PANCREATIC CANCER IN 2015

Precision medicine in pancreatic cancer — fact or fiction?

Thomas Seufferlein and Julia Mayerle

Late diagnosis and an inability to personalize treatment are major problems preventing reductions in pancreatic cancer mortality. In 2015, the identification of a highly discriminatory exosomal biomarker, culture systems that recapitulate human disease and new methods of analysing large data sets to identify prognostic markers have improved the future outlook for patients with this cancer.

Pancreatic cancer is projected to be the third leading cause of cancer-related death by 2030. Multiple factors contribute to the dismal prognosis for patients with pancreatic cancer, but two clinical problems are a major concern: late diagnosis and treatment resistance or lack of personalized treatment stratification[1]. These aspects have been addressed in research published in 2015, and considerable progress has been made towards the use of precision medicine for the treatment of pancreatic cancer (FIG. 1).

> An association between cancer survival and 22 distinct leukocyte subsets has been revealed...

Pancreatic cancer has a very poor prognosis, with a 5-year survival rate of only 6% and 80–85% of patients with pancreatic cancer diagnosed at a stage when the tumour is unresectable. Early detection is projected to increase survival by 30–40%[1]. Serum levels of carbohydrate antigen 19–9 (CA19-9) above 37 U/ml is the best established blood test for the detection of pancreatic cancer. CA19-9 can discriminate between patients with pancreatic cancer and healthy individuals with a sensitivity of 80.3% (95% CI 77.7–82.6) and a specificity of 80.2% (95% CI 78.0–82.3)[2], and between malignant and benign pancreatic disease with a sensitivity of 78.2% (95% CI 72.3–80.4) and a specificity of 82.8%[3]. However, to reduce health-care expenditure and prolong patients' survival, an assay for early diagnosis would have to perform with a minimum sensitivity of 88% at a specificity of 85%[4]. Aiming to identify a marker with a higher diagnostic accuracy than CA19-9, Melo and co-workers established that the detection of the cell surface proteoglycan glypican-1 on circulating exosomes isolated from patient plasma samples enables the discrimination between patients with early-stage and late-stage pancreatic cancer, benign pancreatic disease and healthy individuals. Using a cut-off of 7.6% glypican-1-positive exosomes, patients with pancreatic ductal adenocarcinoma (PDAC) could be distinguished from healthy individuals and those with benign pancreatic disease with a previously unmet sensitivity and specificity of 100%[5]. Identification and isolation of cancer-specific exosomes in body fluids enables a diagnostic marker to be detected without contamination by noncancer proteins, therefore increasing diagnostic accuracy. Questions to be answered in 2016 include whether these findings can be validated independently in a larger set of patients, and whether exosomal glypican-1 is just a marker of pancreatic cancer or whether it has a function in exosome generation or tumour growth and progression.

Isolating exosomes from patients' serum samples is a difficult task in clinical practice; however, a new approach of performing next-generation sequencing on easily obtainable cell-free media, such as plasma, might be more feasible for the detection of cancer-related gene expression in the clinic. In a prospective proof-of-concept study, Zill and colleagues analysed 54 genes concomitantly in tumour tissue and cell-free DNA isolated from a 1 ml plasma sample[6]. The diagnostic accuracy of cell-free DNA sequencing was 97%, with 92.3% mean sensitivity and 100% specificity, over a preselected panel of five genes. If replicated in a larger study, this approach opens new avenues for a tailored and personalized treatment strategy for patients with pancreatic cancer.

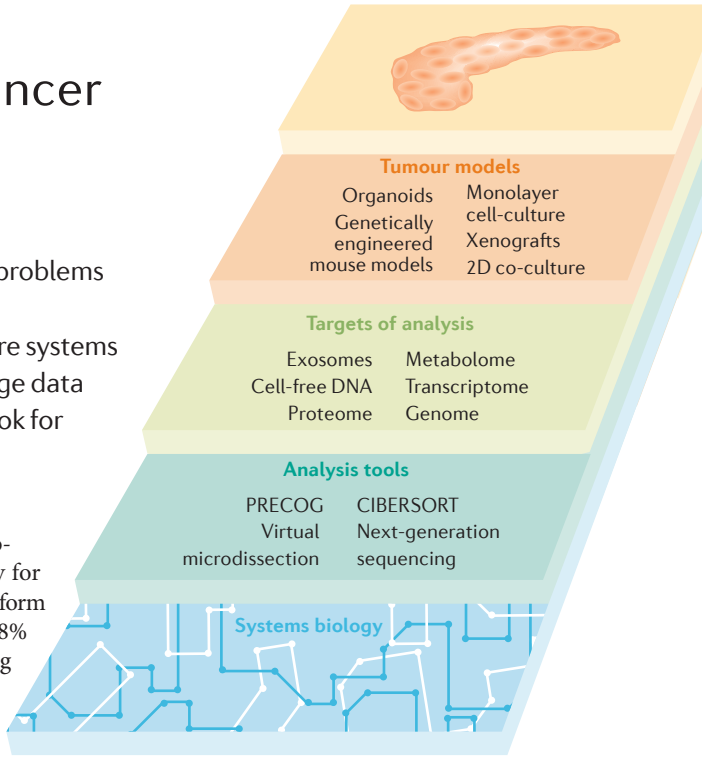

Figure 1 | **Complementary experimental systems can elucidate diagnostic and prognostic biomarkers for pancreatic cancer.** Pancreatic cancer research is multi-layered, with many different approaches possible to understand the disease. An integrated approach, incorporating data from across multiple complex experimental systems, each burdened with limitations if used as a standalone system, will speed up the identification of biomarkers for pancreatic cancer. CIBERSORT, Cell type Identification By Estimating Relative Subsets Of known RNA Transcripts; PRECOG, PREdiction of Clinical Outcomes from Genomic Profiles.

