Acute Pancreatitis-Associated Genes Identified by Integrative Analysis of Gene Expression Data

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Research article

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Abstract

Background Acute pancreatitis (AP) is a common digestive disease. A better understanding of the biology and molecular pathogenesis of AP may provide the basis for the prevention and therapy of AP.

Methods The gene expression datasets GSE109227, GSE3644 and GSE65146, which included gene expression profiles of pancreas samples from AP and control mice, were obtained from the Gene Expression Omnibus database. All differentially expressed genes (DEGs) were identified using GEO2R and annotated using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses.

Results Thirty-eight DEGs were found to overlap among the three datasets. A protein-protein interaction (PPI) network was constructed; hub proteins were identified; and functional modules were extracted. Three genes (Cdh1, Iqgap1 and Actn4), which are involved in the adherens junction pathway, were identified using PPI analysis and may provide potential biomarkers for the diagnosis and treatment of AP.

Conclusions We will further study the effects of the Cdh1, Iqgap1 and Actn4 genes on the occurrence and development of acute pancreatitis, which may provide new targets for the diagnosis and treatment of AP.

Background

The rate of acute pancreatitis (AP) has been increasing in the past years worldwide^[1–3]. According to the Atlanta classification, AP is divided into mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP), and severe acute pancreatitis (SAP). AP was the most common principal discharge diagnosis among patients with gastrointestinal and hepatic diseases in the USA in 2009. Severe acute pancreatitis (SAP), which is associated with multiple organ failure, is especially lethal and poses a major financial burden for the country^[4–8].

AP is characterized by a highly variable clinical course, with a complex pathological process involving a variety of gene expression changes. Therefore, a better understanding of the biology and molecular pathogenesis of AP may provide the basis for the prediction of disease severity or identify markers for the prevention and therapy of AP^[9–11].

Recently, gene expression analysis using high-throughput platforms has become a powerful tool for clinical application, such as elucidation of the molecular mechanism and identification of therapeutic targets for AP^[11–14]. In the present study, integrated analysis of gene expression profiles was conducted to identify differentially expressed genes (DEGs) between AP and control groups in datasets from the Gene Expression Omnibus (GEO) database. KEGG pathways and a protein-protein interaction (PPI) network were also analyzed in this study. Furthermore, significantly enriched functional DEGs, which were screened for their potential role in the progression of AP, will be analyzed.

Methods

Dataset collection and data processing. Gene expression profiles of mice with acute pancreatitis and control mice were obtained from the GEO database (www.ncbi.nlm.nih.gov/geo). The following keywords were used: “acute pancreatitis” and “*Mus musculus*.” The microarray datasets GSE109227, GSE3644 and GSE65146 were downloaded from the website (Table 1) [15–17]. The Affymetrix Mouse Expression 430A Array and Affymetrix Mouse Gene 1.0 ST Array were included in our study. Raw data were processed and analyzed with R software.

Table 1
the 38 DEGs overlapped in the three datasets

Author, year	Datasets	Platforms	Sample no.		(Refs.)
			Acute pancreatitis	Control	
Kowalik AS,2007	GSE3644	Affymetrix Mouse Expression 430A Array	3	3	(1)
IKong B,2017	GSE65146	Affymetrix Mouse Gene 1.0 ST Array	4	5	(2)
Heuchel R,2018	GSE109227	Affymetrix Mouse Gene 1.0 ST Array	6	5	(3)
Note: AP: acute pancreatitis; no.:number; Refs.:References.					

DEG identification. Differentially expressed genes between the normal and acute pancreatitis groups were identified using GEO2R, an interactive web tool. The original P-values were adjusted with BenjaminiHochberg false discovery rate (FDR) correction. In our study, genes with a fold change > 2 and adjusted P-value < 0.05 were considered statistically significant differentially expressed genes.

