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Translational research in pancreatic ductal adenocarcinoma: Current evidence and future concepts

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treatment allocation. This topic highlight is focused on current evidence on potential biomarkers for tumor biology, prognosis and prediction of treatment efficacy.

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Core tip: Recent advances in the treatment of pancreatic ductal adenocarcinoma (PDA) have been made using the intensified chemotherapy regimen folinic acid, irinotecan and oxaliplatin, the recently FDA-approved nab-paclitaxel and the epidermal growth factor receptor-inhibitor erlotinib. Yet overall prognosis of PDA remains poor. To further improve outcome of PDA, innovative strategies are needed to identify patient subgroups that benefit most from specific regimens. This topic highlight focuses on potential biomarkers to identify patients that benefit from treatment with erlotinib (*e.g.* KRAS, AKT, ERK, p53), gemcitabine (hENT1, RRM1, dCK), nab-paclitaxel (SPARC) or angiogenesis inhibitors. Additional biomarkers of tumor biology (like SMAD4 and CXCR4) and future concepts of translational research in PDA are also discussed.

Abstract

Pancreatic ductal adenocarcinoma (PDA) is one of the major causes for cancer death worldwide. Treatment of metastatic disease remains challenging as only certain patients benefit from advances made with the intensified chemotherapy regimen folinic acid, irinotecan and oxaliplatin, the epidermal growth factor receptor inhibitor erlotinib or the recently FDA-approved nab-paclitaxel. Up to date, no established approach for prediction of treatment response or specific treatment allocation exists. Translational research was able to identify a number of potential biomarkers that might help to improve the dismal prognosis of PDA by facilitating upfront

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDA) constitutes

the fifth leading cause of cancer death accounting for approximately 227000 annual deaths worldwide^[1-4]. It is only curable by surgical resection which is feasible in about 15%-20% of all patients^[4]. Non-resectable patients usually receive palliative chemotherapy with gemcitabine-based combinations^[5]. However, these combinations often fail to offer long-term disease control resulting in a poor five-year survival rate of about 4%^[4]. Advances in specific patient populations have been made using the tyrosine kinase inhibitor erlotinib and an intensive treatment regimen consisting of 5-fluorouracil (5-FU), folinic acid, irinotecan and oxaliplatin (FOLFIRINOX). While effects on the overall population are minimal for erlotinib, intensive chemotherapy with FOLFIRINOX is tolerated by certain patients only^[6,7]. Treatment options proven to be beneficial in other cancer entities like the vascular endothelial growth factor (VEGF) inhibitor bevacizumab have failed to improve survival in an unselected PDA population^[8]. Preclinical data propose that innovative agents like the recently FDA-approved albumin bound nab-paclitaxel might be dependent on expression of specific proteins, suggesting predefined patient subgroups as major beneficiaries^[9]. Hence new biomarkers are urgently needed for treatment allocation and identification of patient subgroups that might benefit from alternative treatment strategies^[10]. This topic highlight summarizes and assesses current evidence from translational studies on biomarkers for tumor biology, prognosis and prediction of treatment response in PDA.

Biomarkers for tumor biology and prognosis

SMAD4: SMAD4/DPC4 is a protein involved in intracellular transforming growth factor- β 1 signaling^[11]. In PDA differing functions as biomarker have been ascribed to SMAD4. Based on findings in rapid autopsies performed on patients previously diagnosed with stage I to IV PDA, Iacobuzio-Donahue *et al.*^[12] suggested SMAD4 as a biomarker for metastatic pattern of PDA. They determined presence or absence of intact SMAD4 using immunohistochemistry in PDA of 65 patients. Abnormal immunostaining of SMAD4 was found in 41 patients (63%). Absence of intact SMAD4 was significantly more frequent in metastatic disease (78%) and significantly reduced in locally destructive disease (22%) ($P = 0.007$). Oshima *et al.*^[13] screened 106 patients with PDA who had undergone surgical resection for mutations in different genes including the *SMAD4* gene. Abnormal immunolabeling for SMAD4 was detected in 64 patients (60%). Using univariate analysis, a significant correlation between tumor size ($P = 0.006$), lymphatic invasion ($P = 0.033$), lymph node metastasis ($P = 0.006$) and abnormal immunostaining for SMAD4 was found. Overall survival for patients with intact *vs* mutant SMAD4 was 30.1 mo *vs* 18.3 mo respectively ($P < 0.001$). Within a multivariate analysis mutant SMAD4 was found to be a significant and independent poor prognostic factor for both disease free and overall survival. In a different study Bachet and co-workers examined tumor samples of 471 patients with resected PDA and assessed SMAD4 status by tissue microarray analyses. Patients with mutant

