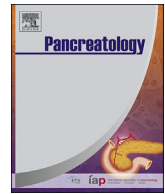




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Overexpression of the adhesion signaling pathway is linked to short-term survival in pancreatic ductal adenocarcinoma

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ABSTRACT

Background and objective: Pancreatic ductal adenocarcinoma (PDAC) is known for its unfavorable prognosis. Gaining insights into the molecular mechanisms that contribute to its progression is crucial for developing effective therapies. In this study, our objective was to investigate the molecular pathways associated with short-term survival in patients with PDAC.

Methods: Immune profiles were analyzed from both long-term survivors (n = 10) and short-term survivors (n = 10) after surgical resection. Pathway scores were calculated to compare the two groups.

Results: The "Adhesion" pathway emerged as the most significant pathway, exhibiting a notably higher score in the samples of short-term survivors (P < 0.009). Within this pathway, significant findings were observed in genes related to integrins and CEACAM.

Conclusion: The role of integrins in the tumor microenvironment of pancreatic cancer is of utmost importance, as they are found to be overexpressed in short-term survivors. These findings provide valuable insights into the underlying biology of PDAC and have potential implications for the development of therapeutic strategies.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) cancer is a highly aggressive and complex malignancy characterized by various genetic alterations that drive tumor development, progression, and resistance to therapy [1]. Despite advancements in treatment, the survival rates for pancreatic cancer remains disappointingly low, necessitating the identification of new therapeutic targets and prognostic markers [2]. Factors such as chronic inflammation, immune evasion mechanisms, antigen presentation, and cytokine signaling significantly contribute to the tumor's immune response [3]. In our previous study, we conducted targeted gene expression profiling of immune-related genes in PDAC samples from both short-term and long-term survivor patients. Our findings provided valuable insights into the significance of B cells and their exclusive infiltration within tumor cells in cases of long-term survivors [4]. However, the underlying pathways contributing to short-term survival remains unknown. This knowledge gap is critical for

comprehensively understanding dysregulated processes and identifying potential therapeutic targets. Therefore, the present study aimed to bridge this gap by exploring the underlying pathways associated with short-term survival in PDAC patients.

2. Methods

2.1. Sample inclusion and RNA expression profiles

The sample inclusion criterium was described in details previously [4]. In short, all patients were diagnosed with resectable PDAC without receiving any preoperative treatment. Clinical Information including tumor grade, location and stage, margin status, lymph node status, CA19-9 and CEA were collected. The main characteristics of the patients were matched to the best of our ability. The Immune-related gene expression profiles were generated from n = 10 long-term survivors (recurrence-free for at least 3 years) and n = 10 short-term survivors (survived less than 6 months due to recurrence). Fresh-frozen samples were used for RNA isolation and profiled using the PanCancer immune profiling panel of NanaString Technology. The inclusion criteria of the patients and details about

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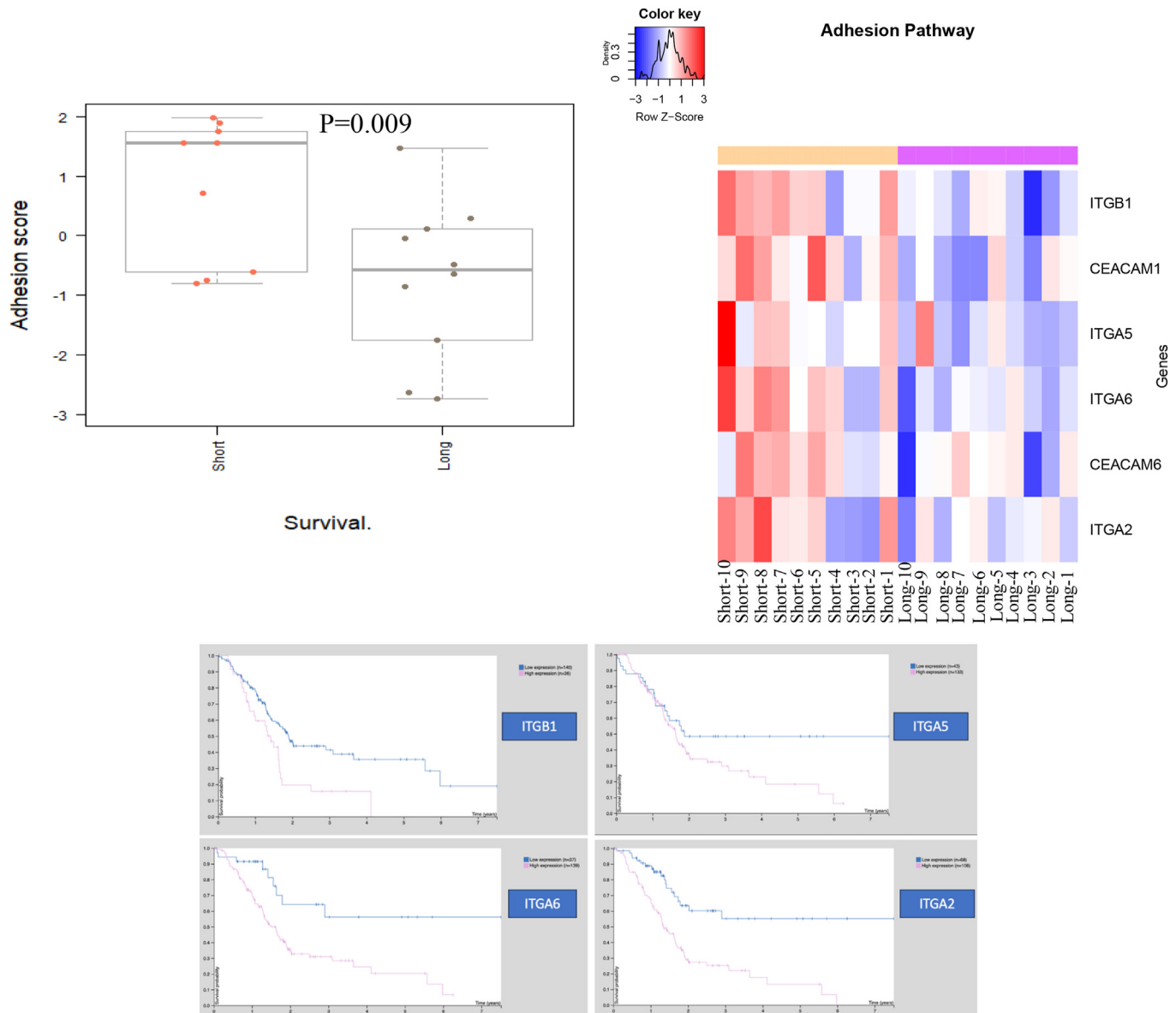


