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Extracellular matrix is the main targeted environment in early stage of pancreatic ductal adenocarcinoma

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ABSTRACT

Aim: Due to weak diagnosis and treatment of pancreatic ductal adenocarcinoma (PDAC), detection of PDAC possible biomarkers in early stage is the main aim of this study.

Background: PDAC is known as an exocrine cancer with a 5-year overall survival of 11%.

Methods: Gene expression profiles of early stage of PDAC tissue and normal tissue are downloaded from gene expression omnibus (GEO) and evaluated via GEO2R. The significant differentially expressed genes (DEGs) are investigated via protein-protein interaction (PPI) network analysis and gene ontology.

Results: Among 104 DEGs, ALB, COL1A1, COL1A2, MMP1, POSTN, PLAU, and COL3A1 were pointed out as hub nodes. "Gelatin degradation by MMP1, 2, 3, 7, 8, 9, 12, 13" group of 52 biological terms were identified as the main affected terms.

Conclusion: In conclusion, ALB, MMP1, and COL1A1 genes were highlighted as possible biomarkers of early stage of PDAC. Dysfunction of extracellular matrix was identified as a main event in patients.

Keywords: Pancreas cancer, Protein, Network analysis, Genes.

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Introduction

Investigations indicate that PDAC is a most common exocrine pancreatic cancer. PDAC is known as an overwhelming disease which is characterized with a 5-year overall survival of only 11%. It is reported that about 80% of PDAC patients are diagnosed at the advanced stages of disease. In plain contrast to the other types of cancer, there are not successful reports about early detection of PDAC (1, 2). Due to low overall survival of patients, the traditional treatments such as surgery, chemotherapy, and radiotherapy have no significant effect on disease improvement (3). There are many efforts to diagnose PDAC at early stage. Caputo D et al have suggested nanoparticle-enabled blood tests to detect PDAC at early stage (4).

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High throughput methods such as proteomics, genomics, and metabolomics in combination with computational approaches have attracted attention of investigators to open new window into preventive medicine (5, 6). Cao F et al published the results of a high throughput functional screen experiment that led to introduction of prominent role of YWHAZ as a player in pancreatic cancer metastasis (7). Network analysis as a bioinformatic approach is used to assess mechanism of many diseases. PPI network analysis is applied to detect new features of molecular mechanism of pancreatic cancers. Yang J et al highlighted the role of COL17A1 as a facilitator of tumor growth in pancreatic cancer via network analysis (8-10). Lu W et al published results of a PPI network analysis related to pancreatic cancer. Based on this document, DNA topoisomerase II alpha, maternal embryonic leucine zipper kinase, thrombospondin 1, syndecan 1, and proto-oncogene receptor tyrosine kinase Met are the hubs of network (11). In the another investigation, CCNB1, DLGAP5, CCNA2, CENPF, UBE2C, KIF23, TPX2, KIF14, RACGAP1, and NEK2 were introduced as hubs of network related to the progression of PDAC (12). Yang S et al implemented an experiment about gene expression alteration in patients with PDAC disease. Based on this research, dysregulation of HNF1B/Clusterin Axis plays a key role in progression of pancreatic cancer (13). In the present study, gene expression profiles of patients in early stage IB of PDAC (from GEO database) are compared with normal tissue to find the differential molecular events.

Methods

Gene expression data related to pancreatic ductal adenocarcinoma (PDAC) patients were extracted from GSE183795 of GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183795). Three sets of gene expression profiles including "stage I tumor tissue", "stage I adjacent non-tumor tissue", and "Normal tissue" were selected for analysis. To find the DEGs that discriminate the studied groups, data were analyzed via GEO2R. Volcano plot and Venn diagrams of analyses were assessed to select the comparable groups. P-adj < 0.05 was considered as statistical parameter. For greater confidence, the comparable sets were evaluated via box plot analysis and UMAP assessment.