SMAD4 significantly benefited from adjuvant chemotherapy (hazard ratio for death compared to untreated patients = 0.59; 95%CI: 0.42-0.82; $P = 0.002$), whereas no significant beneficial effect of adjuvant treatment was witnessed for SMAD4 wild-type status (hazard ratio for death = 0.85; 95%CI: 0.49-1.46; $P = 0.552$). While disputing a correlation between metastatic pattern and SMAD4 status, the authors conclude that SMAD4 might also predict adjuvant treatment response^[14]. Using multivariate analysis Winter *et al.*^[15] recently examined the correlation between SMAD4 status and different clinical criteria in 127 patients with resected PDA. In harsh contrast to earlier findings they found neither a correlation between SMAD4 and metastatic pattern nor a correlation between SMAD4 and overall survival (Table 1).

CXCR4: Chemokines are small cytokines capable of inducing chemotaxis. They exert their effects *via* specific chemokine receptors found on various cell types including immune and tumor cells. The chemokine receptor CXCR4 has been described to be widely expressed in different cancer types^[16]. *Via* interaction with its ligand, the chemokine CXCL12 (SDF-1 α) is believed to promote tumor growth, angiogenesis and tumor dissemination^[17]. In PDA two retrospective studies concluded CXCR4 to be a significant and independent poor prognostic factor for overall survival while a third study found no significant correlation between overall survival and CXCR4^[14,18,19]. The CXCR4 ligand CXCL12 has recently been reported to be a predictive marker for treatment response to bevacizumab as discussed below^[20]. Additional research is clearly necessary to identify the potential of CXCR4 and its ligand in predicting treatment response and prognosis in PDA.

Predictive biomarkers of the epidermal growth factor receptor pathway

The oral epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor erlotinib modestly improves survival in an unselected patient population with metastatic PDA. However, a significant survival benefit from erlotinib treatment is observed for patients developing skin rash^[5]. Erlotinib exerts its effects by inhibiting intracellular receptor transphosphorylation of the ErbB1/HER1 receptor^[21]. Translational studies therefore aimed to identify EGFR polymorphisms and gene amplifications predictive for erlotinib treatment. Despite of promising findings in pre-clinical and early clinical studies, translational subgroup analyses from prospective clinical trials failed to reveal a significant correlation between genetic EGFR alterations or overexpression and treatment response to erlotinib up to now^[22]. Recent investigational approaches on identifying predictive EGFR pathway biomarkers have therefore focused on downstream EGFR signaling such as the PI3K-AKT-PTEN network or the RAS-RAF-MAPK-MEK-ERK cascade.

KRAS: Mutations in members of the *RAS* gene family such as v-Ki-ras2 Kirsten rat sarcoma viral oncogene ho-

Table 1 Summary of current evidence on selected biomarkers in pancreatic ductal adenocarcinoma discussed in this topic highlight

