Gastroenterology and Hepatology From Bed to Bench.
2024 **RIGLD**, Research Institute for Gastroenterology and Liver Diseases

ORIGINAL ARTICLE

p-ISSN: 2008-2258 e-ISSN: 2008-4234

Stage analysis of pancreatic ductal adenocarcinoma via network analysis

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ABSTRACT

Aim: This study aimed to introduce a biomarker panel to detect pancreatic ductal adenocarcinoma (PDAC) in the early stage, and also differentiate of stages from each other.

Background: PDAC is a lethal cancer with poor prognosis and overall survival.

Methods: Gene expression profiles of PDAC patients were extracted from the Gene Expression Omnibus (GEO) database. The genes that were significantly differentially expressed (DEGs) for Stages I, II, and III in comparison to the healthy controls were identified. The determined DEGs were assessed via protein–protein interaction (PPI) network analysis, and the hub-bottleneck nodes of analyzed networks were introduced.

Results: A number of 140, 874, and 1519 significant DEGs were evaluated via PPI network analysis. A biomarker panel including ALB, CTNNB1, COL1A1, POSTN, LUM, and ANXA2 is presented as a biomarker panel to detect PDAC in the early stage. Two biomarker panels are suggested to recognize other stages of illness.

Conclusion: It can be concluded that ALB, CTNNB1, COL1A1, POSTN, LUM, and ANXA2 and also FN1, HSP90AA1, LOX, ANXA5, SERPINE1, and WWP2 beside GAPDH, AKT1, EGF, CASP3 are suitable sets of gene to separate stages of PDAC.

Keywords: Pancreatic ductal adenocarcinoma, Gene, stage, Network analysis.

(Please cite as: **Bahadorimonfared A, Farahani M, Rezaei –Tavirani M, Razzaghi Z, Arjmand B, Rezaei M, Nikzamir A, Ehsani Ardakani MJ, Mansouri V. Stage analysis of pancreatic ductal adenocarcinoma via network analysis. Gastroenterol Hepatol Bed Bench 2024;17(3):297-303. https://doi.org/10.22037/ghfbb.v17i3.2887).**

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is known as an aggressive cancer with poor diagnosis. It detects most frequently at progressive stages. So, the detected cancer is associated with limitations in options of treatment (1). It is noted that the outcomes for pancreatic cancer patients can be enhanced by the implementation of emerging treatment methods, including advanced surgical procedures and novel systemic treatments (2). Considering the heterogeneous and flexible molecular profile of PDAC, responses of

Received: 05 February 2024 *Accepted*: 02 April 2024
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unselected patients to standard chemotherapy and targeted therapies are different (3). The association of PDAC with CDKN2A, SMAD4, KRAS, and TP53 is understood via research (4).

There is much evidence about the application of biomarker panels to effectively detect diseases in the early stage of illness. It is suggested that ovarian cancer, lung cancer, and human traumatic brain injury can be diagnosed via related biomarker panels (5-7). Kim H et al. proposed a biomarker panel for the diagnosis PDAC. The suggested biomarker panel includes APOA1, CA125, CA19-9, CEA, APOA2, and TTR (8).

Since genomics and proteomics provide large numbers of genes, and proteins that dysregulate during disease conditions, bioinformatics as a complementary approach is needed to interoperate the findings. Currently, the combination of proteomics-bioinformatics and genomics-bioinformatics is primarily observed in research (9, 10). PPI network analysis is a bioinformatics approach that is frequently employed in research. The applicability of PPI network analysis in medicine was established many years ago (11, 12). Using the PPI network, the large numbers of genes that are related to certain diseases are screened to find the crucial individuals. The critical genes that dysregulate in disease conditions are responsible for the incidence and progress of illness (13). Well-characterized genes as hubs and bottlenecks are crucial elements of a PPI network. The common hubs, and bottlenecks (hub-bottlenecks) are known as the key nodes in the PPI network (14). Gene expression profiles of PDAC samples in stages I, II, and III are extracted from the GEO database and compared with the samples of healthy controls. The significant DEGs of the analyses are included in the PPI network and analyzed. The central nodes (hub-bottlenecks) are determined, and evaluated to introduce biomarker panels that can be used to detect PDAC in the early stage and differentiate the stages of the disease.

Methods

Data collection

To explore differences among gene expression profiles of various stages of human pancreatic ductal adenocarcinoma, GSE183795 was extracted from the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse183795) (15). Information about 139 tumor sample tissues characterized by various stages of disease is recorded in this gen set. Three normal samples are presented as controls.

