Introduction

The most recent epidemiological data indicate that pancreatic cancer is the fourth leading cause of cancer death in the Western World (1,2) and it is projected to become the second cause by 2030 (3). This evidence depicts a particular troublesome scenario for National healthcare systems given the high mortality rate, the short life expectancy of affected patients, and the absence of effective therapies for this disease (1,2).

The median survival time for PDAC patients is 5 months and the 5 years overall survival is around 5%; even for patients eligible for resection, which is currently the best therapeutic option, the 5 years survival is no more than 20% (2). The usefulness and availability of chemotherapy is limited: despite the huge efforts of the researchers, there have been no breakthroughs in the development of new chemotherapy agents; since its approval and first application in 1996 the nucleoside analogue gemcitabine is the preferred first choice agent and chemotherapy regimens with proven efficacy in other malignancies have substantially failed in clinical trials or gave only limited incremental results (4,5). Nevertheless, compared to previous treatment with Erlotinib, an epidermal growth factor receptor inhibitor, two novel therapeutic regimens, Folfirinox and nab-plactitaxel, have meet limited but significant success (6,7). There are not clear reason has to why these regimen are more effective: for Nab-plactitaxel it has been proposed that it may target the tumor stroma and hence facilitate the delivery of gemcitabine [similar in principle to the targeting of the Hedgehog signaling (8,9)]. Instead Folfirinox is a combination of four drugs (oxaliplatin, 5-FU, folinic acid and irinotecan) and improves the survival to 11 months compared to gemcitabine alone but it is associated to severe toxic effects (7). What is emerging from this evidence is that for reasons unknown some group of patients may take advantage of different therapies: the different responses might be associated to the presence of specific mutations whose analysis and characterization could help defining novel therapies or better target the currently used ones.

PDAC is characterized by a mutational landscape ranging from single point mutations to gross chromosomal alterations. Despite the number of mutations a core set of altered pathways can be identified in TGFβ signaling, DNA repair and remodeling, axon guidance and cell cycle regulation (10-13). An average of 63 mutations are present in each PDAC, but the most frequently mutated genes are the small GTPase Kirsten RAS (KRAS), TP53, CDKN2A and SMAD4 (10,11). More than 90% of PDAC harbor mutations in KRAS, whereas mutation in TP53, CDKN2A and SMAD4 are present in more than 50% and up to 90% of patients; besides these high frequency mutations several other genes or pathways are altered at very low frequency (around 10% or even less).

Nonetheless, given the high frequency of low prevalence mutations, PDAC is a very heterogeneous tumor and not surprisingly a challenge for unselected treatment. For some time KRAS was seen as a reasonable target but after thirty years of failed attempts (14,15), most researchers consider this small GTPase undruggable, prompting the need to the identification of new targets.

The technical development of genomic, karyotyping, exome and methylation analyses, along with gene expression profiles, could help in the rationalization of the therapy and therapeutic targeting. Indeed, a combination of these approaches has allowed the identification of subtypes of...
tumors that could better respond to specific therapies, specifically in lung, breast and colon cancers (16-19). The main hindrance for the genomic analysis of the mutations and its correlation with the clinical outcome in PDAC has always been the paucity and availability of primary samples together with relevant clinical informations. In 2011, Collisson et al. (5) combining their sample collection with the one described in (20) proposed a classification based on the gene signature of PDAC in the following three categories: classical, quasi-mesenchymal and exocrine-like. As their name suggests, the expression of mesenchymal genes is the specific gene signature of quasi-mesenchymal PDAC, whereas expression of epithelial and adhesion genes or digestive enzymes are hallmark of classical and exocrine-like tumors, respectively. What is most interesting is that there was no correlation between tumor subtypes and tumor stage, as opposed to tumor grade but more importantly the subtypes where independent prognostic factors with classical tumors faring better than quasi-mesenchymal and exocrine-like. Gene expression profile allowed the identification of a specific high expression of the transcription factor GATA6 and of KRAS and the KRAS dependency for tumor progression in classical tumors. The authors hypothesized that subtypes could be associated to different responses to the therapy: they identified human and mouse cell lines representative of classical and quasi-mesenchymal tumors (but not of exocrine-like genotype) demonstrating that quasi-mesenchymal were more sensitive to gemcitabine compared to classical that were Erlotinib-sensitive.

In 2015, two different papers have been published that focus on different components of PDAC (21,22). A comprehensive genomic analysis of around 100 PDAC samples was performed and published in Nature by Waddell et al. (21). Performing a deep whole genome sequence analysis they demonstrated that genomic variation in the structure of chromosomes is an important mechanism in PDAC development. The results of Waddell validated and confirmed the role of the previously mentioned genes and gene pathways (such as Wnt) but also identified two genes that weren’t described in PDAC (KDM6A and PREX2) that are mutated in medulloblastoma and melanoma, respectively (21,23). Waddel proposed a classification of PDAC in four groups: stable, locally rearranged, scattered, and unstable. Unstable subtype is described as a tumor having more than 200 and up to 558 structural variation events; genomic analysis demonstrated an association of BRCA mutational signature, BRCA1, BRCA2 and PALPB and the unstable subtype. With extensive follow-up clinical informations regarding the chemotherapy regimen after tumor relapse the researchers suggested that genomic instability and BRCA signature might be a indirect measure of susceptibility to platinum based therapies, such as Folfirinox, and potentially to PARP inhibitors who are supposed to target similar mechanisms of DNA repair.