Ref.	Biomarker	Prognostic	Predictive (for)	Dissemination pattern
Iacobuzio-Donahue <i>et al</i> ^[12]	SMAD4	N/A	N/A	+
Oshima <i>et al</i> ^[13]		+	N/A	+
Bachet <i>et al</i> ^[14]		-	+ (adjuvant chemotherapy)	-
Winter <i>et al</i> ^[15]		-	N/A	-
Bachet <i>et al</i> ^[14]	CXCR4	+	-	+
Maréchal <i>et al</i> ^[18]		+	N/A	+
Gebauer <i>et al</i> ^[19]		-	N/A	-
Lee <i>et al</i> ^[27]	KRAS	+	N/A	N/A
Shin <i>et al</i> ^[28]		+	N/A	N/A
Ogura <i>et al</i> ^[29]		+	N/A	N/A
Boeck <i>et al</i> ^[22,31]		+	? (erlotinib)	N/A
Kim <i>et al</i> ^[24]		-	+ (erlotinib)	N/A
da Cunha Santos <i>et al</i> ^[32]		-	-	N/A
Oliveira-Cunha <i>et al</i> ^[33]		-	N/A	N/A
Farrell <i>et al</i> ^[48]	hENT1	-	+ (adjuvant gemcitabine)	N/A
Morinaga <i>et al</i> ^[49]		-	+ (adjuvant gemcitabine)	N/A
Maréchal <i>et al</i> ^[50]		-	+ (adjuvant gemcitabine)	N/A
Greenhalf <i>et al</i> ^[52]		-	+ (adjuvant gemcitabine)	N/A
Jordheim <i>et al</i> ^[51]		-	+ (review on 18 studies on adjuvant gemcitabine)	N/A
Poplin <i>et al</i> ^[53]		-	- (gemcitabine in metastatic PDA)	N/A

(+): Results suggest that the respective molecule might serve as a biomarker; (-): No evidence found that the respective molecule might serve as a biomarker; (?): Unclear whether the respective molecule could serve as a biomarker. N/A: No data available; in cited study; PDA: Pancreatic ductal adenocarcinoma.

molog (KRAS) are frequently observed in different types of human cancers^[23]. The highest frequency of mutant KRAS can be found in PDA. It has been reported that mutant KRAS is present in up to 90% of all PDA^[10,24]. Single point mutations in codon 12, 13, 59 or 61 of exon 2 and exon 3 of the KRAS oncogene impair intrinsic GTPase activity of KRAS and lead to a permanent active KRAS signaling pathway, resulting in sustained proliferation and survival of cells^[23]. KRAS has been described as a predictive biomarker for treatment success by inhibitors of the EGFR pathway such as the small-molecule drug erlotinib or the monoclonal antibodies cetuximab and panitumumab in metastatic non-small cell lung and colorectal cancer^[25,26]. So far, the value of KRAS as biomarker in PDA has not been clearly established. In a retrospective study performed by Lee *et al*^[27], KRAS status of 66 patients with metastatic ($n = 61$) or locally advanced ($n = 5$) PDA was analyzed. The majority of patients ($n = 64$) had received first-line chemotherapy with gemcitabine alone or gemcitabine in combination with capecitabine, uracil/tegafur (UFT) or cisplatin. In a total of 42 patients (64%) KRAS mutations were found (codon 12: $n = 41$, codon 63: $n = 1$). Comparison between patients with a mutation in codon 12 of the KRAS oncogene and wild-type KRAS showed a significant reduced overall survival for patients with mutant KRAS (9.1 mo *vs* 13.4 mo, $P = 0.03$). In conclusion Lee *et al*^[27] suggested that KRAS might be of value as a prognostic biomarker in PDA. In the largest retrospective study conducted on KRAS as a biomarker in PDA so far, Shin *et al*^[28] analyzed KRAS status of 234 resected PDA patients by polymerase chain reaction. Mutant KRAS was present in 126 patients (55%). Using multivariate analysis mutant KRAS was found to be significantly correlated to poor prognosis. In a different study by Ogura *et al*^[29] similar findings were reported. Of

