

The Italian Rare Pancreatic Exocrine Cancer Initiative

Tumori Journal

1–6

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Nazionale dei Tumori 2019


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DOI: 10.1177/0300891619839461

journals.sagepub.com/home/tmj



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Abstract

Introduction: Exocrine pancreatic cancers include common type pancreatic ductal adenocarcinoma and cystic neoplasms, which account for 85% and 10% of cases, respectively. The remaining 5% are rare histotypes, comprising adenosquamous carcinoma, acinar cell carcinoma, signet ring cell carcinoma, medullary carcinoma, pancreatoblastoma, hepatoid carcinoma, undifferentiated carcinoma and its variant with osteoclast-like giant cells, solid pseudopapillary carcinoma, and carcinosarcoma. Due to their low incidence, little knowledge is available on their clinical and molecular features as well as on treatment choices. The national initiative presented here aims at the molecular characterization of series of rare histotypes for which therapeutic and follow-up data are available.

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Methods: A nationwide Italian Rare Pancreatic Cancer (IRaPaCa) task force whose first initiative is a multicentric retrospective study involving 21 Italian cancer centers to retrieve histologic material and clinical and treatment data of at least 100 patients with rare exocrine pancreatic cancers has been created. After histologic revision by a panel of expert pathologists, DNA and RNA from paraffin tissues will be investigated by next-generation sequencing using molecular pathway-oriented and immune-oriented mutational and expression profiling panels constructed availing of the information from the International Cancer Genome Consortium. Bioinformatic analysis of data will drive validation studies by immunohistochemistry and in situ hybridization, as well as nanostring assays.

Conclusions: We expect to gather novel data on rare pancreatic cancer types that will be useful to inform the design of therapeutic choices.

Keywords

Rare tumors, pancreatic cancer, chemotherapy, biomolecular characterization

Date received: 11 December 2018; revised: 6 February 2019; accepted: 27 February 2019

Introduction

Common type pancreatic ductal adenocarcinoma (PDAC) accounts for 85% of exocrine pancreatic cancers. Cystic tumors represent 10% of cases, but only a minority of these is malignant. According to World Health Organization classification, the remaining exocrine pancreatic cancers are represented by rare histotypes, comprising pancreatic adenosquamous carcinoma, pancreatic acinar cell carcinoma, solid pseudopapillary carcinoma, undifferentiated carcinoma and its variant with osteoclast-like giant cells (UCOGC), signet ring cell carcinoma, medullary carcinoma, pancreatoblastoma, hepatoid carcinoma, and carcinosarcoma.¹ Due to the low incidence of these malignancies, their clinical features are usually reported only in case reports or in small series of retrospective observational studies.^{2,3} Notably, patients with these rare pancreatic cancers are excluded from clinical trials. The clinical dilemma in the management of patients with rare pancreas cancers at advanced stage is as follows: Do chemotherapy regimens recommended for PDAC have a clinical activity or should other therapeutic approaches (different chemotherapy combinations, targeted therapies, immunotherapy) be considered?

To attempt to address this question, we conducted a multicentric retrospective study that evaluated the efficacy of different chemotherapy regimens used over time in patients with advanced stage rare pancreatic cancer types.⁴ Of 4300 exocrine pancreatic cancer patients, 79 advanced cases affected by rare histologic types were identified, including 23 pancreatic acinar cell cancers, 16 pancreatic adenosquamous cancers, and 15 mucinous cystic neoplasms with an associated invasive component. In this study, no statistical significance correlation had been found for survival in relation with the different chemotherapy regimens used, due to the small sample. Moreover, because of their rarity, many clinicopathologic features remain unclear, and prognostic factors should be established. A deeper understanding of the molecular alterations

underpinning these rare cancers may help clinicians make therapeutic choices. Furthermore, the role of immunotherapy in these patients is largely unexplored. Previous studies showed different morphologic features and immunophenotypes in these cancer types,^{5,6} further suggesting the importance to clarify their molecular profiles in order to identify potential novel prognostic biomarkers and therapeutic vulnerabilities.

