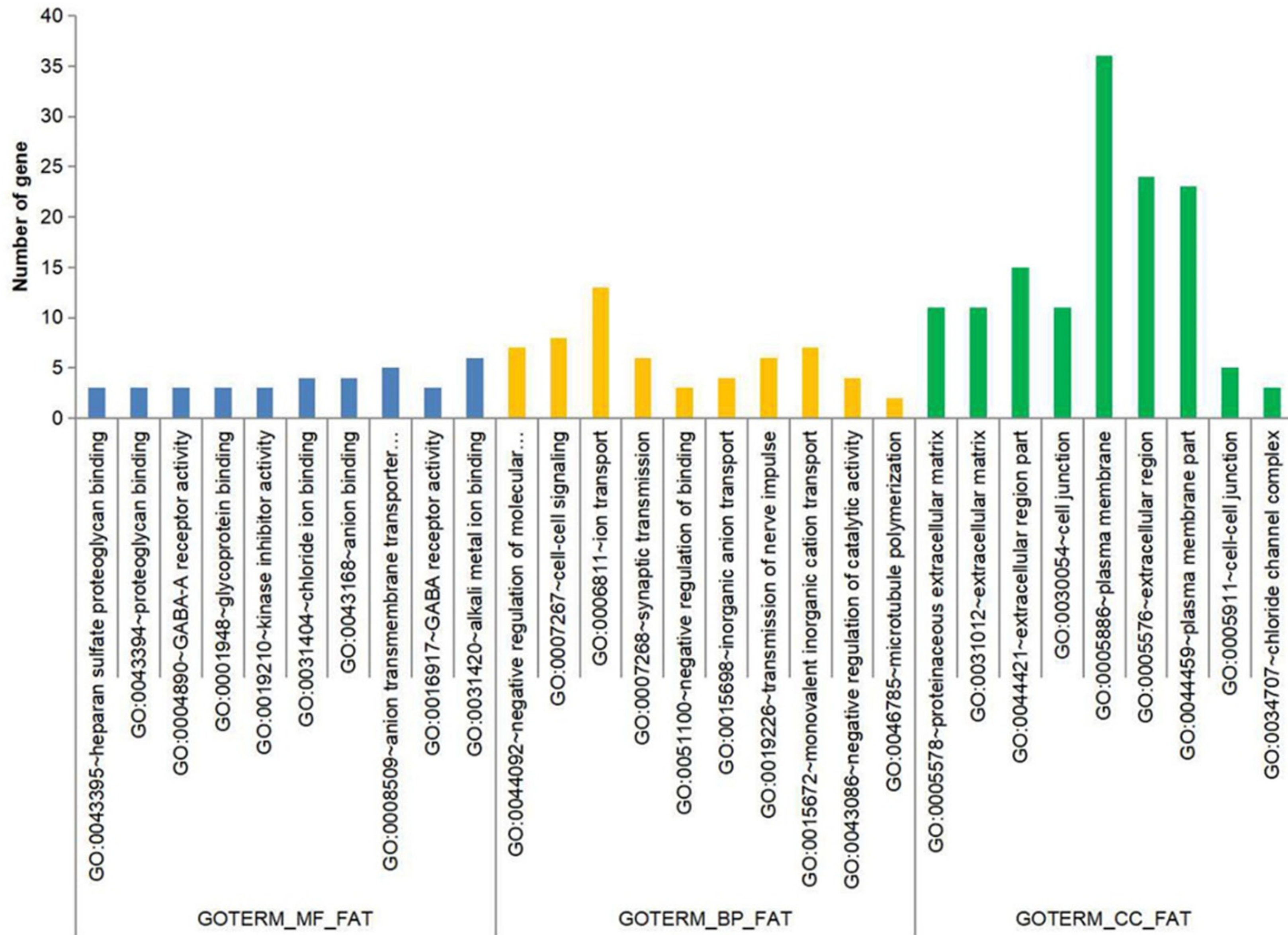

Figure 3. The Gene Ontology (GO) term enrichment analysis of the DEGs from islets cultured 1 h to islets cultured 4 h.