note neither Shin *et al*^[28] nor Ogura *et al*^[29] commented on applied chemotherapy regimens in the study population, making it impossible to distinct between a predictive and merely prognostic correlation. The AIO-PK0104 study was a large, multicenter phase III trial in advanced PDA conducted by the German AIO study group^[30]. In a post-hoc analysis of AIO-PK0104 the wild-type KRAS status was found to be significantly correlated with an improved overall survival (hazard ratio for death for wild-type compared to mutant KRAS = 1.68; $P = 0.005$). Owing to the study design (all patients received erlotinib plus either capecitabine or gemcitabine as first-line chemotherapy) it was impossible to directly distinguish between a prognostic and predictive correlation^[22]. However, within an exploratory analysis no significant correlation of KRAS status with objective response to erlotinib-containing first-line therapy was found, indicating that KRAS may not serve as a predictive but rather as a prognostic biomarker for overall survival in PDA^[31]. Contrary to these observations, Kim *et al*^[24] screened tumor samples of 136 patients with metastatic ($n = 112$) or locally advanced ($n = 24$) PDA, who had received first-line therapy with gemcitabine alone ($n = 22$) or a combination of gemcitabine with either erlotinib ($n = 70$), capecitabine ($n = 31$) or UFT ($n = 13$). In 71 patients (52%) mutations in codon 12 ($n = 70$) or codon 61 ($n = 1$) of the KRAS oncogene were found. Post-hoc analysis showed a significant difference in overall survival between patients with wild-type and mutant KRAS status treated with erlotinib and gemcitabine (9.7 mo *vs* 5.2 mo, $P = 0.002$) whereas no difference in survival was observed in patients treated with regimens without erlotinib (7.0 mo *vs* 7.0 mo, $P = 0.121$). The authors from this Asian study therefore concluded that KRAS might be a predictive but not a prognostic biomarker. In clear contrast to this conclusion

are the findings of da Cunha Santos *et al.*^[32], who analyzed tumor samples of 117 patients from the erlotinib pivotal trial PA.3. Mutant KRAS was present in 92 patients (79%). Comparison of overall survival showed a non-significant survival benefit for wild-type KRAS patients treated with erlotinib plus gemcitabine *vs* patients treated with gemcitabine plus placebo (6.1 mo *vs* 4.5 mo, $P = 0.34$) while patients in the wild-type KRAS arm showed a trend towards reduced overall survival under anti-EGFR therapy (6.0 mo *vs* 7.4 mo, $P = 0.78$). In a study conducted by Oliveira-Cunha and co-workers the correlation between KRAS status and overall survival in 100 patients with resected pancreatic and periampullary cancer was analyzed. The investigators reported a non-significant shorter overall survival for mutant KRAS patients (22.8 mo *vs* 28.1 mo, $P = 0.88$) and concluded that there is no correlation between KRAS and overall survival^[33]. Noteworthy limitations of mentioned studies are retrospective design, lack of data on systemic therapy and vague definition of the cancer subtype investigated (*e.g.* periampullary cancer *vs* PDA). Additional research using prospective biomarker studies with clearly defined patient populations is crucial to clarify the possible use of KRAS as a prognostic or (even more important) as a predictive biomarker for treatment response to erlotinib.

ERK: EGFR signaling through KRAS is dependent on a complex interplay of intracellular proteins like the extracellular signal-regulated-/mitogen-activated protein kinase (ERK/MAPK). Because of its location downstream the RAS-RAF-MEK cascade, ERK might be useful in predicting success of anti-EGFR treatment^[34]. Additionally some previous studies in PDA suggested that high ERK expression might be a poor prognostic factor while other studies found no correlation between ERK expression and survival^[35-37]. The AIO-PK0104 investigators also examined the correlation between ERK expression and overall survival. Archival tumor tissue samples of 153 patients with advanced PDA who had received an erlotinib-based 1st-line regimen were analyzed using a grading system of cytoplasmic and nuclear phospho(p)-ERK expression ranging from 0 (no expression) to 12 (high expression). A significant increase in the hazard ratio for death by a factor of 1.06 for each pERK score level (0 to 12) was observed (HR = 1.06; 95%CI: 1.0-1.12; $P = 0.05$). As for KRAS (see above) it was not possible to definitely determine whether this correlation is solely prognostic or predictive for erlotinib efficacy due to the design of the trial^[38].