The recent whole genome sequencing of 456 PDAC coupled with transcriptomic profiling has furnished information on their most frequently altered genes and the pathways in which they participate, in addition to the identification of four different cancer subtypes (ADEX, progenitor, squamous, and immunogenic) with potentially diverse prognosis and response to therapy.^{7,8} PDAC molecular subtypes, some of which also were defined using immunohistochemical markers, have been associated with differing response to chemotherapies.^{9–11} Since tissue DNA and RNA sets of biomarkers able to identify molecular subtypes of PDAC were identified in previous studies,^{7,8} we are generating clinically applicable tests working on DNA and RNA from paraffin tissue specimens, which are the sole source of material for rare pancreatic cancer. The molecular subclassification of cancers entails the molecular characterization of both the cancer cells and the cells populating cancer tissue microenvironment. Tissue biomarkers include next-generation sequencing (NGS) mutational panels and expression profile nanostring panels capable of classifying these tumours into discrete molecular subgroups. The design and application of NGS mutational panels interrogating the molecular asset of cancer cells applicable to paraffin tissues has been amply applied by Scarpa et al.¹² and others.^{13,14} Moreover, PDAC is characterized by a microenvironment of varying cell composition, often rich with cancer-associated fibroblasts (CAFs) and tumour-associated macrophages (TAMs) that play a crucial role in immune regulation through CAF-derived extracellular matrix components and modulators. CAFs are known to be significant in the

inflammatory response as well as immune suppression in tumours and it has been shown that pancreas cancer cells obtain nutrients through metabolic crosstalk with CAFs.¹⁵ TAMs are important regulators of the tumour microenvironment and their abundance correlates with a worse prognosis in PDAC.¹⁶

Moreover, in a recent whole-exome sequencing of UCOGC, Luchini et al.¹⁷ have shown that the study of somatic mutations alone cannot provide sufficient data to characterize the unique microenvironment of PDAC variants, suggesting the need to address studies on the immunologic microenvironment to better understand the biology of this tumor type.

Silvestris et al.¹⁸ have performed a preliminary molecular and angiogenetic/immunogenetic characterization of adenosquamous cancer. The angiogenetic pathway in this histotype was compared to PDAC unveiling a putative role of miR-21-5p, miR-181a-5p, miR-122-5p, and miR-27a-3p in the regulation of this process. Indeed, miR-21-5p, miR-181a-5p, and miR-27a-3p were upregulated in adenosquamous pancreatic cancer with respect to PDAC since the first holds a more developed immunophenotypic aspect than the second; on the contrary, miR-122-5p is downregulated, revealing an antiangiogenic role. Assuming that the genotype/phenotype of adenosquamous cancers overlap those of the squamous phenotypes of PDAC⁸ and of other solid tumors, which have been shown to be sensitive to immune checkpoint inhibitors, Silvestris et al.¹⁹ performed immunogenomic analysis through a next-generation multigene sequencing custom panel including immunity checkpoint, inflammation, B- and T-cells activation genes.

We suggest applying tests that the proposing team has developed for common type PDAC availing of the studies of the International Cancer Genome Consortium, in which the proposers have participated,^{2,3,5} to obtain a molecular characterization of rare exocrine histotypes of pancreatic cancer based on a selection of DNA, RNA, and immunohistochemical biomarkers. The aim of this study is to perform a genomic and transcriptomic analysis of a multicentric cohort of patients with rare variants of exocrine pancreatic cancer.

Methods

This multicentric retrospective study involves the collection of histologic material and clinical data referring to patients with rare histotypes of exocrine pancreatic neoplasm, radically operated or locally advanced, recurrent or metastatic disease at the onset.

In particular, the objectives are as follows:

1. Creation of a nationwide Italian Rare Pancreatic Cancer (IRaPaCa) task force whose first objective will be the collection of tissue samples of these cancers from resected (surgical specimens) or

unresected patients (endoscopic biopsies) that have traceable therapeutic and follow-up data. The Italian centers dedicated to the management of pancreatic cancer patients that will be participating are summarized in Table 1.

2. Assessment of the molecular genotype/phenotype of the different rare pancreatic cancer subtypes using pathway-oriented NGS multigene DNA mutational panels and nanostring assays assessing multigene RNA expression panels capable of discerning the molecular phenotypes described in Bailey et al.⁸ Moreover, identification of immunologic alterations and immunophenotype may represent prognostic and potentially predictive factors of response to immunotherapy.