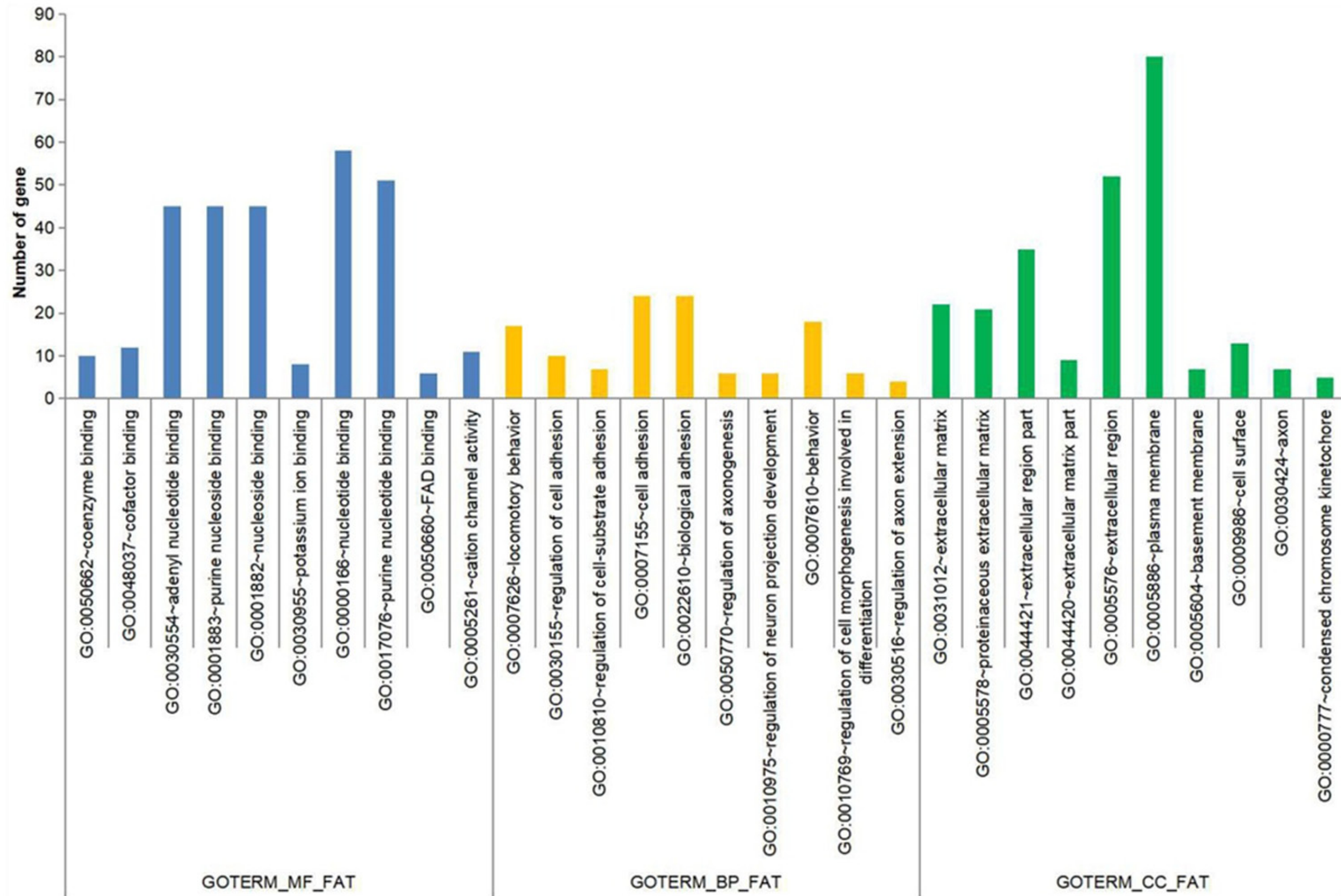

Figure 4. The Gene Ontology (GO) term enrichment analysis of the DEGs from fresh islets to islets cultured 4 h. Note: In **Figures 2-4** above, the GO term enrichment analysis all showed the top 10 DEGs. The blue indicates the molecular function (MF), the yellow indicates biologic processes (BP) and the green indicates cellular components (CC).

