

Using Molecular Subtyping of Pancreatic Ductal Adenocarcinoma for Multimodal Treatment Selection in Resectable Disease

Simona O Dima^{1,2}, Traian Dumitrascu^{1,3}, Irinel Popescu^{1,2*}, Dan G Duda⁴

¹Center of Digestive Diseases and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania

²Center of Excellence for Translational Medicine, Fundeni Clinical Institute, Bucharest, Romania

³Department of Surgery, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

⁴Steele Laboratories for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

*Corresponding author:

Irinel Popescu, MD, FACS, FEBS
Professor of Surgery
"Dan Setlacec" Center of General
Surgery and Liver Transplantation
"Fundeni" Clinical Institute
Sos. Fundeni, 258, 022328
Bucharest, Romania
E-mail: irinel.popescu@icfundeni.ro

Rezumat

Utilizarea subtipării moleculare pentru selecția tratamentului multimodal în adenocarcinomul ductal pancreatic rezecabil

Adenocarcinomul ductal pancreatic se caracterizează prin heterogenitate ridicată; astfel, chiar și după o intervenție chirurgicală cu intenție curativă, există o variabilitate semnificativă în ceea ce privește supraviețuirea pacienților, reflectând diferite comportamente biologice. Selectarea terapiei adecvate, personalizate pentru fiecare pacient cu adenocarcinom ductal pancreatic rezecabil, în terapia multimodală, de către o echipă multidisciplinară cu experiență, este de cea mai mare importanță pentru a obține un beneficiu clinic maxim, evitând tratamentele potențial dăunătoare. Identificarea pacienților cu adenocarcinom ductal pancreatic rezecabil care ar beneficia de rezecția chirurgicală în contextul terapiei multimodale rămâne un subiect de interes pentru practica clinică. Pentru a îmbunătăți rezultatele pacienților cu adenocarcinom ductal pancreatic, un pas semnificativ înainte ar fi integrarea subtipurilor moleculare în procesul decizional clinic în ceea ce privește efectuarea intervenției chirurgicale per primam sau folosirea tratamentului neoadjuvant. Integrarea cu succes a cunoștințelor privind subtipul de adenocarcinom ductal pancreatic poate ghida în mod corespunzător această selecție de tratament pentru a îmbunătăți în continuare rezultatele în ceea ce privește supraviețuirea pe termen lung. În această lucrare de tip review, prezentăm cunoștințele actuale cu privire la rolul subtipării moleculare în deciziile chirurgicale pentru pacienții cu adenocarcinom ductal pancreatic.

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Cuvinte cheie: carcinom ductal pancreatic; subtipuri moleculare; tratament neoadjuvant; supraviețuire

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is characterized by high heterogeneity; thus, even after a curative intent surgery, there is significant variability in the survival of patients, reflecting different biological behaviors. The selection of proper, personalized therapy for each patient with resectable PDAC, in multimodal therapy, by an experienced multidisciplinary team is of utmost importance to get maximal clinical benefit avoiding potentially harmful treatments. Identifications of patients with resectable PDAC that would benefit from surgical resections in the context of multimodal therapy remain a topic of interest for clinical practice. To improve PDAC patient outcomes, a significant step forward would be the integration of the molecular sub-types in the clinical decision-making between upfront surgery versus neoadjuvant treatment. Successful integration of the preoperative knowledge of the subtype of PDAC can properly guide this treatment selection to further improve patient outcomes. In this review, we present an overview of the current knowledge on the role of molecular subtyping in surgical decisions for PDAC patients.