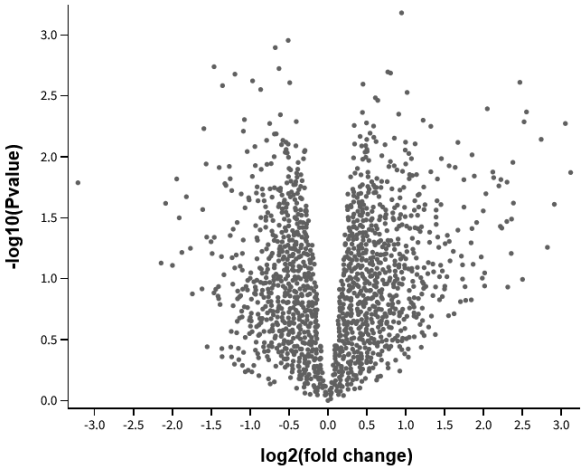

Figure 1. Volcano plot of ["stage I tumor tissue" - "stage I adjacent non-tumor tissue"] analysis. P-adj < 0.05

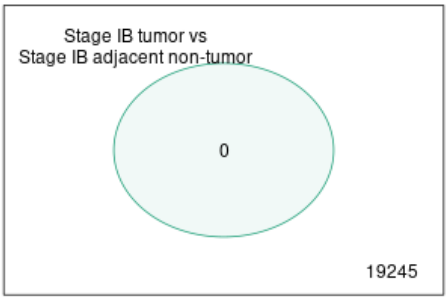

Figure 2. Venn diagram of ["stage I tumor tissue" - "stage I adjacent non-tumor tissue"] analysis. P-adj < 0.05

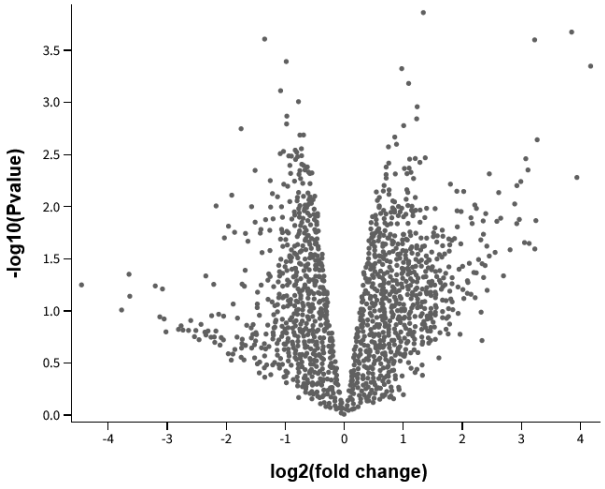

Figure 3. Volcano plot of ["stage I adjacent non-tumor tissue" - "Normal tissue"] analysis. P-adj < 0.05

The significant DEGs (based on P-adj < 0.05 and FC > 1.5) were included in a PPI network by STRING database via Cytoscape software v 3.7.2. Optimum numbers of first neighbors from STRING database

were added to the queried significant DEGs to maximize connections between the assessed nodes. The PPI network was evaluated by "Network analyzer" application of Cytoscape. Top 10% of the queried DEGs based on degree value and betweenness centrality were identified as hubs and bottlenecks respectively. The common hubs and bottlenecks were determined as hub-bottlenecks.

The hub nodes were investigated via ClueGO v.2.5.7 plugin of Cytoscape software. The related biological terms were extracted from REACTOME_Pathways_08.05.2020, REACTOME_Reactions_08.05.2020, WikiPathways_08.05.2020, GO_MolecularFunction-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020, GO_CellularComponent-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020, and GO_BiologicalProcess-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020 sources. The terms were determined painstaking based on, Term P-value, Term P-value corrected with Bonferroni step down, Group P-value, and Group P-value corrected with Bonferroni step down ≤0.01.

Results

Based on Figures 1-4, there are not significant DEGs related to the analyses of ["stage I tumor tissue" - "stage I adjacent non-tumor tissue"] and ["stage I adjacent non-tumor tissue" - "Normal tissue"].

The only comparable analysis refers to ["stage I tumor tissue" - "Normal tissue"] assessment. As depicted in Figures 5-6 there are 104 DEGs that discriminate "stage I tumor tissue" from "Normal tissue". Box plot results (see Figure 7) indicate that gene expression profiles of the "stage I tumor tissue" and "Normal tissue" follow a similar statistical pattern. Suitable clustering of gene expression profiles is shown in Figure 8. The studied samples are grouped in two distinct clusters.