Table 1. KEGG pathway analysis of genes in fresh islets compared to islets cultured 1 h

Category	Term	Count	*P* Value	Genes
KEGG_PATHWAY	Pathways in cancer	15	7.7×10^{-3}	COL4A4, PRKCA, STAT5B, FGF11, IGF1, ACVR1C, CBLC, CDKN1A, HHIP, LAMC1, IKBKB, MMP1B, AKT3, AKT2, FN1.
KEGG_PATHWAY	MAPK signaling pathway	14	3.6×10^{-3}	PRKCA, DUSP4, MAPK14, NTRK2, PLA2G1B, FGF11, HSPA1A, HSPA1B, IKBKB, SRF, DDIT3, AKT3, AKT2, ACVR1C, CACNA2D4.
KEGG_PATHWAY	Focal adhesion	11	8.6×10^{-3}	COL4A4, PRKCA, PARVG, VAV3, IGF1, LAMC1, AKT3, MYL9, AKT2, FN1, PARV.
KEGG_PATHWAY	Chemokine signaling pathway	10	1.4×10^{-2}	CCR9, DOCK2, CCL22, VAV3, CCR5, CXCL16, STAT5B, IKBKB, AKT3, AKT2.
KEGG_PATHWAY	VEGF signaling pathway	7	5.1×10^{-3}	PRKCA, MAPK14, SPHK1, PLA2G1B, NFAT5, AKT3, AKT2.
KEGG_PATHWAY	Chronic myeloid leukemia	7	5.1×10^{-3}	CBLC, CDKN1A, STAT5B, IKBKB, AKT3, AKT2, ACVR1C.
KEGG_PATHWAY	Fc epsilon RI signaling pathway	7	7.4×10^{-3}	PRKCA, VAV3, MAPK14, PLA2G1B, MS4A2, AKT3, AKT2.
KEGG_PATHWAY	Erb B signaling pathway	7	9.8×10^{-3}	PRKCA, CBLC, CDKN1A, STAT5B, AREG, AKT3, AKT2.
KEGG_PATHWAY	T cell receptor signaling pathway	7	3.8×10^{-2}	CBLC, VAV3, MAPK14, NFAT5, IKBKB, AKT3, AKT2.
KEGG_PATHWAY	Small cell lung cancer	6	3.3×10^{-2}	COL4A4, LAMC1, IKBKB, AKT3, AKT2, FN1.
KEGG_PATHWAY	Glioma	5	4.5×10^{-2}	PRKCA, CDKN1A, IGF1, AKT3, AKT2.

Table 2. KEGG pathway analysis of genes in islet culture for 1 h compared to 4 h

Category	Term	Count	*P* Value	Genes
KEGG_PATHWAY	Calcium signaling pathway	7	6.5×10^{-3}	ADRB2, HRH1, ERBB4, CAMK4, RYR3, PLCD4, PTGFR.
KEGG_PATHWAY	Neuroactive ligand-receptor interaction	7	2.7×10^{-2}	GABRD, S1PR3, ADRB2, HRH1, GABRA2, GABRA5, PTGFR.
KEGG_PATHWAY	ECM-receptor interaction	4	3.7×10^{-2}	COL4A4, LAMA3, ITGA5, HMMR.

Table 3. KEGG pathway analysis of genes in fresh islets compared to those cultured 4 h

Category	Term	Count	*P* Value	Genes
KEGG_PATHWAY	Pathways in cancer	15	2.2×10^{-3}	PRKCA, WNT5A, BMP4, HSP90AA1, XIAP, IGF1, ZBTB16, CTNNA3, ACVR1C, PTK2, CDKN1A, LAMA3, LAMC1, AKT3, FN1.
KEGG_PATHWAY	Focal adhesion	14	6.25E-05	PRKCA, VAV3, XIAP, ITGA1, IGF1, MYL9, PTK2, LAMA3, COL6A2, COL1A1, LAMC1, AKT3, PARVA, FN1.
KEGG_PATHWAY	ECM-receptor interaction	6	1.8×10^{-2}	LAMA3, COL6A2, ITGA1, LAMC1, COL1A1, FN1.
KEGG_PATHWAY	Small cell lung cancer	6	2.0×10^{-2}	PTK2, LAMA3, XIAP, LAMC1, AKT3, FN1.
KEGG_PATHWAY	TGF-beta signaling pathway	6	2.2×10^{-2}	BMP4, INHBA, ACVR2B, INHBE, ID3, ACVR1C.
KEGG_PATHWAY	Drug metabolism	5	4.9×10^{-2}	GSTA4, FMO1, ALDH1A3, GSTO1, CYP2B10.
KEGG_PATHWAY	Aldosterone-regulated sodium reabsorption	4	4.1×10^{-2}	PRKCA, HSD11B2, IGF1, ATP1A2.