Fig. 1. “Adhesion” pathway is over-expressed in short-compared to long-term survival samples of patients with PDAC. **A**-Boxplot of the adhesion pathway score calculated by the sum of the averages of all genes associated with the pathway. Every dot represents a sample. **B**- heatmap of the significant genes in the adhesion pathway ($P < 0.05$). Log₂ values of the normalized genes expression were used. The expression is scaled/gene. Red represents the highest gene expression and blue represents the lowest gene expression. **C**- results of the in-silico validation using data from the Protein Atlas. Kaplan-Meier curves validating that the higher expression of ITGB1, ITGA5, ITGA6, and ITGA2 was significantly associated with a lower survival in PDAC samples ($P < 0.05$).

the methodology were published previously [4].

2.2. Pathway analysis

The expression of measured genes was linked to predetermined pathways, including adhesion, antigen processing, B-cell functions, cell cycle, chemokines, cytokines, and more that are described in the package of the nSolver Advanced Analysis software (Version 2.0). Pathway scores were calculated based on average gene expression. Pathway scores summarize the gene expression profile of each sample by reducing it to a concise set of scores. Higher scores indicate predominantly increased expression, as each pathway score assigns positive weights to at least half of its associated genes. Gene set analysis and heatmaps were performed using differentially

expressed genes. Validation was conducted using an in-silico approach with protein atlas data. R version 4.1.1 was utilized for analysis.

3. Results

3.1. Patient characteristics

Twenty PDAC samples were included, evenly distributed between long-term and short-term survival groups [4]. There were no significant differences in age, gender, tumor location, operation procedure, tumor differentiation, lymph node status (positive vs. negative), margin status (R0 vs. R1), T-stage (T1-T2 vs. T3), adjuvant systemic therapy, SIII, CEA, or CA19-9.

3.2. Overexpression of the adhesion pathway in short-term survivors

In our analysis, the “adhesion” pathway emerged as the only pathway that exhibited significant differences between short-term and long-term survival samples. Specifically, it was found to be overexpressed in short-term survival samples compared to long-term survival samples ($P < 0.009$), Fig. 1A. The adhesion pathway consists of 23 genes, with 13 of them being integrin associated genes. Remarkably, 69 % of the integrin genes were overexpressed in the short-term survival group, as shown in Table 1. Notably, four integrin genes (ITGB1, ITGA5, ITGA6, and ITGA2) displayed the highest fold of change (FOC), as indicated in Table 1 and Fig. 1B, which demonstrates the overrepresentation of integrins within the adhesion pathway, along with CEACAM-1 and -6. Our findings of the four integrin genes with the highest FOC were supported by the validation process conducted in the protein atlas. Interestingly, ITGB1, ITGA5, ITGA6, and ITGA2 were mostly overexpressed in pancreatic cancer compared to other cancer types. Furthermore, the overexpression of these integrins was associated with poorer survival, as illustrated in Fig. 1C.

4. Discussion

This study focused on investigating molecular pathways associated with short-term survival in PDAC patients. Notably, the findings revealed that the adhesion pathway exhibited overexpression in short-term survivors, suggesting its potential contribution to the aggressiveness of PDAC.