A total of 100 genes among the 104 queried DEGs were recognized by STRING database. A network was formed including 100 nodes and 158 edges. After adding 70 first neighbors, the network was reformed. The new network was constructed with 170 nodes (28 isolated genes, 6 paired DEGs, and a main connected component of 136 nodes) and 2909 edges. The main connected component is shown in Figure 9.

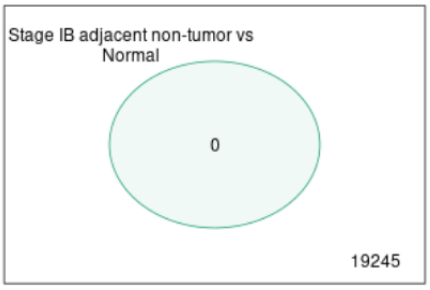

Figure 4. Venn diagram of ["stage I adjacent non-tumor tissue" - "Normal tissue"] analysis. P-adj < 0.05

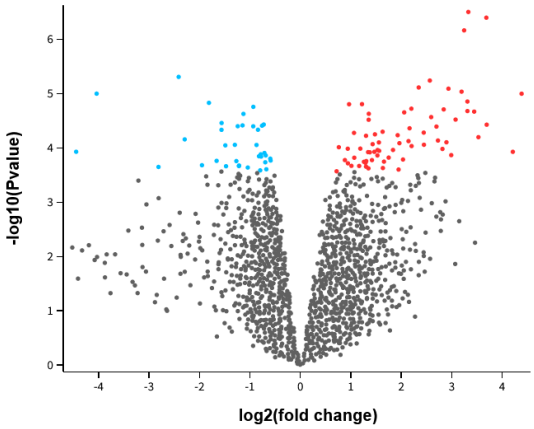

Figure 5. Volcano plot of ["stage I tumor tissue" - "Normal tissue"] analysis. P-adj < 0.05

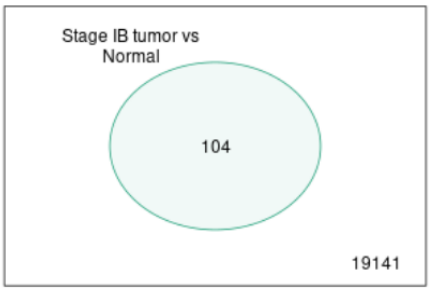

Figure 6. Venn diagram of ["stage I tumor tissue" - "Normal tissue"] analysis. P-adj < 0.05

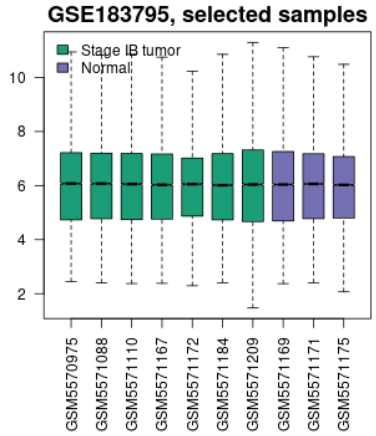

Figure 7. Box plot of ["stage I tumor tissue" - "Normal tissue"] analysis. P-adj < 0.05

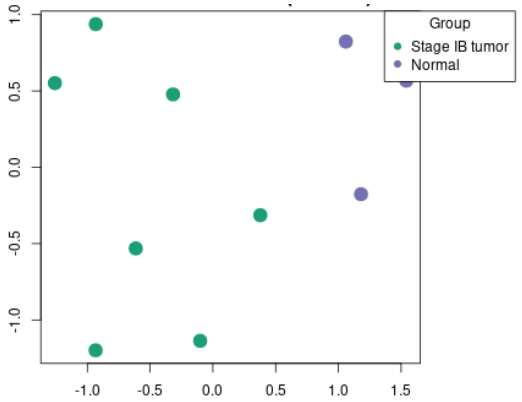

Figure 8. UMAP diagram of ["stage I tumor tissue" - "Normal tissue"] analysis. P-adj < 0.05

Gene ontology revealed that 52 biological terms are related to the hub nodes. All biological terms were grouped as "Gelatin degradation by MMP1, 2, 3, 7, 8, 9, 12, 13" group (see Table 2). Except "Collagen type I degradation by MMP1, 2, 8, 13, PRSS2", all biological terms are related to COL1A1, COL1A2, and COL3A1.