Key words: pancreatic ductal adenocarcinoma, molecular sub-types, neoadjuvant therapies, survival

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal diseases, with a 5-year survival rate of approximately 10% (1). Currently, the only potentially curative option is surgical resection, but only 10–20 percent of the patients present with resectable disease (2). Unfortunately, most patients present with advanced-stage disease, either locally advanced (30%) or metastatic (50%) at the time of diagnosis (3). PDAC is characterized by high heterogeneity; thus, even after a curative intent surgery, there is significant variability in the survival of patients, reflecting different biological behaviors. The selection of proper, personalized therapy for each patient with resectable PDAC, in multimodal therapy, by an experienced multidisciplinary team is of utmost importance to get maximal clinical benefit avoiding potentially harmful treatments. It is worth

mentioning that pancreatic resections are widely associated with increased morbidity and non-neglectable mortality rates. Thus, identifications of patients with resectable PDAC that would benefit from surgical resections in the context of multimodal therapy remain a topic of interest for clinical practice.

Based on the experience in other cancer types, there is great interest in using prognostic tools for treatment selection for patients with PDAC. To improve PDAC patient outcomes, a significant step forward would be the integration of the molecular sub-types in the clinical –decision-making between upfront surgery versus neoadjuvant treatment (4). Successful integration of the preoperative knowledge of the subtype of PDAC can properly guide this treatment selection to further improve patient outcomes. In this review, we present an overview of the current knowledge on the role of molecular subtyping in surgical decisions for PDAC patients.

Multimodal Approaches Based on Disease Classification in PDAC

To date, the clinical staging of PDAC is determined by radiological findings, including tumor size and local and distant extension. Contrast-enhanced computed tomography scanning (using pancreas-specific protocols) of the chest, abdomen, and pelvis can assess the degree of tumor involvement in surrounding vasculature such as a superior mesenteric artery, and superior mesenteric vein, portal vein, and celiac axis, or common hepatic artery. According to this approach, multidisciplinary assessments of the tumor's resectability status are usually based on its anatomical features. Different consensus criteria stratify the primary non-metastatic PDAC into three categories: resectable, borderline resectable, and locally advanced (5, 6). The widely used standard of care for tumors considered resectable usually involves surgery followed by adjuvant chemotherapy (7,8); for borderline resectable and locally advanced diseases, neoadjuvant therapy (NAT) is used for disease downstaging followed by surgery when feasible (9,10).

The Multimodal Approach in Resectable PDAC

Patients with upfront surgical PDAC resection who successfully received adjuvant therapy have a median overall survival (OS) of less than 25 months (11) and a 5-year survival rate approaching 30% (12,13). Adjuvant FOLFIRINOX (mFFX) has improved median OS after complete (R0) and incomplete (R1) PDAC resection (14). However, only 55-75% of patients who start adjuvant therapy complete the entire chemotherapy course (15,16). Moreover, for various reasons, including early recurrence, postoperative complications, and inadequate recovery after surgery – 30-50 % of patients do not undergo adjuvant therapy at all (11,17-19).

In addition, data show that up to 50% of patients have incomplete resection with positive surgical margins. For those patients,

the overall 5-year survival sharply decreases to 7% (12).

Previous studies have shown that even when deemed radiologically resectable, up to 31% of patients are found with occult metastases in surgical exploration or staging laparoscopy (20).

In this context, one question arises: "How to improve the survival rate of these patients in the future?" To answer this question, we must first see if the NAT increases the resectability rate in resectable PDAC.

Is NAT Justified in Resectable PDAC?

Ongoing studies are investigating the use of NAT (which is the standard of care for anatomically borderline resectable PDAC) (21, 22) versus upfront surgery in patients with resectable PDAC (23-25).

Although published data on NAT's role in resectable PDAC tumors have been inconsistent (26), the latest NCCN Guidelines supported NAT in "high-risk" resectable PDAC cases (27). High-risk is defined according to the biological factors, including elevated CA 19-9 (cut-off values were set as high as 500 IU/ml), large tumors, large regional lymph nodes, and conditional factors such as excessive weight loss and extreme pain (27). However, when assessing NAT's feasibility in PDAC resectable tumors, potential benefits, as well as potential disadvantages, should be kept in mind.