AKT: Besides activation of KRAS, dimerization of EGFR activates phosphoinositol-3-kinase (PI3K), resulting in activation of the serine/threonine-specific protein kinase AKT. The active form of AKT, phosphorylated AKT (pAKT) is an important mediator of cell survival and protein synthesis^[34]. Its activity is negatively regulated by the tumor suppressor protein phosphatase and tensin homolog (PTEN). A deregulated AKT/PTEN pathway leads to

resistance of cancer cell lines against anti-EGFR treatment *in vitro*^[39]. Additionally, a correlation between expression of AKT and overall survival has been described in previous small PDA studies^[35,36]. Within AIO-PK0104 tumor samples of 35 patients were categorized based on their pAKT expression level: no difference in progression free or overall survival between PDA patients expressing low or high levels of pAKT was observed^[38].

p53: Mutations in the tumor suppressor gene TP53 are an important step in the oncogenesis of most human cancer types. Including PDA, approximately 80% of all malignant tumors embody mutated TP53. Its transcriptional product p53 has been described to interact with the EGFR/KRAS signaling pathway in PDA^[34,40,41]. Additionally, p53 was recently also found to potentially serve as an independent predictive biomarker for treatment success with the monoclonal anti-EGFR antibody cetuximab in locally advanced rectal cancer^[42]. Pre-liminary findings from 50 patients treated within AIO-PK0104 showed that overall survival was independent of p53 expression; however, progression free survival was significantly reduced in patients with p53 loss (1.8 mo) or overexpression of p53 usually resulting in dominant negative p53 (2.5 mo) compared to normal levels of p53 expression (6 mo)^[38]. These pre-liminary findings may provide further evidence that not only a loss of p53 but also its overexpression is an important step in carcinogenesis and might be correlated to poor prognosis as also suggested by other studies^[43]. Further research in PDA is necessary to confirm these findings and to clarify whether the observed correlation is of prognostic or predictive nature.

Predictive biomarkers of the VEGF pathway

Angiogenesis inhibitors like the VEGF inhibitor bevacizumab have proven to be beneficial as add-on treatment in multiple cancer entities like colorectal and non-small cell lung cancer^[44]. In PDA *antiangiogenic* treatment has failed to show a significant effect in unselected patient populations so far^[8]. Lambrechts *et al.*^[44] identified a possible predictive biomarker to select patients who might benefit from anti-VEGF treatment with bevacizumab: using blood samples collected within the AVITA trial, they genotyped a set of 157 single nucleotide polymorphisms (SNP) in patients who had received gemcitabine and erlotinib plus either bevacizumab ($n = 77$ patients) or placebo ($n = 77$ patients). They identified the rs9582036 SNP in the VEGF receptor 1 region, which significantly correlated with progression-free and overall survival in the bevacizumab-treated group but not in the placebo group. Bevacizumab-treated AA carriers of the rs9582036 SNP showed a median overall survival of 10.2 mo (95%CI: 7.8-14.9) while AC and CC carriers showed a median overall survival of 5.9 mo (95%CI: 4.0-11.5) and 4.7 mo (95%CI: 4.3-NA), respectively. Using a novel multiplex ELISA system Nixon *et al.*^[20] recently analyzed 31 different factors in plasma samples of 328 patients with metastasized or locally advanced PDA who had re-

ceived gemcitabine plus either bevacizumab or placebo within the CALGB 80303 study; after multivariate analysis three factors were identified as possible predictive biomarkers: while low levels of VEGF-D were found to be predictive for improved outcome in the bevacizumab group, below median levels of CXCL12 (SDF-1 α) and angiopoietin 2 (Ang2) predicted a lack of benefit in the bevacizumab group. Further prospective biomarker studies are clearly necessary to confirm these pre-liminary findings and to assess the possible benefit of add-on treatment with bevacizumab in a pre-selected PDA population.