Integrins are fundamental regulators of cell adhesion and migration processes. These cell surface receptors play a crucial role in mediating interactions between cells and their surrounding extracellular matrix. In the context of cancer, integrins have been implicated in various aspects of tumor progression and metastasis. Integrin beta-1 (ITGB1) forms a complex with integrin alpha subunits (ITGA5, ITGA6, and ITGA2) [5–7]. In pancreatic cancer, ITGB1 overexpression leads to increased cancer cell adhesion to the extracellular matrix, enhanced invasiveness, and facilitated tumor cell dissemination [8]. ITGA2 dysregulation in pancreatic cancer is associated with increased invasiveness and metastasis. Similarly,

ITGA5 and ITGA6, alpha subunits of integrins that heterodimerize with ITGB1, contribute to elevated invasiveness and metastatic potential in pancreatic cancer [9]. Altered integrin expression in pancreatic cancer enhances cancer cell adhesion, promotes tumor invasion, and facilitates metastasis. Integrins also modulate downstream signaling pathways involved in cancer cell survival, angiogenesis, and immune evasion, promoting tumor growth and progression [10]. Furthermore, CEACAMs (CEACAM1 and CEACAM6), cell surface glycoproteins, play vital roles in cell adhesion, immune response, and tumor development. Dysregulated CEACAM expression is observed in pancreatic cancer, with CEACAM1 promoting tumor cell survival, angiogenesis, and metastasis, and CEACAM6 enhancing tumor cell invasion and resistance to apoptosis [11,12]. These results align with our own findings, which showed an overexpression of CEACAM1 and CEACAM6 in the short-term survivors.

To conclude, our findings provide valuable insights into the underlying biology of PDAC and emphasize the significance of the adhesion pathway, particularly in short-term survivors. This revelation has multiple applications, including molecular subtyping of PDAC tissues. Furthermore, high integrin expression serves as a potential prognostic biomarker, indicating a link to aggressive tumor behavior and unfavorable patient outcomes. The elevated levels of integrins also present promising avenues for pioneering personalized and combination therapies aimed at improving outcomes for PDAC patients. However, one of the limitations to our present study is the small sample size and the limited validations. Unfortunately, finding samples of long-term survivors of PDAC patients poses a significant challenge, primarily contributing to the study's limited sample size. However, this also highlights the significance of elucidating potential pathways associated with the shorter survival of these patients. It is worth noting that the Pan-Cancer Immune profiling panels we used in this study contains in total 14 integrin-related genes. While other integrins could also be influential in PDAC progression, our emphasis was on those specified by this panel. Further investigations into additional integrins would be beneficial for a comprehensive understanding. In addition, functional studies are needed to uncover the specific roles of these genes in pancreatic cancer development. Understanding their mechanisms will offer insights for targeted therapies and personalized treatments.

Table 1
Genes included in the adhesion pathway.

Genes	Mean Short	St. Deviation	Mean Long	St. Deviation	Linear fold of change	P-value
CEACAM1	288.17	173.66	121.50	63.37	2.48	0.001
CEACAM6	17860.77	13734.88	8083.58	5322.99	2.21	0.036
ITGA5	3869.29	4682.59	1824.74	1504.42	2.13	0.024
ITGA2	2932.79	2063.37	1559.03	538.15	1.89	0.018
ITGB1	26472.09	9407.46	14778.72	4698.43	1.79	0.002
ITGA6	2793.68	947.09	1826.13	366.84	1.53	0.002
ITGB3	185.65	125.67	119.10	66.65	1.60	0.073
ITGB4	3281.99	2213.14	2154.31	950.46	1.52	0.087
EPCAM	6145.66	3906.42	4249.28	2042.46	1.45	0.135
ITGA1	3693.97	828.84	2849.33	919.69	1.30	0.067
ALCAM	1314.43	590.52	1089.16	393.58	1.21	0.343
ICAM1	1338.35	496.44	1140.89	793.29	1.17	0.473
ITGB2	1167.62	468.55	1046.69	294.15	1.12	0.469
ITGAE	315.41	106.29	289.42	99.76	1.09	0.58
MCAM	248.05	127.80	239.07	110.96	1.04	0.858
VCAM1	1846.11	803.06	1864.94	1017.91	1.01	0.962
ITGAM	317.18	137.30	321.12	155.79	1.02	0.938
ICAM4	30.29	8.82	31.36	12.08	1.04	0.973
ITGAX	926.75	364.39	1049.64	479.88	1.14	0.485
ICAM2	324.18	155.93	366.09	179.85	1.14	0.525
ICAM3	633.35	224.54	721.13	224.69	1.14	0.332
ITGA4	314.26	110.15	386.51	191.70	1.24	0.231
ITGAL	207.68	162.42	275.58	144.54	1.35	0.338

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Contribution of authors

MHA, LS, DM analyzed data. DM, CE conceived and supervised the project. All authors wrote, revised, contributed to the article, and approved the submitted version.

Declaration of competing interest

The authors declare no conflict of interests regarding any financial disclosures nor commercial associations.

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