Pancreatology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

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

M.H. Aziz [a], L. Saida [b], C.H.J. van Eijck [a], D.A.M. Mustafa [b, *]

[a] Department of Surgery, Erasmus University Medical Center, Rotterdam, Netherlands
[b] Department of Pathology & Clinical Bioinformatics, The Tumor Immuno-Pathology Laboratory, Erasmus University Medical Center, Rotterdam, Netherlands

ARTICLE INFO

Article history:
Received 30 June 2023
Received in revised form
30 October 2023
Accepted 1 November 2023
Available online xxx

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.
© 2023 The Authors. Published by Elsevier B.V. on behalf of IAP and EPC. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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

* Corresponding author.
E-mail address: d.mustafa@erasmusmc.nl (D.A.M. Mustafa).

https://doi.org/10.1016/j.pan.2023.11.001
1424-3903/© 2023 The Authors. Published by Elsevier B.V. on behalf of IAP and EPC. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite this article as: M.H. Aziz, L. Saida, C.H.J. van Eijck *et al.*, Overexpression of the adhesion signaling pathway is linked to short-term survival in pancreatic ductal adenocarcinoma, Pancreatology, https://doi.org/10.1016/j.pan.2023.11.001

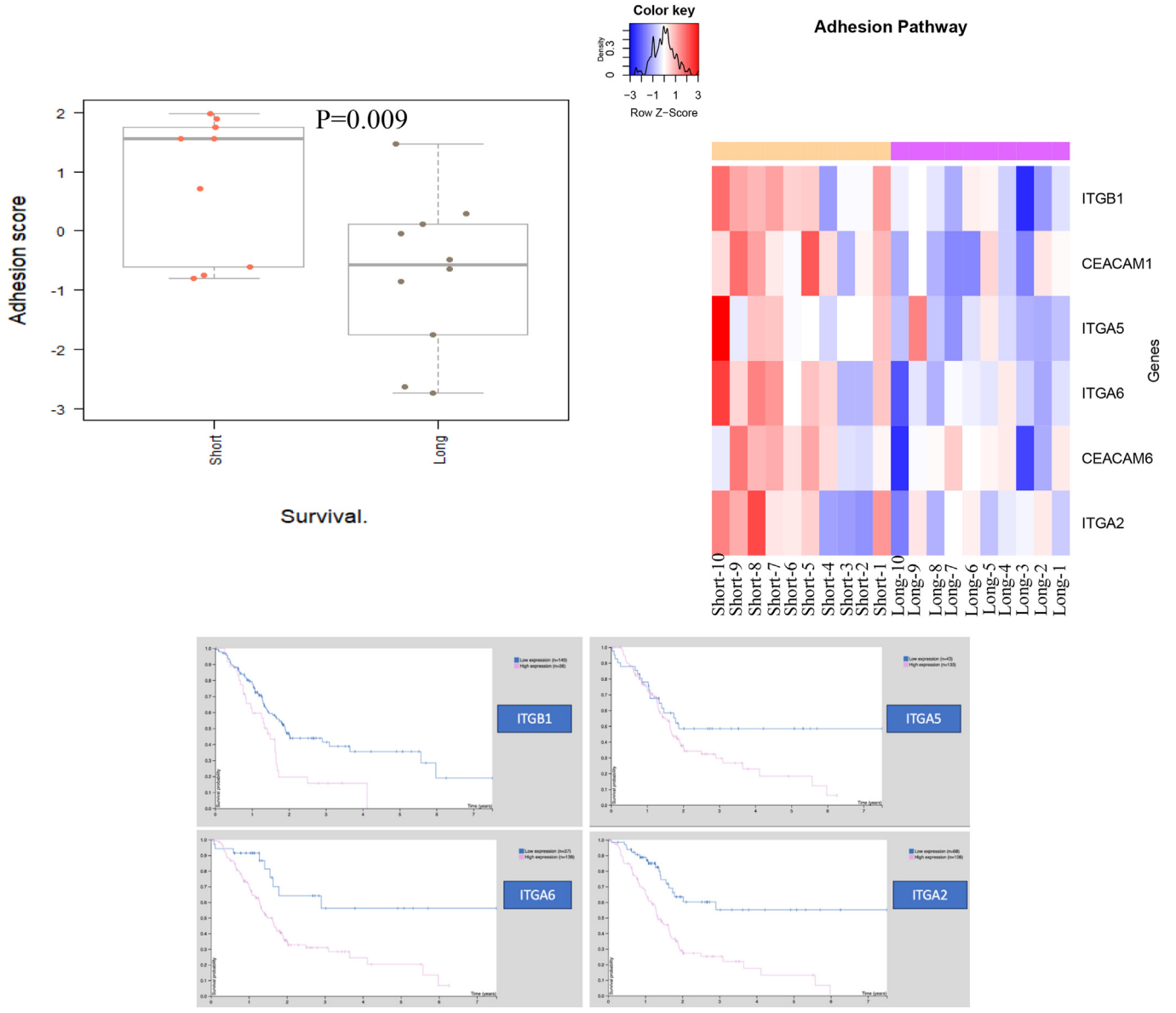

Fig. 1. "Adhesion" pathway is over-expressed in short-compared to long-term survival samples of patients with PDAC. A-Boxplot of the adhesion pathway score calculated by the sum of the averages of all genes associated with the pathway. Every dot represents a sample. **B-** heatmap of the significant 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 highlight 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

Source of funds

Survival with Pancreatic Cancer Foundation.

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.

References

[1] Polireddy K, Chen Q. Cancer of the pancreas: molecular pathways and current advancement in treatment. J Cancer 2016;7(11):1497–514.