Key advances

- Exosomal glypican-1 is a highly specific and sensitive biomarker of pancreatic cancer diagnosis, and holds promise for early cancer detection, at least in high-risk cohorts[5]
- Next-generation sequencing of cell-free DNA allows identification of tumour-derived mutations without directly sampling the tumour[6]
- Pooling large data sets that include data on tumour gene expression and clinical outcomes aids the identification of cancer-specific signatures that are predictive of disease[7]
- Virtual microdissection of RNA microarray data is feasible and can classify tumour and stroma subtypes[8]
- Organoid models of human and mouse pancreatic cancer recapitulate the key features of the disease more accurately than monolayer cell cultures or patient-derived xenografts[9]

Genomic features of cancer and its microenvironment represent promising candidates for predictive and prognostic biomarkers. During the past decade, emerging high-throughput genomic technologies have generated large amounts of data with apparently inconsistent results, owing to tumour heterogeneity and low individual patient prevalence of distinct mutations. In 2015, Gentles and co-workers have defined a "prognostic landscape of genes and infiltrating immune cells across human cancer". The study authors assembled, curated and integrated cancer gene expression and clinical outcome data into a new resource called PRECOG (PREdiction of Clinical Outcomes from Genomic Profiles), encompassing 30,000 expression profiles from 166 cancers. An association between cancer survival and 22 distinct leukocyte subsets has been revealed by applying a computational approach known as CIBERSORT. CIBERSORT estimates the abundances of cell types from gene expression data to detect and characterize leukocyte contamination in bulk tumour transcriptomes[7]. The prognostic potential of leukocyte signatures has been demonstrated by numerous studies, including the work published this year by Gentles and colleagues. However, concerns remain that heterogeneity of the extent of stromal involvement and the scarcity of tumour cells reduces the value of prognostic biomarkers from whole tumour tissue samples.

To overcome these limitations, Moffitt *et al.*[8] developed a computer-based algorithm to perform virtual microdissection on microarray data from local and metastasized pancreatic cancer tissue, cell lines and normal pancreatic tissue. In line with previous findings, two tumour subtypes were identified, validated and defined as classic and basal-like, with the basal-like subtype associated with poorer clinical outcomes. Furthermore, they delineated two stromal types: normal stroma and activated stroma. The activated stroma type, which was associated with reduced median survival time, was characterized by increased macrophage expression of genes such as *ITGAM*, *CCL13* and *CCL18*, and members of the *SPARC*, *WNT* and *MMP* families. Findings from this study are interesting for several reasons. Firstly, traditional cell lines often used in preclinical studies lack the classic phenotypic subtype, which is far more common in patients than the basal-like subtype. Secondly, tumour subtypes can be recapitulated in patient-derived xenografts, but grafts from patients with an activated stroma subtype are more likely to survive and grow, meaning results have to be interpreted accordingly. Lastly, the heterogeneity between primary tumour and metastasis is markedly lower than expected. Overall, the study suggests that an RNA-derived signature characterizes the tumour better than somatic mutations, which in the past have been advocated for guiding personalized tumour therapy.

A major breakthrough of the past year has been the development of organoid cultures from human and mouse ductal pancreatic cancer[9]. Orthotopic transplantation of pancreatic tumour organoid cultures into syngeneic mice recapitulates the full spectrum of disease progression, forming early and advanced pancreatic intraepithelial neoplasia (PanIN)-like stages and progressing towards invasive and metastatic PDAC. How PDAC organoids form PanIN-like structures still remains to be determined; the organoids might preserve tumour neoplastic cell heterogeneity and recover a variety of stem cell characteristics that reflect the different stages of disease progression. Alternatively, an attractive explanation is that transplanted PDAC organoids retain the cellular plasticity and epigenetic changes that result from organoid culture conditions. Huang and co-workers made use of the advantages of an organoid culture system for drug screening and tested the effect of histone-lysine *N*-methyltransferase EZH2 inhibition on organoids generated from either pluripotent stem cells from KPC mice or from patient-derived tumour cells bearing a *TP53* mutation[10]. The systems developed by Boj *et al.*[9] and Huang *et al.*[10] resemble tractable and transplantable systems to study the genetic, molecular and cellular properties of pancreatic tumour development in mice and humans. One hopes that these systems will speed up the development of personalized approaches to pancreatic cancer therapy.

In summary, although the predominant question in pancreatic cancer research in 2014 was whether stroma was friend or foe, the focus of research in 2015 has been the identification and validation of diagnostic biomarkers. If the findings reported in 2015 can be reproduced in the clinical setting, tests based on glypican-1 hold promise for the early detection of pancreatic cancer, at least in high-risk cohorts. Organoid models of PDAC are an improvement on previous preclinical models for the study of disease pathogenesis and treatment response. In addition, pooling large data sets enables the identification of cancer-specific signatures that are predictive of disease outcome, potentially paving the way to precision medicine. It remains to be seen whether these research efforts will be able to alter the pessimistic projections for the burden of pancreatic cancer.

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Competing interests statement
The authors declare that there are no competing interests.