The potential benefits of NAT versus upfront surgery are the following:

- enables early treatment of micro-metastatic disease, often present but undetectable;
- can shrink tumors and increase R0 resection rates (83.7% in NAT versus 76.8% in the US) (28);
- can decrease lymph node positivity rate (45.0% in NAT versus 69.3% in the upfront surgery) (28);
- can decrease the need for associated vascular resections;
- can decrease the rates of postoperative pancreatic fistula (29);
- may have a higher chemotherapy comple-

tion rate than adjuvant treatment (25).

The potential downside of preoperative NAT in resectable PDAC is that about 20% of tumors progress during NAT and may become unresectable (30), making the of NAT questionable in the management of resectable PDAC (31). These patients may lose the opportunity for radical surgery. At the same time, the rapid tumor progression during NAT might be a tool for selecting patients with aggressive diseases who would not have benefited from radical surgery. A more significant progress in the molecular understanding of resectable PDAC will be critical to improve the ability to identify aggressive disease and high-risk patient populations and optimize the selection of patients (26) and to improve NAT efficacy by using novel molecular targeted agents in addition to chemotherapy.

Although several prospective trials and meta-analysis studies have shown promising median OS and R0 resection rates, conclusive results for using NAT over upfront surgery are awaited from the ongoing trials (27,32,33). One limitation is that most studies include patients with borderline resectable PDAC and resectable PDAC, with only a few studies focused specifically on the subgroup of resectable PDAC (33-35). However, it is worth mentioning that recent analyses from the PREOPANC trial have associated NAT in resectable PDAC with statistically significant increased OS rates compared with upfront surgery (33). Moreover, there was no detrimental influence of NAT on postoperative complications after resection for PDAC (29).

Future directions for preoperative therapy in resectable PDAC

The most common NAT chemotherapy regimens recommended by current guidelines are based on: FOLFIRINOX (i.e., 5-fluorouracil /5-FU, leucovorin, oxaliplatin, and irinotecan), mFOLFIRINOX (mFFX), gemcitabine, or gemcitabine/nab-paclitaxel (GnP) (36). To allow for individualized NAT, future studies should identify and validate biomarkers in biopsy tissues or blood samples to select the patients most likely to experience tumor

downsizing, reduced need for vascular resection, and potentially decreased local recurrence rates.

Molecular PDAC Subtypes Using Transcriptomic Data

Given the genotypic and phenotypic heterogeneity of PDAC, the optimal treatment strategy for patients—i.e., NAT versus surgery-first—is likely to vary from patient to patient. Recent data have shown the prognostic role of molecular subtypes based on transcriptomic analyses. Unfortunately, only limited data are available from patients treated with surgery for PDAC (37).

Collisson et al. were the first to attempt PDAC subtyping by integrating microarray information from resected tumors in 2011 (38). The authors combined transcriptional profiles generated from the dataset generated by their group at UCSF (n=27 microdissected tumors), and a dataset generated by our group at ICF (n=39 primary resected PDAC) (39), made available on GEO (GSE 15471). From the combination of these two clinical datasets, three transcriptional PDAC subtypes emerged: classical, quasi mesenchymal (QM-PDA), and exocrine-like (38). Moffitt et al. digitally separated tumor from stromal gene expression. They identified two subtypes of pancreatic cancer tumors, "classic" and "basal," as well as two subtypes of stroma, "normal" and "active," in another study published in 2015 (40). This study highlights the significance of stroma in pancreatic cancer tumorigenesis. Another molecular classification, published by Bailey et al. in 2016, was based on the analysis of 456 resected primary PDAC samples using whole-genome sequencing, deep exome sequencing, and gene expression. In this classification, the classical subtype was subdivided into pancreatic progenitor and immunogenic subtype and defined into four subtypes: "squamous" "pancreatic progenitor", "immunogenic", and "aberrantly differentiated endocrine exocrine (ADEX)" (41). Apart from the aforementioned classification subtypes, Puleo et al. studied

formalin-fixed paraffin-embedded PDAC samples and identified five subtypes „pure classical”, „immune classical”, „desmoplastic”, „stroma activated”, and „pure basal-like” subtypes. This study was focused on primary resected PDAC rather than a metastatic disease (42).