Discussion

Early detection of pancreatic cancer is targeted by many researchers. However, achievements have not been considerable (14, 15). As displayed in Figures 1-2, there are no significant differences between gene expression profiles of "stage I tumor tissue" and "stage I adjacent non-tumor tissue". However, regarding expression of 19245 genes, there are no DEGs which discriminate the tumor tissue from the adjacent non-tumor tissue. It can be concluded that molecular events in the early stage of tumor tissue may be similar in adjacent non-tumor tissue. This fact refers to possible incorrect diagnosis of local place of tumor.

It is reported that early stage of pancreatic cancer is detected (16).

Comparison between gene expression profiles of "stage I adjacent non-tumor tissue" and "normal tissue" is presented in Figures 3-4. Again, there is no significant alteration between gene expression profiles of compared

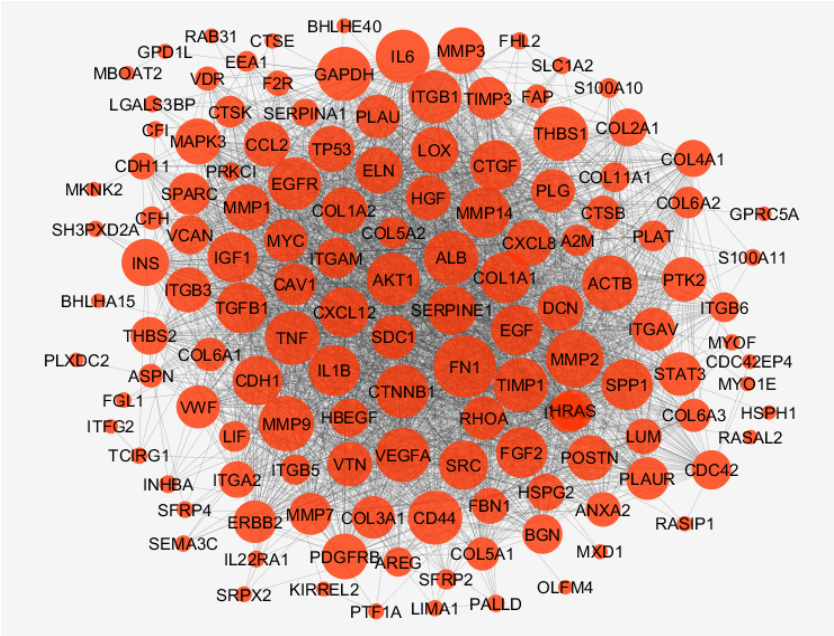

Figure 9. Main connected component of ["stage I tumor tissue" - "Normal tissue"] analysis PPI network. A total of 66 queried DEGs plus 70 added first neighbors are included. Nodes are layout based on degree value, where the larger size of nodes refers to increment of degree.

Table 1. Hub nodes of the analyzed PPI network. The hub-bottleneck nodes are bolded.

Display name	Degree	Betweenness centrality	Closeness centrality	Stress
ALB	81	0.020	0.703	5218
COL1A1	75	0.014	0.672	4496
COL1A2	65	0.006	0.622	2568
MMP1	64	0.004	0.634	1870
POSTN	64	0.006	0.631	2696
PLAU	60	0.005	0.634	2148
COL3A1	58	0.005	0.595	2034

samples. It seems gene expression index is not suitable tool to detect molecular events in early stage of PDAC. The last experiment was done by comparison of "stage I tumor tissue" profiles with "normal tissue". As displayed in Figure 5-6, 104 significant DEGs appeared as up and down-regulated genes. Box plot analysis confirmed the assessment (see figure 7). UMAP plot of the analysis indicated that gene expression index separated the two

Table 2. Biological terms related to the hub nodes. All terms are grouped as "Gelatin degradation by MMP1, 2, 3, 7, 8, 9, 12, 13". REACTOME_Pathways_08.05.2020, REACTOME_Reactions_08.05.2020, WikiPathways_08.05.2020, GO_MolecularFunction-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020, GO_CellularComponent-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020, and GO_BiologicalProcess-EBI-UniProt-GOA-ACAP-ARAP_08.05.2020 are sources of terms. Term P-value, Term P-value corrected with Bonferroni step down, Group P-value, and Group P-value corrected with Bonferroni step down ≤0.01 were considered.