Functional enrichment analysis of DEGs. The DEGs between the two groups (i.e., control and acute pancreatitis) were analyzed using an online tool Database for Annotation, Visualization and Integrated Discovery (DAVID; david.abcc.ncifcrf.gov/knowledge-base), as well as the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. A P-value of 0.05 was selected as the cutoff criterion.

PPI network construction. To identify the interactions among DEGs, we mapped protein-protein interactions (PPIs) using the STRING database, which was applied to predict protein-protein interactions (PPIs) and construct a PPI network.

Results

DEG Identification. Three datasets from the GEO database of the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/gds/>) were included in this study (Table 1). A total of 291, 922, and 358 DEGs were identified in GSE3644 (Fig. 1A), GSE65146 (Fig. 1B), and GSE109227 (Fig. 1C), respectively. The matched heatmaps are shown in Fig. 1. Among the genes, 38 DEGs

overlapped in the three datasets (Fig. 2), including 35 upregulated and 3 downregulated genes (Cckar, Sostdc, and Klf15) (Table 2).

Table 2
the 38 DEGs overlapped in the three datasets

GENE	logFC value in 3 series			Expression change
	GSE109227	GSE3644	GSE65146	
Cckar	-4.05	-2.02	-2.46	down
Sostdc1	-2.62	-6.03	-3.30	down
Klf15	-2.21	-2.61	-2.78	down
Zfp52	1.96	2.02	2.01	up
Sdc1	2.03	2.31	2.77	up
Epcam	2.16	2.00	2.48	up
Slc2a1	2.17	2.97	2.35	up
Cttn	2.18	2.45	2.70	up
Iqgap1	2.19	1.96	2.36	up
Ano1	2.25	2.60	2.45	up
Got1	2.40	2.01	1.99	up
Tagln2	2.45	3.89	3.35	up
Nabp1	2.47	2.85	1.98	up
Actn4	2.48	1.98	2.20	up
Ywhag	2.53	2.05	2.36	up
Myo1c	2.59	2.15	2.23	up
Clic4	2.65	2.03	2.03	up
Zwint	2.65	2.10	2.52	up
Osmr	2.95	2.60	2.70	up
Efhd2	2.95	2.57	2.84	up
Slc7a3	3.03	2.42	2.92	up
Tubb6	3.06	4.50	3.16	up
Ceacam1	3.09	4.27	4.16	up
Cdc42ep5	3.20	2.45	2.54	up
Tgif1	3.21	3.63	2.19	up
Ier3	3.26	3.21	2.06	up

GENE	logFC value in 3 series			Expression change
	GSE109227	GSE3644	GSE65146	
Kel	3.30	4.22	2.07	up
Rtn4	3.32	2.17	2.30	up
Cdh1	3.47	2.70	2.60	up
Dstn	3.61	3.06	2.03	up
Srxn1	3.66	2.37	2.66	up
Tmsb10	3.71	2.67	2.08	up
Krt18	4.03	2.87	2.60	up
Tac1	4.13	3.81	4.22	up
Gclc	4.28	4.46	2.14	up
Krt8	4.34	3.34	2.65	up
Krt19	4.39	2.13	3.62	up
Rcan1	5.40	5.28	2.33	up

Functional enrichment and pathway analysis. For DEG-related biological functional enrichment, we used the DAVID database to analyze the genes, which are shown in Fig. 3. The top 10 enriched functional terms for the 38 DEGs were mainly involved in the cell process. As shown in Fig. 4D, the top 4 KEGG pathways for the 38 DEGs that overlapped in the three datasets are adherens junction, cell cycle, tight junction and regulation of actin cytoskeleton.

PPI network construction. The 38 DEGs that overlapped in the three datasets were imported into STRING software to construct a PPI network, which included 38 nodes and 29 edges. The genes are shown in Table 2. The hub genes were associated with the KEGG pathway “adherens junction,” which included the Cdh1, Iqgap1 and Actn4 genes, with a false discovery rate (FDR) of 0.0174.