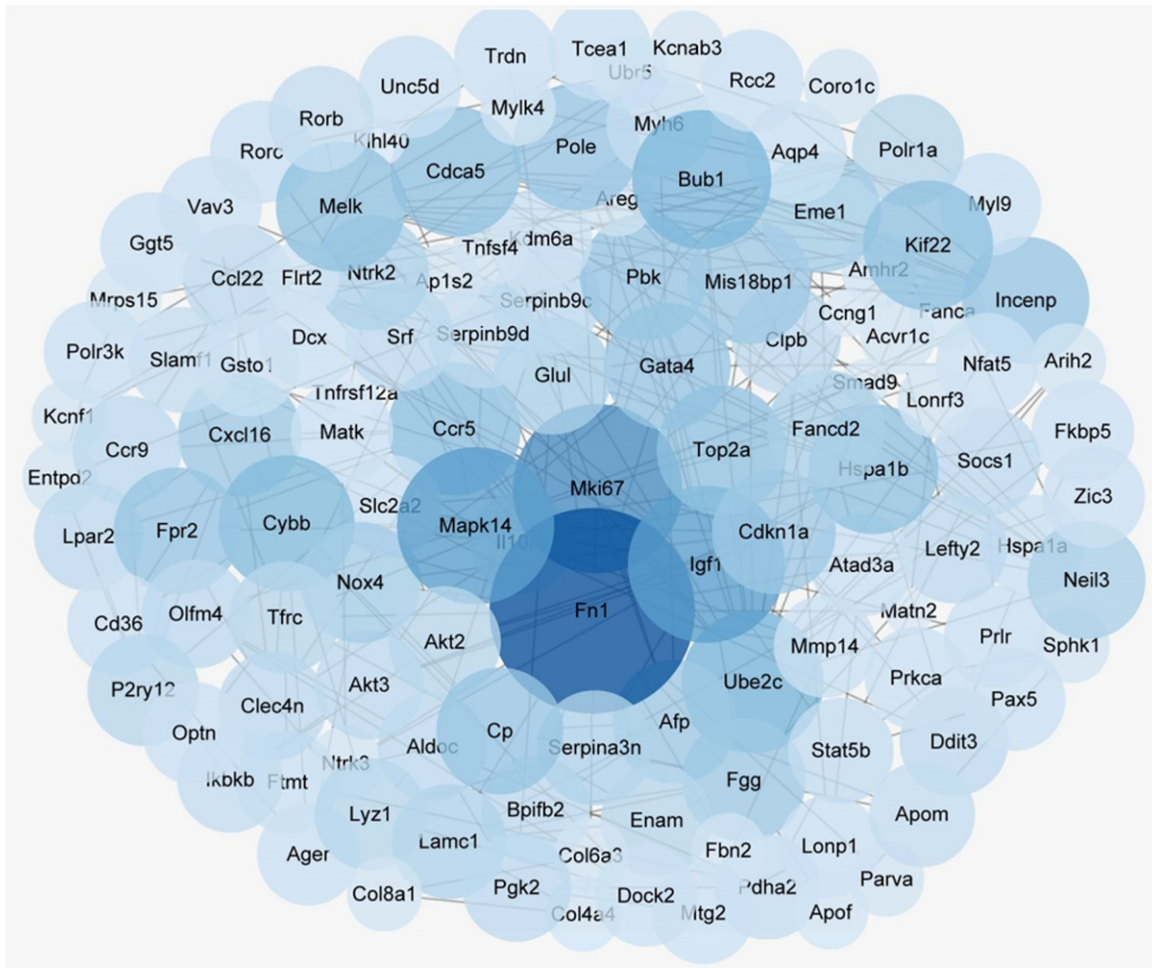

Figure 5. PPI network of islet-mediated proteins between fresh islets and islets cultured 1 h.

ion binding and anion binding in MF, ion transport, cell-cell signaling, negative regulation of molecular function and monovalent inorganic cation transport in BP, and plasma membrane, extracellular region, and plasma membrane part in CC (**Figure 4**).

