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SERPINB7 Expression Predicts Poor Pancreatic Cancer Survival Upon Gemcitabine Treatment

Daniela Bianconi[*], Merima Herac[†], Daniel Spies[‡,§], Markus Kieler[*], Robert Brettner[*], Matthias Unseld[*], Katrin Fürnkranz[*], Barbara Famler[†], Margit Schmeidl[†], Christoph Minichsdorfer[*], Christoph Zielinski[*], Gerwin Heller[*] and Gerald W. Prager[*]

[*]Department of Internal Medicine I, Comprehensive Cancer Center Vienna, Medical University of Vienna, Waehringer Guertel 18-20, 1090, Vienna, Austria; [†]Clinical Institute of Pathology, Medical University of Vienna, Waehringer Guertel 18-20, 1090, Vienna, Austria; [‡]Swiss Federal Institute of Technology Zurich, Department of Biology, Institute of Molecular Health Sciences, Otto-Stern Weg 7, 8093 Zurich, Switzerland; [§]Life Science Zurich Graduate School, Molecular Life Science Program, University of Zurich, Institute of Molecular Life Sciences, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Abstract

Stratification of patients with pancreatic ductal adenocarcinoma (PDAC) remains a key challenge in the field of clinical oncology. No predictive biomarkers have yet been found for any available treatment options. Previously, we identified *SERPINB7* as a putative biomarker for PDAC and thus, herein, we aimed to validate our previous findings and assessed the predictive value of *SERPINB7.* Patients who underwent surgery and received gemcitabine (gem) or gemcitabine plus nab-paclitaxel (gem/nab) as adjuvant therapy, between 2011 and 2017, were included in this study (n = 57). Expression level of *SERPINB7* was assessed in tumor tissue by immunohistochemistry (IHC) and RNA in situ hybridization (RNA ISH). Its association with disease-free survival (DFS) and overall survival (OS) was investigated. While IHC did not show any correlation between survival and the protein level of *SERPINB7*, RNA ISH revealed that expression of *SERPINB7* was associated with a poor DFS ($P = .01$) and OS ($P = .002$) in the gem group but not in the gem/nab. Adjusted Cox-regression analysis confirmed the independent predictive value of *SERPINB7* on OS ($P = .006$, HR: 3.47; 95% CI: 1.49–8.09) in the gem group. In conclusion, *SERPINB7* was identified as the first predictive RNA biomarker for PDAC. This study suggests that patients who expressed *SERPINB7* might receive another treatment than gem alone.

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Background

In the last decade, it was possible to translate groundbreaking scientific discoveries into oncologic therapies in order to improve patients´ lives. Representative examples of these breakthroughs are ipilimumab (the first anti-cancer immunotherapy) and talimogene laherparepvec (the first oncolytic virus therapy); both approved to treat metastatic melanoma [1,2]. Even if these technological and medical advances are irrefutable, not all oncologic patients have benefited from these major improvements. Pancreatic ductal

Address all correspondence to: Gerald Prager, Department of Internal Medicine I, Comprehensive Cancer Center Vienna, Währinger Gürtel 18-20, 1090, Medical University of Vienna, Austria.
E-mail: gerald.prager@meduniwien.ac.at
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adenocarcinoma (PDAC) represents one of these exceptions: it is projected to become the second deadliest type of cancer in the United States by 2030 [3] and among all other cancer types, only its mortality rate is predicted to increase in the European Union for both sexes [4].

Latest research studies have shown that PDAC exhibits some special features that differentiate it from other cancer types [5]. Briefly, PDAC seems not to follow the classical step-by-step cancer development model but to follow a development path similar to a 'big bang model', in which a single phenomenon called chromothripsis originates several chromosomal rearrangements [6]. Furthermore, thanks to the advancement of new technologies, such as Next Generation Sequencing (NGS), the genetic and transcriptomic landscapes of pancreatic cancer have started to be elucidated, enabling a deeper insight into the tumor biology of this complex disease. For instance, it was shown that patients with PDAC indeed exhibit recurrent gene mutations but these classical mutations (e.g., mutations in *TP53* and *KRAS*) appear together with other rare mutations, in combinations that seem to be unique for each patient [7–10]. This apparent futility of profiling somatic mutations to predict outcome was later supported by Dal Molin et al., who demonstrated that mutations at the DNA-level were not useful predictors of overall survival in a long-survival cohort [11]. Notwithstanding these discouraging findings, results of gene expression analyses have been very promising so far. More recent evidence has revealed that transcriptome analysis enables the identification of subgroups of PDAC that might have clinical implications [12–15]. Although there is a high discrepancy between the differentially expressed genes of the three classifications, it was demonstrated that there is a high concordance in predicting patient's outcome [12–15]. For example, Bailey's squamous subtype includes genes, which are involved in e.g. inflammation and metabolic reprogramming and according to unsupervised analysis of RNA-seq data, this subtype corresponds to Moffitt's basal subtype [13–15]. In both cases, a poor overall survival (OS) was associated with these subtypes [13–15]. Overall, these data strongly suggest that there is an urgent need for changing the research strategies in order to finally identify predictive biomarkers for pancreatic cancer that might improve survival and the quality of life of patients.