Data pre-evaluation

Our pre-evaluation using the GEO2R tool in the first phase showed that there are no significant DEGs that distinguish between the gene expression patterns of the different stages of pancreatic ductal adenocarcinoma in humans. In the second step gene expression profiles of samples of stages I, II, and III versus controls were assessed via the GEO2R program to find possible differences.

Network analysis

The significant DEGs were included in the "protein query" of STRING database via Cytoscape software to form PPI network. PPI networks were analyzed via the "Network analyzer" application of Cytoscape. The top 10% of nodes based on degree value and betweenness centrality were selected as hub and bottleneck nodes. The common hubs and bottlenecks were introduced as hub-bottlenecks.

Statistical analysis

The significant DEGs were selected based on $P_{adj}<0.05$. PPI network was formed considering a confidence score = 0.2. The top 10% of nodes based on degree value and betweenness centrality were introduced as hubs and bottlenecks respectively.

Results

Results of Stage I analysis indicate that among 19105 dysregulated genes, there are 140 DEGs (Figure 1). Among 140 queried DEGs that discriminate stage I from control samples, 132 individuals were recognized by the STRING database. The six hub-bottlenecks that are connected are ALB, CTNNB1, COL1A1, POSTN, LUM, and ANXA2. These are shown in Table 1. Out of the 18,371 dysregulated genes, a total of 874 were identified as significant DEGs in stage II compared to the control samples (Figure 2). The significant DEGs were assessed via PPI network analysis. There were 52 hub bottlenecks among the hubs and bottleneck nodes. As shown in Table 2, hub-bottleneck node that ranked as the top 10 hubs and bottlenecks (including CTNNB1, ALB, FN1, HSP90AA1, LOX, ANXA5, SERPINE1, and WWP2) were introduced as critical hub-bottlenecks.

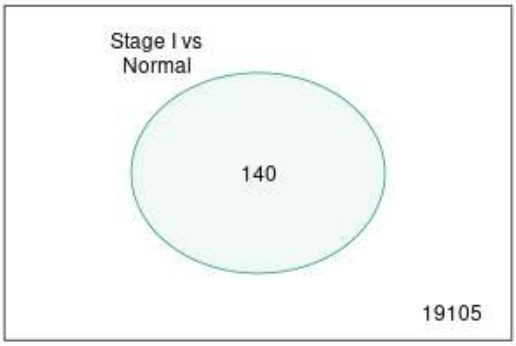

Figure 1. Venn diagram for Stage I analysis. The significant DEGs that discriminate patient samples from health controls are presented.

Table 1. Hub-bottlenecks related to Stage I of human pancreatic ductal adenocarcinoma analysis.

No.	Display name	Degree	Betweenness centrality
1	ALB	48	0.207
2	CTNNB1	48	0.208
3	COL1A1	34	0.046
4	POSTN	34	0.064
5	LUM	30	0.043
6	ANXA2	27	0.049

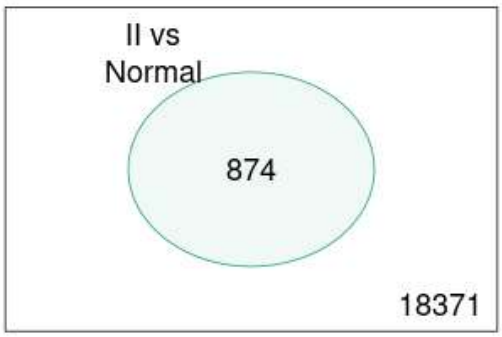

Figure 2. Venn diagram for Stage II analysis. The significant DEGs that discriminate patient samples from health controls are presented.

Table 2. The critical hub-bottlenecks related to Stage II of human pancreatic ductal adenocarcinoma analysis.

No.	Display Name	Degree	Betweenness centrality
1	CTNNB1	167	0.058
2	ALB	158	0.049
3	FN1	141	0.027
4	HSP90AA1	141	0.045
5	LOX	89	0.006
6	ANXA5	86	0.011
7	SERPINE1	84	0.006
8	WWP2	81	0.023

As depicted in Figures 3 and 4, 1519 significant DEGs separate stage III tumor samples from controls. Numbers of 1380 DEGs were documented by STRING database, and 93 hub-bottleneck nodes were determined. The top 10 hubs and bottlenecks (GAPDH, AKT1, CTNNB1, ALB, HSP90AA1, FN1, EGF, CASP3, HIF1A, and EEF2K appeared as crucial hub-bottleneck nodes (Table 3). To investigate the significance of hub-bottlenecks, Table 4 shows the rate of expression changes of these genes in stages I, II, and

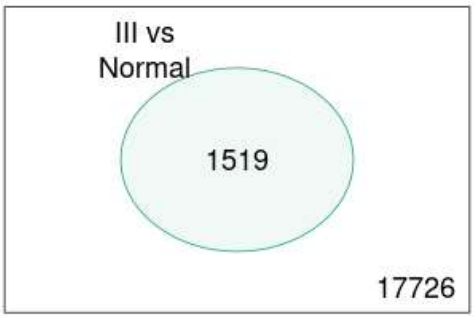

Figure 3. Venn diagram for Stage I analysis. The significant DEGs that discriminate patient samples from health controls are presented.