Gene ontology term	Associated genes
Collagen degradation	[COL1A1, COL1A2, COL3A1, MMP1]
Gelatin degradation by MMP1, 2, 3, 7, 8, 9, 12, 13	[COL1A1, COL1A2, COL3A1, MMP1]
Collagen type I degradation by MMP1,2,8,13, PRSS2	[COL1A1, COL1A2, MMP1]
Formation of collagen fibrils	[COL1A1, COL1A2, COL3A1]
Prolyl 4-hydroxylase converts collagen prolines to 4-hydroxyprolines	[COL1A1, COL1A2, COL3A1]
Collagen biosynthesis and modifying enzymes	[COL1A1, COL1A2, COL3A1]
Collagen prolyl 3-hydroxylase converts 4-Hyp collagen to 3,4-Hyp collagen	[COL1A1, COL1A2, COL3A1]
Procollagen lysyl hydroxylases convert collagen lysines to 5-hydroxylysines	[COL1A1, COL1A2, COL3A1]
Galactosylation of collagen propeptide hydroxylysines by procollagen galactosyltransferases 1, 2.	[COL1A1, COL1A2, COL3A1]
Galactosylation of collagen propeptide hydroxylysines by PLOD3	[COL1A1, COL1A2, COL3A1]
Glucosylation of collagen propeptide hydroxylysines	[COL1A1, COL1A2, COL3A1]
Removal of fibrillar collagen N-propeptides	[COL1A1, COL1A2, COL3A1]
Removal of fibrillar collagen C-propeptides	[COL1A1, COL1A2, COL3A1]
P4HB binds Collagen chains	[COL1A1, COL1A2, COL3A1]
Procollagen triple helix formation	[COL1A1, COL1A2, COL3A1]
Assembly of collagen fibrils and other multimeric structures	[COL1A1, COL1A2, COL3A1]
Secretion of collagens	[COL1A1, COL1A2, COL3A1]
Binding and Uptake of Ligands by Scavenger Receptors	[ALB, COL1A1, COL1A2, COL3A1]
Formation of collagen fibres	[COL1A1, COL1A2, COL3A1]
FN1 binds Collagen types I-V, VII	[COL1A1, COL1A2, COL3A1]
DDR1 binds collagen type I, II, III, IV, V, XI fibrils	[COL1A1, COL1A2, COL3A1]
DCN binds collagen I, II, III, VI fibrils	[COL1A1, COL1A2, COL3A1]
DDR2 binds collagen type I, II, III, V, X fibrils	[COL1A1, COL1A2, COL3A1]
Gelatin degradation by MMP19	[COL1A1, COL1A2, COL3A1]
Syndecan-1 binds collagen types I, III, V	[COL1A1, COL1A2, COL3A1]
Syndecan interactions	[COL1A1, COL1A2, COL3A1]
Non-integrin membrane-ECM interactions	[COL1A1, COL1A2, COL3A1]
Scavenging by Class A Receptors	[COL1A1, COL1A2, COL3A1]
MSR1 (SCARA1) binds collagen	[COL1A1, COL1A2, COL3A1]
OSCAR binds collagen and SP-D	[COL1A1, COL1A2, COL3A1]
LAIR2 binds collagen	[COL1A1, COL1A2, COL3A1]
PTK2 binds activated MET	[COL1A1, COL1A2, COL3A1]
MET activates PTK2 signaling	[COL1A1, COL1A2, COL3A1]
MET promotes cell motility	[COL1A1, COL1A2, COL3A1]
Collagen chain trimerization	[COL1A1, COL1A2, COL3A1]
PLOD3 binds Lysyl hydroxylated collagen propeptides	[COL1A1, COL1A2, COL3A1]
PLOD3:Fe2+ dimer:Galactosyl-hydroxylysyl collagen propeptides dissociates	[COL1A1, COL1A2, COL3A1]
PLOD3:Fe2+ dimer:Glucosyl-galactosyl-hydroxylysyl collagen propeptides dissociates	[COL1A1, COL1A2, COL3A1]
Prolyl 3-hydroxylases:Fe2+:3,4-Hyp collagen propeptides dissociates	[COL1A1, COL1A2, COL3A1]
COLGALT1,COLGALT2 bind Lysyl hydroxylated collagen propeptides	[COL1A1, COL1A2, COL3A1]
P3HB binds 4-Hyp-collagen propeptides	[COL1A1, COL1A2, COL3A1]
COLGALT1,COLGALT2:Galactosyl-hydroxylysyl collagen propeptides dissociates	[COL1A1, COL1A2, COL3A1]
Lysyl hydroxylated collagen propeptides dissociate from Lysyl hydroxylases	[COL1A1, COL1A2, COL3A1]
P4HB:4-Hyp collagen propeptides dissociates	[COL1A1, COL1A2, COL3A1]
Inflammatory Response Pathway	[COL1A1, COL1A2, COL3A1]
extracellular matrix structural constituent conferring tensile strength	[COL1A1, COL1A2, COL3A1]
platelet-derived growth factor binding	[COL1A1, COL1A2, COL3A1]
complex of collagen trimers	[COL1A1, COL1A2, COL3A1]
banded collagen fibril	[COL1A1, COL1A2, COL3A1]
fibrillar collagen trimer	[COL1A1, COL1A2, COL3A1]
collagen fibril organization	[COL1A1, COL1A2, COL3A1]
cellular response to amino acid stimulus	[COL1A1, COL1A2, COL3A1]