To date, only our and few other research groups have focused on the identification of RNA-biomarkers for pancreatic cancer [16–18]. Recently, we have performed a retrospective analysis of a small cohort of patients with metastatic PDAC who were treated with capecitabine plus nab-paclitaxel and participated in a phase II clinical trial [16]. In this study, patients were divided into short- and long-term survivors according to the median OS and we analyzed the differential gene expression between these two groups by RNA-seq [16]. These results were then validated using publically available data from the The Cancer Genome Atlas (TCGA) database, revealing that SERPINB7 (serine protease inhibitor, clade B, member 7) expression was an independent predictor of OS [16,19]. Based on our aforementioned study (see *Supplementary Methods*), we hypothesized that SERPINB7 overexpression in the primary tumor might correlate with a poor prognosis among patients who underwent surgery for tumor resection and received gemcitabine as adjuvant therapy, a treatment considered to be the standard of care for this disease. Thus, to address this issue, we examined the RNA- and protein level of SERPINB7 by RNA in situ hybridization (RNA ISH) and immunohistochemistry (IHC), respectively, and evaluated the association between SERPINB7 expression level and disease-free survival (DFS) and overall survival (OS).

Methods

Study Cohort

Patients with PDAC presented in our multi-disciplinary tumor board meeting between 2011 and 2017 were included in this retrospective study. All patients underwent surgery and received gemcitabine (n = 46) or gemcitabine plus nab-paclitaxel (n = 11) as adjuvant chemotherapy at our institution. This study was approved by the Ethics Committee of the Medical University of Vienna (1794/2017). (More information in *Supplementary Methods*).

RNA ISH

RNA ISH was performed according to the manufacturer's instructions (RNAscope 2.5 Brown Assay, Advanced Cell Diagnostics (ACD), Hayward, CA) [20]. Briefly, FFPE tissue was deparaffinized in xylene and ethanol. Samples were incubated in citrate buffer, rinsed in water and treated with protease at 40 °C for 30 minutes in a HybEZ Oven (Advanced Cell Diagnostics, Hayward, CA). After that, samples were incubated with a custom designed sample probe and then with the probes AMP1 to AMP6. Chromogenic detection was performed using 3,3′-diaminobenzidine (DAB). Sections were counterstained with diluted Gill III (EMD Millipore, Billerica, MA, HX55589174) followed by an incubation in Scott's tap water (Morphisto, Germany, 11,192.01000). Slides were dehydrated using ethanol and xylene and then mounted with Neo-Mount (MerckKGaA, Germany, HX69711216). Positive and negative control probes (Polr2A (DNA-directed RNA polymerase II subunit RPB1) and dapB (bacterial dihydrodipicolinate reductase), respectively) were acquired from ACD. The target probe was designed by our group based on our previous results and level-1 data from TCGA (access Request #59059–3) [16,21]. If dots were detected by the pathologist in tumor cells or reactive pancreatic ducts, samples were considered as positive. Stained samples were scanned using the Aperio ScanScope (Leica Biosystems) at 40× objective magnification.

Immunohistochemistry

Sections were deparaffinized and rehydrated by washing in xylene, ethanol and water. Endogenous peroxidase activity was blocked by

Table 1. Baseline characteristics of patients included in the study before surgery. All patients underwent surgery and received gem or gem/nab at our institution.

		Gemcitabine (n = 46)	Gemcitabine + nab-paclitaxel (n = 11)*
Age at diagnosis (median)		65.72 y	65.48 y
Gender	Female	18 (39.13%)	3 (27.27%)
	Male	28 (60.87%)	8 (72.73%)
T	1	1 (2.17%)	0
	2	6 (13.04%)	0
	3	38 (82.61%)	11 (100%)
	4	1 (2.17%)	0
N	0	9 (19.57%)	2 (18.18%)
	1	37 (80.43%)	9 (81.82%)
M	0	41 (89.13%)	10 (90.91%)
	1	2 (4.35%)	1 (9.09%)
Grade	x	3 (6.52%)	0
	1	1 (2.17%)	0
	2	31 (67.39%)	6 (54.55%)
	3	14 (30.43%)	5 (45.45%)
Metastasis at diagnosis		2 (4.35%)	1 (9.09%)
	Lung	1	0
	Liver	1	0
	Other	0	1
Resection of tumor		46 (100%)	11 (100%)
R	0	32 (69.57%)	8 (72.73%)