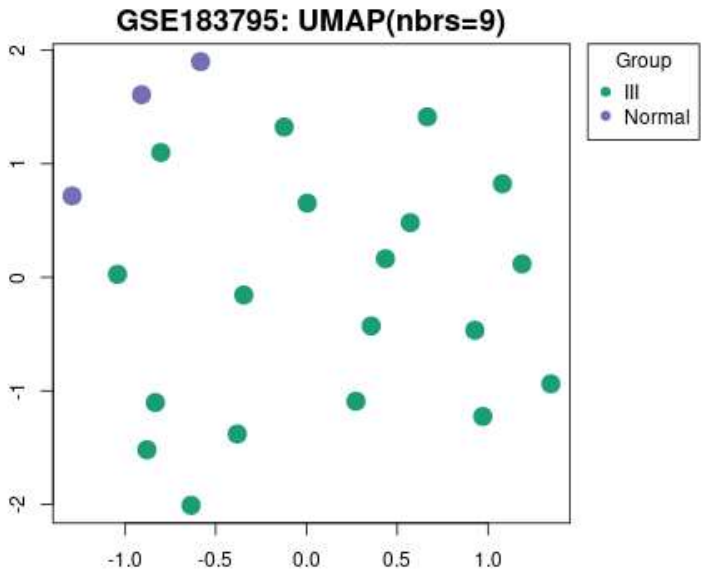

Figure 4. UMAP plot for Stage III analysis. The patient samples and health controls are separated.

III of ductal pancreatic adenocarcinoma, as taken from the associated gene expression profile of GSE183795.

Discussion

Despite fast developments in modern medical technology, and substantial progresses in survival rates of numerous cancers, early detection of pancreatic cancer is still difficult (16). It seems more understanding of pancreatic cancer molecular mechanism is required to establish a suitable diagnostic method for early detection of the disease. Significant differences among stages I, II, and III were not detectable through comparative analyses. The aforementioned phases exhibit significant differences from normal samples, as illustrated in Figures 1-3. The gene expression profiles of stages I, II, and III are distinguished from those of normal individuals by 140, 874, and 1519 DEGs, respectively. This finding indicates that advances in diseases are associated with larger alteration in the gene expression process. Luan H et al published a document about 159 upregulated and 53 downregulated genes that differentiate PDAC from controls (17). Totally 212 dysregulated genes are near 140 dysregulated individuals for stage I analysis. Piao J et al reported a document using data from the GEO database. Based on this investigation CDK1 and BUB1 involve in PDAC progression (18).

As shown in Tables 1-4, 18 dysregulated genes are identified as critical DEGs for stages I, II, and III of PDAC. ALB, CTNNB1, COL1A1, POSTN, LUM, and ANXA2 are the key dysregulated genes in stage I of PDAC. Except for ALB, the other genes are upregulated. ALB is a common hub-bottleneck for all studied stages and it is downregulated extremely (above

Table 3. The crucial hub-bottlenecks related to Stage III of human pancreatic ductal adenocarcinoma analysis.

No.	Display Name	Degree	Betweenness centrality
1	GAPDH	491	0.058
2	AKT1	440	0.052
3	CTNNB1	336	0.025
4	ALB	319	0.023
5	HSP90AA1	294	0.017
6	FN1	279	0.015
7	EGF	258	0.010
8	CASP3	235	0.009
9	HIF1A	228	0.007
10	EEF2K	189	0.008

Table 4. Log (fold change) all hub-bottlenecks related to Stages I, II, and III of human pancreatic ductal adenocarcinoma analysis.