Biomarkers for the efficacy of gemcitabine

hENT1: Gemcitabine has been established as standard agent in the adjuvant and palliative chemotherapy setting of PDA more than a decade ago^[45]. Gemcitabine uptake by PDA cancer cells is thought to be dependent on human equilibrative nucleoside transporter 1 (hENT1), suggesting hENT1 as a possible predictive biomarker for treatment response to gemcitabine^[46,47]. In PDA patients receiving adjuvant treatment with gemcitabine or 5-FU within the RTOG 97-04 study, Farrell *et al*^[48] indeed demonstrated that patients treated with gemcitabine showed significant better overall survival if hENT1 was expressed in cancer cells as determined by immunohistochemistry in resected tumors (hazard ratio for death for hENT1 expression *vs* no hENT1 expression: 0.40; 95%CI: 0.22-0.75; $P = 0.03$). No correlation between overall survival and hENT1 expression was found in the 5-FU treated group indicating that hENT1 is a predictive biomarker for treatment response to gemcitabine. In line with these recent findings, Morinaga *et al*^[49] previously reported superior overall survival for patients with high levels of hENT1 (22.2 mo) *vs* patients with low hENT1 levels (11.8 mo) in a population that had received adjuvant gemcitabine chemotherapy. In the largest retrospective study to date, Maréchal *et al*^[50] collected tumor samples from 434 surgically resected PDA patients among whom 243 had received gemcitabine-based regimens. They found that high hENT1 expression was a strong predictive factor for superior overall survival in the gemcitabine treated group (hazard ratio for death high *vs* low hENT1 expression = 0.43; 95%CI: 0.29-0.63; $P < 0.0001$). In a recent review on 18 (mainly retrospective) clinical studies Jordheim and co-workers concluded that “it has been clearly shown that hENT1 expression is a predictive marker for patient outcome after (adjuvant) gemcitabine therapy” in resectable PDA^[51]. This conclusion is also supported by the recently reported translational hENT1 data from the ESPAC studies: a retrospective subgroup analysis on 352 patients with resected PDA treated with either adjuvant 5-FU or gemcitabine found that hENT1 serves as a predictive marker for the efficacy of gemcitabine but not for 5-FU^[52]. To overcome the poor prognosis in low hENT1 expressing PDA, CO-101, a chemically modified gemcitabine molecule thought to be capable of entering the cell independent of hENT1, was developed^[53]. In

the ‘low hENT1 and adenocarcinoma of the pancreas (LEAP)’ trial, the efficacy and safety of CO-101 was investigated in chemo-naïve metastatic PDA patients. Enrolling 360 patients, this international randomized phase II trial was also the first to prospectively assess the value of hENT1 as a predictive biomarker for treatment response to gemcitabine. Astonishingly, the LEAP trial not only demonstrated that CO-101 had no additional value over standard gemcitabine treatment, it also indicated that hENT1 expression does not correlate with overall survival in gemcitabine-treated patients with metastatic PDA^[53]. Further research will have to elucidate whether these contrasting findings are due to different research approaches (retrospective *vs* prospective studies), differences in methodology (*e.g.* use of different antibodies) or if hENT1 expression has differing functions as a biomarker in the adjuvant and metastatic PDA setting.

RRM1 and dCK: Several other molecules involved in the metabolism of gemcitabine are currently under investigation as potential biomarkers in PDA: retrospective evidence suggests that - besides hENT1 - also deoxycytidine kinase (dCK) may be able to predict benefit from adjuvant gemcitabine in resected PDA^[54]. dCK is responsible for the intracellular phosphorylation of the prodrug gemcitabine to its mononucleotide in a rate-limiting manner. Thus high expression levels of dCK may enhance the efficacy of the drug. RRM1 (ribonucleotide reductase M1) is a cellular target for gemcitabine and may additionally also act as a tumor suppressor. Preliminary evidence in resectable PDA showed that RRM1 may potentially serve as both a prognostic (in non-gemcitabine treated patients) and a predictive (in gemcitabine treated patients) biomarker^[54].

Biomarker for the efficacy of nab-paclitaxel

SPARC: Adjacent stromal tissue is a hallmark of PDA believed to be an important contributor to poor treatment outcome by reducing drug delivery to cancer cells^[55]. New treatment strategies aim on facilitating drug delivery to cancer cells by reducing tumor stroma. A promising candidate is the recently FDA-approved albumin bound nab-paclitaxel, which was originally developed to avoid toxicities observed in treatment with solvent-based paclitaxel^[9]. In addition to a favorable safety profile, nab-paclitaxel has been shown to deplete tumor stroma and increase intratumoral gemcitabine concentration by a factor of 2.8 in mice bearing xenograft PDA tumors^[56]. Further, co-administration of gemcitabine and nab-paclitaxel reduced levels of the gemcitabine metabolizing enzyme cytidine deaminase, making PDA cells more sensitive to gemcitabine treatment^[9]. Recent results from the phase III ‘metastatic pancreatic adenocarcinoma clinical (MPACT) trial showed a statistically significant increase in overall survival from 6.7 mo in patients receiving single-agent gemcitabine to 8.5 mo in patients receiving the combined nab-paclitaxel/gemcitabine regimen^[57]. Recent findings in humans confirmed that