Study population

This multicentric retrospective study includes all patients in the centers (Table 1) participating in the study with rare histotypes of exocrine pancreatic neoplasia currently in treatment protocol and/or in follow-up situations at the same centers. Inclusion and exclusion criteria are reported in Table 2.

Sample collection and evaluation

A total of 100 rare pancreas cancers will be studied. All cases will be histologically reviewed by a panel of expert pathologists. DNA and RNA will be isolated from formalin-fixed, paraffin-embedded sections of surgical specimens or bioptic samples. Molecular profiling of potentially targetable core signaling pathways will be performed using NGS multigene panels. The following core signaling pathways will be analyzed: DNA damage repair, chromatin remodeling, tumor growth factor- β (TGF β), and WNT spliceosome, examining the mutational and copy number status of 87 genes, as previously described.^{12,13} The panels are intended to identify the altered pathways in single cancers. It has been reported that cancers bearing mutations in chromatin remodeling genes have a better outcome, while cancers with inactivation of DNA damage repair genes respond to platinum therapies.⁷ These panels have been already used to characterize large series of classic type PDAC from the Verona biobank and to address specific questions, as in a set of cases showing differential hMena expression that interacts with TGF β pathway. Molecular profiling of immune-related genes will be performed using NGS multigene panels. A custom panel examining the entire coding regions of 41 genes involved in immune checkpoints, inflammation, and B- and T-cells activation will be used as described,¹⁹ using antibodies against CD3, CD4, CD8, CD20, CD68, and CD163. The expression of molecules targetable with immunotherapy will be assessed, including PD-1 and PD-L1, VISTA, and LAG-3.

Table 1. Cancer centers and principal investigators.

Cancer centers	Principal investigators
1. Cancer Institute of Bari "Giovanni Paolo II" IRCCS	Nicola Silvestris (coordinating center)
2. Careggi Hospital, Florence	Francesco di Costanzo
3. Casa Sollievo della Sofferenza, San Giovanni Rotondo	Evaristo Maiello
4. Hospital of Vicenza	Giuseppe Aprile
5. Sant'Andrea Hospital, University of Rome La Sapienza	Paolo Marchetti
6. European Institute of Oncology IRCCS, Milan	Nicola Fazio
7. Istituto Nazionale Tumori IRCCS, Milan	Vincenzo Mazzaferro
8. Istituto Oncologico Veneto, IRCCS, Padua	Sara Lonardi
9. Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola	Andrea Casadei Gardini
10. Regina Elena Institute, Rome	Michele Milella
11. San Raffaele Institute, Milan	Michele Reni
12. Hospital of Treviglio	Fausto Petrelli
13. University of Ancona	Rosanna Berardi
14. Campus Biomedico of Rome University	Daniele Santini
15. Fondazione Policlinico Universitario A. Gemelli, IRCCS, Catholic University of the Sacred Heart, Rome	Felice Giuliani
16. University of Bologna	Giovanni Brandi
17. University of Cagliari	Mario Scartozzi
18. University of Modena	Stefano Cascinu
19. University of Naples	Fernando De Vita
20. University of Pisa	Enrico Vasile
21. University of Verona	Aldo Scarpa

Table 2. Inclusion and exclusion criteria.

Inclusion criteria	<ol style="list-style-type: none"> 1. Patients over the age of 18 2. Patients with rare resectable or advanced (locally advanced and/or metastatic) histotypes 3. Histologic diagnosis performed on an operative sample and/or biopsy of pancreatic carcinoma including the following rare histotypes: acinar, adenosquamous, carcinosarcoma, hepatoid carcinoma, medullary, osteoclastic-like carcinoma, pancreatoblastoma 4. Availability of tumor tissue from an operative and/or biopsy specimen fixed and included in paraffin in the pathological anatomy archive of the various centers participating in the study 5. Patients currently living and effectively available able to understand and sign a regular written declaration of consent to clinical trial without drugs using biological material for scientific purposes and the processing of personal data, in compliance with the new European regulation on data protection effective from 25 May 2018 6. Patients deceased or not available in any way, without the need to obtain a regular declaration of consent, in accordance with the new European Data Protection Regulation in force since 25 May 2018
Exclusion criteria	<ol style="list-style-type: none"> 1. Patients with a histologic diagnosis doubtful or different from the rare variants of pancreatic carcinoma specified in the inclusion criteria 2. Patients currently living and effectively available refusing to sign a regular written declaration of consent to the clinical trial without drugs using biological material for scientific purposes and the processing of personal data, in compliance with the new European data protection regulation in from 25 May 2018