As a result, two consensus transcriptomic-based tumor subtypes have emerged consistently across various classification systems: (1) "classical" (overlapping with Collisson's "classical" and Bailey's "pancreatic progenitor") and (2) "squamous"/ basal-like (overlapping with Collisson's "QM-PDA", "Moffit's „basal-like”, " and Bailey's "squamous") (43).

In addition to the most known expression-based subtyping studies, another study demonstrated – using single-cell RNA sequencing – that a subset of tumors harbor the „basal-like” and „classical subtypes and define them as „hybrid” basal-like/classical PDAC tumors (44). Interestingly, this study suggested that resectable PDAC tumors were frequently of classical-A/B subtypes. In contrast, the „basal-like” subtype A was rarely detected in resectable disease (44). More recently, Hwang et al. used single-nucleus RNA sequencing and whole-transcriptome digital spatial profiling (DSP) of 43 primary PDAC tumor specimens that either received NAT or surgery to construct a high-resolution molecular landscape of the cellular subtypes and spatial communities that compose PDAC (45). They uncovered recurrent expression programs across malignant cells and fibroblasts, including a newly identified neural-like progenitor malignant cell program enriched after NAT and associated with poor prognosis. Integrating spatial and cellular profiles revealed three multicellular communities with distinct contributions from malignant, fibroblast, and immune subtypes: classical, squamoid-basaloid and treatment enriched.

Biomarkers Associated with Specific Transcriptomic Subtypes

However, genomic analysis of all patients for PDAC subtype classification is cumbersome

and expensive. Integrating molecular subtyping in clinical practice will require more simple and cost-effective techniques to measure specific biomarkers to select PDAC patients likely to respond to a NAT chemotherapy regimen (46).

To this end, our group is pursuing genomic and proteomic studies of specific biomarkers generated by microarray or RNA sequencing analysis and validated by RT-PCR or immunohistochemistry (IHC).

So far, the protein expression of GATA 6, S100A2, and S100A4 by IHC may be used as surrogate biomarkers for the "classical" and "squamous" transcriptome subtypes, respectively (47).

Expression of the transcription factor GATA6 aligned with the classical subtype (38). The Collisson et al. study showed a significantly higher expression of GATA6 in the classical subtype compared to QM-PDA and exocrine-like subtypes in our Badea et al. dataset ($p=0.013$, Kruskal-Wallis Test). Considering Collisson's classification as a reference, we validated GATA6 expression by RT-PCR. The relative expression from 36 resected PDAC patients was significantly higher in the classical subtype compared to the QM-PDA subtype ($p<0.05$). Similar to other genomic studies, most patients from our group were from stages I, IIA, and IIB (unpublished data). This agrees with the study by Collisson et al., which showed that the classical tumor subtype had higher GATA6 mRNA expression and was associated with a significantly superior outcome (38). Consistent with these findings, Martinelli et al. showed that GATA6 loss in resected PDAC was associated with a „basal-like” phenotype in the ESPAC-3 phase III trial and shorter survival after adjuvant 5-FU treatment (48). Thus, GATA6 detection using formalin-fixed, paraffin-embedded needle biopsies at diagnosis might be an attractive surrogate biomarker for disease subtypes (49).

Collisson et al. have shown that the classical subtype also expresses relatively higher levels of KRAS than the QM-PDA or exocrine-like subtypes. However, this finding was not significant in the PDAC samples from

the study by Badea et al. ($p=0.2$, Kruskal Wallis Test).