KEGG enrichment analysis showed that the DEGs between group A and B mainly involved pathways in cancer, MAPK signaling pathway, and focal adhesion (**Table 1**). Groups B to C also take part in pathways of calcium signaling pathway, neuroactive ligand-receptor interaction and ECM-receptor interaction (**Table 2**). The groups A to C most showed pathways in cancer, focal adhesion, and others (**Table 3**).

DEGs of PPI network analysis

The physiologic functions of biologic systems are mainly dependent on interactions of proteins and genes. It is important to research the interaction of islet DEGs with different culture conditions. The DEGs in the three groups were respectively implemented in a STRING database to identify their associated proteins. There were 338 PPI pairs whose combined scores were higher than 0.5 in the network of groups A to B (**Figure 5**), 65 PPI pairs existed in groups B to C (**Figure 6**), and 386 PPI pairs were in the network of groups A to C (**Figure 7**). We input the results of STRING analysis into Cytoscape 3.7.2 to obtain the PPI network. The node degree was evaluated to investigate the centrality of proteins and represented by size, and low values to the small size. Meanwhile, we chose the top 10 of nodes found as the hub genes from each PPI network (**Table 4**).

Discussion

The survival time of islets cultured in vitro is short and they have a low mass, which would

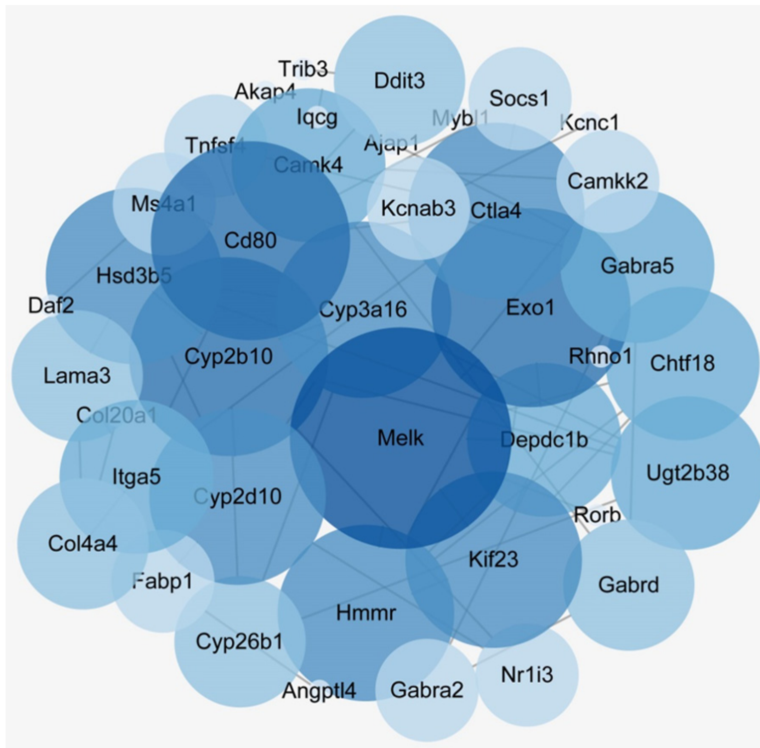

Figure 6. PPI network of islet-mediated proteins between islets cultured 1 h and 4 h.

result in poor treatment in diabetics. Therefore, it is important to find an internal cause that could prolong the survival time of islets [7]. Through a bioinformatics analysis, we disclosed a series of biologic changes and screened the regulatory factors that play a role in these processes, so as to provide candidate genes for further study of particular phenomena [8].

In our study, firstly, we used the online tool of GEO2R to compare the gene chip data in GEO database of islet culture of fresh, 1 h and 4 h, and obtained the expression levels of different genes among different groups. For example, there 434 genes were overexpressed and 114 genes underexpressed in fresh compared to culture for 4 h.

Secondly, GO enrichment analysis was conducted. We found many upregulated genes of biologic processes mainly associated with cell adhesion, and downregulation of genes that were primarily involved in apoptosis and inflammatory responses. Among them, the biologic process of apoptosis was found in data analysis of islet culture for 4 hours, but this process did not occur in other groups, indicating that apoptosis began to occur only 4 hours after islet exposure in vitro. Moreover, according to molecular function, we found out that changes of molecular function were mainly concentrated in: actin binding, transcription factor activity, sodium symporter activity and cation symporter activity. Therefore, we speculated that the biologic process of apoptosis after 4 hours of islet culture might be related to stress stimuli.