Immunohistochemistry will be used to validate selected markers. Transcriptomic analysis has identified PDAC subgroups based on both cancer cell differentiation and composition of stroma.⁸ Based on this, we designed nanostring assays that are able to define these subgroups using a few dozen genes and RNA from paraffin-embedded cancer tissues (unpublished). A second nanostring assay will be used to assess the specific expression of 95 genes involved in immune response and inflammation.

The Ion PGM Hi-Q View Sequencing Kit will be used for sequencing loaded with the Ion 318 Chip v2. All generated reads will be aligned to human genome hg19 using the Torrent Suite Server. Variant calling will be performed through IonReporter workflow and confirmed by Vardict. Deregulated genes will be evidenced by DESeq2 R package. RNA and DNA data will be integrated to evidence the causal relation between mutations and gene expression through appropriate bioinformatic tools. Nanostring assays

will be performed following manufacturer instructions and interpretation tools are in place at Verona and Bari units.

Study monitoring

The study will be performed in accordance with good clinical practice. All information will be reported in the case report form/study database, in an accurate manner according to the instructions provided.

The principal investigator (PI) has responsibilities to the health authorities to take all reasonable steps to ensure the proper conduct of the study as regards ethics, protocol adherence, integrity, and validity of the data recorded on the case report forms/study database.

The study falls under the conditions foreseen by the new European Data Protection Regulation in force since 25 May 2018 for which the request for informed consent to patients enrolled in the retrospective analysis is not required. The PI ensures that this study will be conducted according to the procedures and methods indicated in this protocol, respecting the privacy and rights of the patient set in the Helsinki Declaration (1964) and in accordance with the current legislation in Italy. The PI also guarantees that any modification or amendment to the proposed protocol after acceptance by the Ethics Committee will be submitted to the same Committee in accordance with current procedures so as to allow them to be evaluated for further approval. The PI will be sent requests for missing data or clarification of inconsistencies or discrepancies.

Statistical analysis

Continuous variables will be presented as means \pm SD, while categorical variables will be expressed with numbers and percentage. Pearson correlation will be used to assess the correlation between molecular subtypes in biopsy and surgical specimens. Kaplan-Meier analyses will be used to compare disease-free, progression-free, and overall survival assessed on molecular subtypes. Cox proportional hazards regressions will be used to evaluate the effect of molecular subtypes on time to death, in patients with resectable and unresectable PDAC, adjusting for potential confounders. In addition to Cox analyses, the interaction between molecular subtypes and chemotherapy treatments will be formally tested, to understand if molecular subtypes can modify the effect of chemotherapy on survival. Mixed model of covariance will be adopted to compare change in number of DNA mutations according to subtyping profile.

Conclusions

To our knowledge, this is the first time that a large series of rare pancreas cancers will be characterized for their

molecular and immunologic profiles based on a curated collection of samples from patients for which clinical, therapeutic, and follow-up data are available. We expect to define the molecular/phenotypic profiles of these cancers highlighting their potential therapeutic vulnerabilities according to the core signaling pathways specifically altered in each cancer type. This and the immunologic characterization will address immunotherapy multi-institutional trials.

Being a retrospective study, it is possible that DNA or RNA analyses could not be efficiently performed. Thus we have chosen to have 100 cases available for the study and a preliminary list of more than 250 cases from the different institutions that expressed their interest in joining the IRaPaCa task force.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

The molecular tests developed or under development for the molecular classification of pancreas neoplasms are part of a project financed by the Associazione Italiana Ricerca sul Cancro (AIRC 5x1000 n. 12182)

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