A recent study used a triple IHC biomarker panel (S100A4, Ca-125, and mesothelin) to stratify PDAC patients based on prognosis after upfront resection or NAT. The Bailey et al. transcriptomic classification of PDAC subtypes was available for a sub-group of these patients ($n=8$). The patients with triple-negative biomarker status represented those with a „low-risk” phenotype, which was associated with the „non-squamous” PDAC genotype. By contrast, patients with triple-positive biomarker status represented those with a „high-risk” phenotype. S100A4 expression individually demonstrated a non-significant trend toward association with the squamous PDAC subtype (50). We and others previously observed that the increased S100A4 level is associated with tumor aggressiveness, metastasis, and poor survival (47,51). Previous studies showed that increased S100A4 gene expression (measured by two-step qPCR using TaqMan hydrolyzed probes) was correlated with increased tumor stage, tumor differentiation degree, and many lymph nodes as well as with the lymph node ratio (LNR). Furthermore, in the same study, high protein levels of S100A4 determined by IHC correlated with poor OS after pancreatectomy in PDAC patients ($p=0.043$) (51). However, prospective validation of these findings will be required for their clinical applicability.

In addition, a molecular „preoperative prognostic nomogram” incorporating two biomarkers (S100A2 and S100A4) was tested and validated in multiple independent cohorts of patients with resectable PDAC ($n=1,184$) using IHC and mRNA analysis. Moreover, IHC for S100A2 and S100A4 was performed on the preoperative endoscopic ultrasound fine-needle aspiration (EUS-FNA) cell blocks ($n=17$). High S100A2 and S100A4 mRNA expression correlated with the „squamous subtype” of PDAC ($n=96$ patients who underwent whole transcriptome sequencing analysis) and with poorer OS (47).

The Role of Transcriptomic Molecular Subtypes as a Tool for a Multimodal Approach

The previously defined molecular PDAC subtypes were associated with significant differences in survival. Based on published data, the „squamous” subtype is associated with a worse survival and a more aggressive clinical behavior (38,40,41). Moreover, in the Collison et al. study, a multivariate Cox regression model showed that this subtype was an independent predictor of OS ($p=0.024$) (38). The presence of the „activated stroma” subtype identified by Moffit et al. conferred a worse prognosis (40). Analysis of the resection margin status, a surrogate of aggressive tumor biology, in a high cohort of PDAC patients showed no association between the margin status (R0 vs. R1) and the „squamous” subtype. In this study, lymph node involvement and R1 status did not associate with prognosis (52).

However, there is currently no agreement on the optimal treatment regimens for each subtype, and PDAC continues to face a significant challenge from chemotherapy resistance. Recent evidence suggests that the „basal-like” and „basal-like A” subtype PDAC subtypes respond poorly to mFFX (44,49). Consistent with this hypothesis, a study by Tiriac et al. demonstrated that patient-derived organoid signatures might predict chemotherapy response (53). Moffitt et al. also demonstrated that patients with „basal-like” tumors benefitted more from adjuvant therapy, although the effect was not significant (40). According to Collison et al., the QM-PDA subtype cell lines are relatively more sensitive to gemcitabine than those with the classical subtype. The classical subtype PDAC cell lines are more sensitive to the anti-EGFR drug erlotinib in vitro (38). These results indicate that gemcitabine and erlotinib may be more active in different PDAC subtypes, which need to be identified to increase efficacy and avoid unnecessary toxicity for patients with non-responsive PDACs.

The prospective trial „GATA6 Expression as a predictor of response to perioperative chemotherapy in resectable pancreatic adenocarcinoma: A multicenter Canadian phase II study (NeoPanONE)” will evaluate clinical outcomes and molecular biomarkers including GATA6 and radiomic biomarkers in patients with resectable PDAC treated with mFFX in preoperative setting (54).

Future studies should validate whether patients with triple-negative biomarker status, associated with “non-squamous” PDAC, benefit from a surgery-first approach rather than NAT and, conversely, whether patients with triple-positive biomarker status, associated with a “squamous” genotype, benefit from NAT followed by resection (50).

Conclusion

Answering whether NAT is the optimal approach to resectable PDAC remains a timely one to create a consensus in the multidisciplinary management of this aggressive malignancy. Defining „high-risk” PDAC patients using molecular subtypes and specific biomarkers may prove an essential tool for finding the answer to this critical question, and potentially to design more effective NAT approaches.

Conflicts of Interests

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