stromal depletion by nab-paclitaxel might be responsible for the reported survival benefit^[58]. For intracellular uptake of nab-paclitaxel into PDA stromal cells, specific albumin-binding proteins are necessary. Secreted protein acidic and rich in cysteine (SPARC) has been proposed to be one of them^[9]. SPARC is a matricellular glycoprotein involved in different biological processes like wound repair or angiogenesis^[59]. Its overexpression and correlation with poor prognosis independent of the therapeutic agent has been described in different human cancers like colon, esophageal, breast and lung cancer^[60]. In PDA it was demonstrated that increased SPARC expression in adjacent fibroblasts but not in cancer cells conversely correlates to overall survival^[60]. Results from a phase I / II nab-paclitaxel trial suggested that elevated SPARC levels in fibroblasts adjacent to PDA might be a predictive marker for treatment success with nab-paclitaxel^[56,61]. Yet a very recent study using SPARC knockout mice reported drug delivery and antitumoral effects of murine nab-paclitaxel to be independent of SPARC expression^[62]. Thus further research will be necessary to elucidate the potential use of SPARC as a predictive biomarker for nab-paclitaxel treatment in humans; specifically the translational results on SPARC from the large international MPACT study are urgently awaited in this context.

Future directions

Translational research studies conducted so far have failed to identify reliable prognostic or predictive biomarkers for PDA. Besides methodological limitations like retrospective design and heterogeneous study populations, most trials focused only on specific mutations in a small number of genes. Yet prognosis and treatment response might depend on the interaction of a large variety of genes and mutations as proposed in a work of Collisson *et al*^[63]. In this study microdissected DNA of resected PDA was analyzed using gene expression microarray analysis. A 62-gene signature for PDA was defined by means of different statistic models. Subsequently tumor probes were divided into three different subgroups depending on their genetic signature. Subgroups were classical type for PDA expressing high levels of adhesion-associated and epithelial genes, quasi-mesenchymal type for PDA expressing high levels of mesenchyme-associated genes and exocrine-like type for PDA expressing high levels of tumor cell derived digestive enzyme genes. Prognosis between these three subtypes differed significantly with classical type having the best and quasi-mesenchymal type having the worst prognosis regarding overall survival. In further experiments Collisson *et al*^[63] analyzed human and murine PDA cell lines using the 62-gene microarray technique. Dependence on KRAS was analyzed using RNAi. Proliferation of classical subtype PDA cell lines was more prone to inactivation of KRAS by RNAi (of note this approach did not distinguish between wildtype and mutant KRAS alleles). Additionally, classical PDA cell lines were more sensitive to treatment with erlotinib while quasi-mesenchymal cell lines were more sensitive

to gemcitabine^[63]. Further clinical research is required to translate these findings into clinical practice. As cancer genome sequencing becomes more available and less expensive^[64], analyzing large subsets of genes appears to be a promising future approach to predict treatment response and prognosis in PDA.

CONCLUSION

In this topic highlight several potential biomarkers for prognosis, tumor biology and treatment response of PDA were identified and discussed (as summarized within Table 1). Despite promising pre-liminary results, translational research has failed to establish reliable biomarkers for clinical practice so far. Main limitations for most trials on potential biomarkers conducted in PDA were: non-comparable patient cohorts, retrospective design and non-consistent treatment protocols and molecular methods used. Besides the general need for more accompanying translational studies in pancreatic cancer trials, future studies on potential biomarkers should be conducted prospectively in well-defined patient populations, using standardized molecular methods and profound biostatistical analysis. Furthermore, innovative technologies like cancer genome sequencing and multiplex ELISA platforms might help to identify new options in predicting prognosis and facilitating treatment allocation in PDA.

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