N0.	Gene	Stage I	Stage II	Stage III
1	ALB	-4.29	-3.60	-4.43
2	CTTNB1	0.77	0.73	0.89
3	COL1A1	3.01	2.95	3.07
4	POSTN	3.40	3.90	4.36
5	LUM	2.38	2.19	2.24
6	ANXA2	1.28	1.31	1.32
7	FN1	-	2.01	2.36
8	HSP90AA1	-	0.64	0.75
9	LOX	-	1.76	1.91
10	ANXA5	-	0.62	N
11	SERPINE1	-	2.35	2.24
12	WWP2	-	-0.62	-0.63
13	GAPDH	-	-	1.01
14	AKT1	-	-	-0.49
15	EGF	-	-	-2.82
16	CASP3	-	-	0.80
17	HIF1A	-	-	1.35
18	EEF2K	-0.73	-0.57	-0.61

10-fold). The literature survey refers to the importance of ALB level in the progress of PDAC. Based on the investigation of Fan Z et al, PDAC patients with baseline CRP/ALB < 0.180 presented a remarkably better clinical consequence than patients with baseline CRP/ALB ≥ 0.18 (19). This report emphasizes the significance of the CRP/ALB index in the prognosis of patient survival. ALB is significantly downregulated in all three stages of the disease, as illustrated in Table 4. It is possible that ALB deficiency is a member of the panel that corresponds to the incidence and progression of PDAC. The second hub of stage I analysis is CTTNB1. As with albumin, CTTNB1 is a common hub-bottleneck in the other analyses. Inverse ALB, CTTNB1 is upregulated in all analyses. Chai W et al.'s investigation showed that "tumor suppressor long noncoding RNA on chromosome 8p12" linked with human antigen R (HuR), promoted the binding of HuR with CTNNB1 mRNA and amplified the stability of CTNNB1 mRNA. The stability of CTNNB1 via HuR-mediated mRNA leads to positive regulation of pancreatic cancer growth and metastasis (20). This finding indicated that CTNNB1 can be introduced as the second element of the diagnostic biomarker panel for the detection of PDAC in the early stage.

COL1A1 is 3rd hub-bottleneck of stage I analysis. This gene is upregulated about 8-fold in all studied stages, but does not appear as a hub-bottleneck for stages I and II analyses. Based on Yang J et al investigation, considerable upregulation of hsa_circRNA_0007334 leads to enhance the expression and function of COL1A1 in PDAC (21). It seems that COL1A1 is a suitable biomarker to detection PDAC in the early stage. We suggested ALB and COL1A1 as

possible biomarkers to detect PDAC in the early stage (In pressed data).

POSTN, LUM, and ANXA2 are three hub-bottlenecks of stage I assessment which are characterized with the same expression changes in stages II and III of PDAC. However, similar to COL1A1, these genes do not serve as hub-bottlenecks in the analyses of Stages II and III. Several studies (22-24) describe the upregulation of ANXA2 in pancreatic cancer, the relationship between dramatically elevated POSTN expression and advanced disease stage in PDAC, and the significantly higher levels of LUM in pancreatic cancer tissue. Like ALB, CTNNB1, and COL1A1, these three genes can be considered as the other members of the suggested biomarker panel to detect PDAC early stage.

As presented in Table 4, FN1, HSP90AA1, LOX, ANXA5, SERPINE1, and WWP2 are possible biomarkers that discriminate stage I from stages II and III. If PDAC is diagnosed via the suggested biomarker panel including ALB, CTNNB1, COL1A1, POSTN, LUM, and ANXA2, but FN1, HSP90AA1, LOX, ANXA5, SERPINE1, and WWP2 levels were normal, there are sufficient evidences about the incident of stage I of PDAC. Upregulation of FN1 and significant correlation with pancreatic progress and metastasis is confirmed by previous investigations (25). GAPDH, AKT1, EGF, CASP3, and HIF1A are hub-bottlenecks of stage III analysis and do not appear as dysregulate genes in Stages I and II. It seems that these dysregulated genes can provide possible evidences to separate stage III from stages I and II. The involvement of EGF, AKT1, and CASP3 in cell migration and metastasis in cancers is investigated and discussed by researchers (26-28).

Conclusion

In conclusion, a possible biomarker panel, including ALB, CTNNB1, COL1A1, POSTN, LUM, and ANXA2 is suggested to early detect PDAC. It was discussed if PDAC was diagnosed, the normal expression of FN1, HSP90AA1, LOX, ANXA5, SERPINE1, and WWP2 refer to the stage I of the illness. Normal expression of GAPDH, AKT1, EGF, CASP3, and HIF1A in PDAC patients refers to stage III of the disease. Complementary investigation can validate these finding to applied findings in clinics.

Acknowledgements

Shahid Beheshti University of Medical Sciences supported this research.

Conflict of interests

The authors declare that they have no competing interests.

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