Discussion

Acute pancreatitis is known to involve common inflammatory cascades^[18–19]. Understanding the gene expression changes at the beginning of AP would facilitate the diagnosis and treatment of AP. As shown in Fig. 2 and Table 1, 38 DEGs overlapped in the three datasets, for which enriched functional terms mainly involved biological process and cellular component (Fig. 3D). The top 4 KEGG pathways detected by enrichment analysis were adherens junction, cell cycle, tight junction, and regulation of actin cytoskeleton (Fig. 4D). To further clarify the molecular mechanism of acute pancreatitis, we analyzed the

protein-protein interaction network of the 38 DEGs (Fig. 5) and found that the only KEGG pathway referred to was “adherens junction,” which involved three genes, Cdh1, Iqgap1 and Actn4.

The Cdh1 gene encodes E-cadherin, a calcium-dependent cell adhesion molecule that plays an important role in the establishment and maintenance of epithelial cell morphology. E-cadherin might participate in the progression of acute pancreatitis to severe acute pancreatitis through the adherens junction pathway [20]. Adhesion molecules such as E- and P-selectins were found to be indicators for the assessment of the severity of acute pancreatitis [21].

The Iqgap1 gene encodes the Ras GTPase-activating-like protein. The absence of the Iqgap1 protein leads to insulin resistance [22]. The Iqgap1 gene is involved in cell-cell adhesion, transcription, cytoskeletal architecture, and insulin signaling [23–24].

The Actn4 gene encodes the α -actinin-4 protein, which can bind F-actin to modulate cytoskeletal organization and cell motility [25]. Actn4 is essential for NF- κ B mediated transcriptional activation and may thus be targeted for anti-inflammatory treatment [26].

Conclusions

Once the adhesion between pancreatic acinar cells and/or endothelial cells is loosened, this leads to interstitial edema, which would promote inflammatory cell migration and disrupt tissue structure, resulting in the development of AP [20]. We will further study the effects of the Cdh1, Iqgap1 and Actn4 genes on the occurrence and development of acute pancreatitis, which may provide new targets for the diagnosis and treatment of AP.

Abbreviations

AP: acute pancreatitis; DEGs: differentially expressed genes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction; FDR: Benjamini-Hochberg false discovery rate; DAVID: Database for Annotation, Visualization and Integrated Discovery.

Declarations

Consent for publication

Not applicable.

Declarations

None.

Availability of data and material

All analyzed data are included in this published article. The original data are available from the GEO database

Competing interests

The authors have no conflicts of interest to declare.

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Author contributions

CZ and YZ contributed equally to this work; CZ and YZ designed the study and drafted the manuscript; CZ collected the data; CZ and YZ analyzed the data; CZ and YC revised the manuscript for important intellectual content and made substantial contributions to conception, design, and coordination of the study and gave final approval of the version to be published. All authors have read and approved the final manuscript.

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Figures

Figure 1

Heat map of differentially expressed genes. Heat maps showed top 30 upregulated and downregulated genes from 3 datasets GSE3644 (A), GSE 65146 (B) and GSE109227 (C) which were determined with the GEO2R tool. Limitations were set as adjusted P<0.05 and fold change >2. AP: acute pancreatitis.

Figure 2

Venn diagram showing the number of DEGs in the mRNA microarray expression profile datasets GSE109227, GSE3644, GSE 65146, which were determined with the GEO2R tool. Limitations were set as adjusted $P < 0.05$ and fold change > 2 .

Figure 3

Functional enrichment results. The functional enrichments showed top 30 of GO enrichments from 3 datasets GSE3644 (A), GSE 65146 (B) and GSE109227 (C). Figure 3 D showed the top 30 of GO enrichments from 38 DEGs overlapped in the three datasets.

Figure 4

KEGG pathway results. The figures showed top 30 of KEGG pathways from 3 datasets GSE3644 (A), GSE 65146 (B) and GSE109227 (C). Figure 3 D showed the top 30 of KEGG pathways from 38 DEGs overlapped in the three datasets.