Then, KEGG pathway enrichment analysis also was performed to indicate a total of 21 pathways. These mainly included MAPK signaling pathway, VEGF signaling pathway, and TGF-beta signaling pathway. A study had shown that vascular endothelial growth factor (VEGF) was one of the major regulatory molecules in diabetics. The MAPK signaling pathway can mediate expression of VEGF [9]. VEGF signaling pathway also plays an important role in the treatment of diabetes by promoting cell proliferation to regulate islet cells [10]. In addition, transforming growth factor β (TGFβ) belongs to a signaling superfamily that governs pancreatic development, and the control of β-cell development, proliferation, and function [11, 12]. Inhibition of the TGF-β signaling pathway can result in increased β-cell replication [13]. These studies show that the signal pathways screened by bioinformatic analysis are a reference value for the further study of islets in the future.

Finally, the hub genes of each group islet were screened by PPT network. The most important core genes were FN1, MKI67, IGF1, and MAPK-14. Their degrees in the PPI network were all above 20. One of the most prominent was FN1 (Fibronectin 1). Research showed that FN1 is associated with cell adhesion, growth, migration, apoptosis, and differentiation. Down-regulation of FN1 expression can inhibit proliferation and induce cell apoptosis [14]. Other studies had shown that FN1 gene was significantly upregulated in islets with a high survival