evaluated of sample sets. Application of UMAP plot to visualize gene expression alteration effects has been reported previously (17).

The identified DEGs were investigated via PPI network analysis. As demonstrated in Figure 9, a network including different types of nodes (regarding centrality properties of nodes) is formed. ALB, COL1A1, COL1A2, MMP1, POSTN, PLAU, and COL3A1 have appeared as hubs of the studied network. Central properties of the introduced hubs are presented in Table 1. ALB and COL1A1 as the top hubs are identified as top bottlenecks. Xu SS et al have highlighted hemoglobin, albumin, lymphocyte, and platelet as predictors of postoperative survival in pancreatic cancer (18). Fang L et al suggested "fibrinogen to albumin ratio" as a predictive prognosis in pancreatic cancer patients (19). Fold change of ALB is reported as 21.77. The severe reduction of albumin in early stage of PDAC can lead to gross dysfunction of many hormones and ligands. The significant role of COL1A1 in malignancies and pancreatic cancers has been pointed out by Li X et al and Yang J et al (20, 21).

Results of gene ontology assessment are presented in Table 2. A total of 52 biological terms were related to COL1A1, COL1A2, COL3A1, MMP1, and ALB. As mentioned earlier, ALB and COL1A1 are the two identified hub-bottlenecks. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases involved in degradation of extracellular matrix in connective tissues (22). Investigations indicate that MMP1 and MMP14 recruit COL1 fiber degradation in the microenvironment of tumor (23). Gene ontology results indicate that the 52 biological terms are grouped as "Gelatin degradation by MMP1, 2, 3, 7, 8, 9, 12, 13". It can be concluded that extracellular matrix is the critical target in PDAC. The complex relationship between pancreatic cancer and extracellular matrix has been investigated and discussed by several researchers (24, 25). The amount of fold change for MMP1 and COL1A1 was recorded as 2.76 and 9.50 respectively. Up-regulation of MMP1 and COL1A1 is consisted with other discussed results.

Conclusion

In conclusion, ALB, MMP1, and COL1A1 can be suggested as possible biomarkers of early stage of PDAC. Due to more sensitivity of COL1A1 relative to MMP1 (fold change 9.50 versus 2.76), COL1A1 is a suitable biomarker candidate for early detection of PDAC. The analysis revealed that extracellular matrix is the main environment of PDAC in the early stage. "Gelatin degradation by MMP1, 2, 3, 7, 8, 9, 12, 13" was highlighted as a crucial set of biological terms in the tissue of PDAC early stage. It can be concluded that size of tumor is larger than the pathological report.

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Conflict of interests

The authors indicate no potential conflicts of interest.

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