[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136(5):E359–86.
[3] Sideras K, Braat H, Kwekkeboom J, van Eijck CH, Peppelenbosch MP, Sleijfer S, et al. Role of the immune system in pancreatic cancer progression and immune modulating treatment strategies. Cancer Treat Rev 2014;40(4):513–22.
[4] Aziz HM, Saida L, de Koning W, Stubbs AP, Li Y, Sideras K, et al. Spatial genomics reveals a high number and specific location of B cells in the pancreatic ductal adenocarcinoma microenvironment of long-term survivors. Front Immunol 2022;13:995715.
[5] Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer 2010;10(1):9–22.
[6] Mierke CT, Frey B, Fellner M, Herrmann M, Fabry B. Integrin alpha5beta1 facilitates cancer cell invasion through enhanced contractile forces. J Cell Sci 2011;124(Pt 3):369–83.
[7] Liu F, Wu Q, Dong Z, Liu K. Integrins in cancer: emerging mechanisms and therapeutic opportunities. Pharmacol Ther 2023;247:108458.
[8] Li J, Peng L, Chen Q, Ye Z, Zhao T, Hou S, et al. Integrin beta1 in pancreatic cancer: expressions, functions, and clinical implications. Cancers 2022;14(14).
[9] Grzesiak JJ, Bouvet M. The alpha2beta1 integrin mediates the malignant phenotype on type I collagen in pancreatic cancer cell lines. Br J Cancer 2006;94(9):1311–9.
[10] Seguin L, Desgrosellier JS, Weis SM, Cheresh DA. Integrins and cancer: regulators of cancer stemness, metastasis, and drug resistance. Trends Cell Biol 2015;25(4):234–40.
[11] Zinczuk J, Zareba K, Romaniuk W, Kaminska D, Niziol M, Baszun M, et al. Expression of chosen carcinoembryonic-related cell adhesion molecules in pancreatic intraepithelial neoplasia (PanIN) associated with chronic pancreatitis and pancreatic ductal adenocarcinoma (PDAC). Int J Med Sci 2019;16(4):583–92.
[12] Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. CEACAM6 gene silencing impairs anoikis resistance and in vivo metastatic ability of pancreatic adenocarcinoma cells. Oncogene 2004;23(2):465–73.