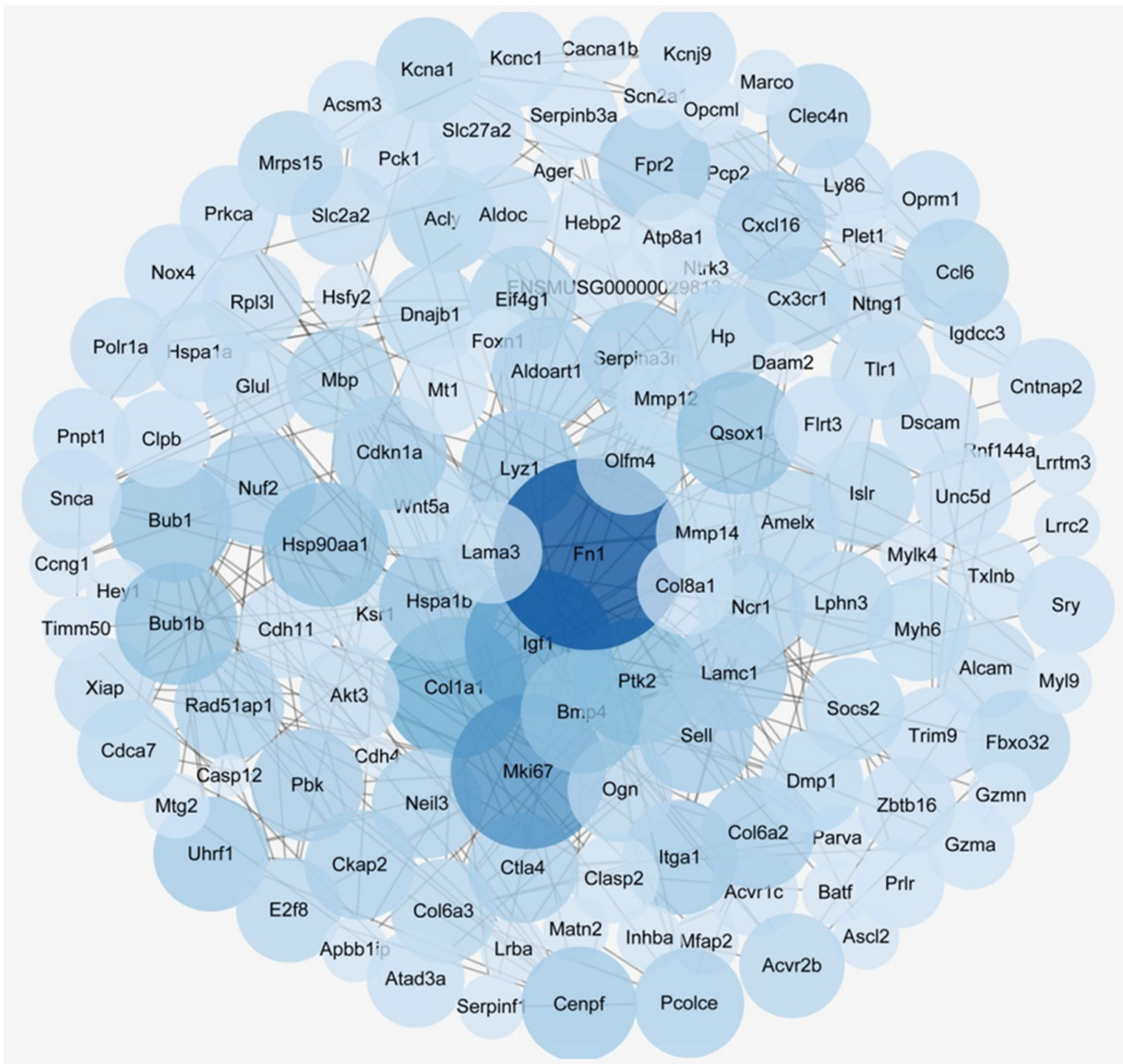

Figure 7. PPI network of islet-mediated proteins between fresh islets and those cultured 4 h. Note: In **Figures 5-7** above, the stronger the regulation with islet culture, the larger the circle and the darker the color.

Table 4. Hub genes of each PPI network

Group	Gene	Degree	Group	Gene	Degree	Group	Gene	Degree
A to B	Fn1	38	B to C	Melk	7	A to C	Fn1	43
	Mki67	26		Cd80	6		Mki67	28
	Igf1	22		Cyp2b10	6		Igf1	25
	Mapk14	22		Exo1	6		Col1a1	23
	Ube2c	17		Ctla4	5		Ptk2	17
	Bub1	16		Cyp3a16	5		Hsp90aa1	16
	Cybb	15		Cyp2d10	5		Bub1	15
	Ccr5	14		Kif23	5		Bub1b	15
	Cdca5	13		Hsd3b5	5		Bmp4	15
	Hspa1b	13		Hmmr	5		Qsox1	15

Note: group A is fresh islets, group B is islets cultured 1 h, group C is islets cultured 4 h.

rate [15]. Therefore, we believe that the FN1 gene has a great influence on the survival state of islets in the process of islet culture. The MKI67 (Marker Of Proliferation Ki-67) is also an important candidate gene. It is a nuclear protein that can code cell proliferation and is a proliferation marker [16]. A study showed that culturing islets with Ki67 could promote islet proliferation [17]. Another study showed that the expression of Ki67 increased with the enhancement of islet function [18]. We also analyzed another islet culture data GSE27547 from GEO database and found that MKI67 was also a hub gene. Insulin-like growth factor 1 (IGF1) is a β-cell mitogen and pro-survival factor that plays an important role in β-cells maturation and function. IGF1 overexpression in β-cells also prevents islet infiltration and helps mediate β-cell death, reducing β-cell apoptosis [19]. Another study using mice with IGF-1 gene deficiency showed that IGF-1 may inhibit islet cell growth and probably has a negative regulatory effect on islets in culture [20]. Therefore, we believe that up-regulated IGF1 expression may prolong the survival time of islets. Mitogen-activated protein kinases 14 (MAPK14) is more implicated in the control of cell survival and cell death [21]. A study showed that inhibiting the MAPKs could preserve islet beta cell mass, suggesting that MAPK14 might have a negative regulatory effect on islets. Up-regulated MAPK14 expression may reduce the survival time of islets [22].

These genes are not only related to cell proliferation and apoptosis, but also play an important role in regulating islet survival and apoptosis.

Conclusion

Bioinformatic analysis based on a series of online tools including GEO2R, STRING, and DAVID was applied to mine and integrate knowledge of islet survival biologic action at different culture times. We screened four key genes that play a major role in islet culture. Although we are not sure whether the regulation of these four key genes can regulate the survival time of islets, we believe that these four genes must have some regulatory effect on islet function, which we will further explore in a follow-up study.

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Disclosure of conflict of interest

None.

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