One of the key feature of PDAC is the presence of a desmoplastic reaction caused by the excessive production of extracellular matrix mainly by fibroblasts and activated pancreatic stellate cells (24). The desmoplastic reaction is believed to be important in PDAC chemoresistance, as demonstrated by a seminal work published in 2009 (8). Component of the extracellular matrix and not epithelial cancer cells constitute the bulk of PDAC: consequently, proper cancer cells often are only a minimal fraction of the totaling tumor, a characteristic that may limit genome analyses. To overcome this issue and find specific stromal and tumor signature Moffitt et al. (22) performed a virtual microdissection of tumor specimens by blind source separation of gene expression microarray data from primary tumor, metastatic and normal samples. Following the array analysis a classification of PDAC in “classical” and “basal-like” tumors and “activated” and “normal” tumor stroma was proposed. Activated stroma tumors express at high levels fibroblast markers such as fibroblast activation protein (FAP) and collagen at had a worst outcome, underlying the importance of tumor stroma in tumor progression. Tumors belonging to the basal like group derive their name from the expression of laminins and keratins and to similarity with breast and bladder basal tumors (22); basal-like patients had a lower survival time compared to classical subtypes. Stromal and epithelial subtype classification are independent and both classical and basal-like tumors were found in normal and stromal-activated subtypes. Survival analysis demonstrated a cumulative effect with basal-like and stromal activated having the greatest hazard ratio and the classical and normal stroma having the lowest. Nonetheless, basal-like subtypes showed a better response to adjuvant therapy.

Very recently a new paper has been published by the journal Nature in the 2016 March issue (25). This paper come from the same international group responsible for the paper published in Nature in 2015 (21) which proposed the classification of tumors in term of genomic events in stable, locally rearranged, scattered, and unstable. The authors return to the analysis of pancreatic cancer samples combining whole genome and deep-exome...
sequencing of an outstanding collection of 456 pancreatic cancers. Tested samples were acquired through Australian Pancreatic Cancer Genome Initiative (APGI) as part of the International Cancer Genome Consortium (ICGC); 74 were previously published pancreatic cancers exomes. The APGI samples were from treatment-naive resected tumors representing PDAC, PDAC variants (e.g., adenosquamous carcinoma) and rare acinar cell carcinomas.

The mutational landscape of this cohort substantially validates the involvement of already known genes and gene pathways (e.g., KRAS, TGFβ and Wnt pathways, G1/S checkpoints, DNA repair such as BRCA, KDM6A for histone modification). Fifty gain and 73 of loss in chromosomes regions spanning known oncogenes and tumor suppressors were also identified and BRCA deficiency and DNA deamination among others where re-affirmed as the essential mutational driving forces in PDAC.

Focusing on a restricted group of 96 tumors with an epithelial content higher than 40% the authors proposed a novel classification of pancreatic cancer based on gene expression profile. In this new system tumors are classified in: squamous; pancreatic progenitor; immunogenic; and aberrantly differentiated endocrine exocrine, or ADEX. The presence of the subtypes was then confirmed in 232 tumors with different contribution of stromal tissue (cellularity ranging from 1% to 100%). Interestingly, squamous subtype patients have a lower cumulative survival whereas the other three subtypes do not substantially differ.

The differences among classes is restricted to the expression of 10 gene programs: squamous cancers are characteristically enriched in inflammation, ECM, hypoxia response, TGFβ and metabolic gene programs; progenitor tumor in gene networks depending of transcription factor and nuclear receptors important in pancreas development and differentiation such as PDX1 or HNF4A; ADEX class can be considered a subclass of the progenitor class with enrichment of transcription factors implicated in the late phases of pancreas development such as NR5A2, that could either inhibits or facilitates tumor development (4); finally, the immunogenic class is enriched in gene networks characteristically associated to B cell signaling, CD4+ and CD8+ T cell, and antigen presentation and it is probably the more interesting group for future therapeutic choices.

Indeed the newly proposed classification is nearly in agreement with the Collisson’s (5) and partially with Moffitt’s (22), who already only partially overlapped with Collisson due to a mixed signature (stromal and basal-like of Moffitt) in the Collisson’s quasi-mesenchymal type. Specifically, the classical subtype of Collisson overlap with the progenitor, the quasi-mesenchymal with the squamous, the exocrine-like with the ADEX. Approximately 50% of squamous tumors are instead Moffitt’s basal-like. No clear association of the new classification with the one described in (21) is made, although the Authors did perform a structural variant analysis, but this may be explained on the focus on transcriptomic classification. Moreover, although experimental data could be obtained from the published literature, the paper is missing an in vivo demonstration of the consequences of the identified genomic modifications in defining the tumors types, with the notable exception of squamous tumors, where the Authors analyzed the contribution of TP53 and TAp63.

A very interesting topic addressed by Bailey is the role of the immune system in tumor progression. They identify three different genetic programs (GP6, GP7 and GP8) in the immunogenic class that are associated with difference in survival. Specifically, whereas there is no difference regarding GP8, a high module eigengene (ME) value in GP7 is associated with a shorter survival, whereas high ME GP6 correlates with longer survival. Intriguingly, GP6 is associated with B and T cell signatures whereas GP7 is enriched for Toll-like receptor, antigen processing and presenting genes, and generally with inflammation and macrophage immune suppression.

Conclusions

PDAC (and its variants) is a difficult to treat disease for a combination of causes ranging from late diagnosis, rapid metastatization, high chemoresistance; the latter could be a consequence of the tumor desmoplastic reaction that could reduce the delivery of drugs and at the same time transmit pro-survival signals. There are limited therapeutic strategies for PDAC treatment, with the best hope still residing in surgical resection, that nonetheless may be performed in a limited number of patients. The advancement and the increasing sophistication of genomic studies are providing novel insights in pancreatic cancer development and may help in the identification of new targets and in the definition of patients subgroups that could respond to specific therapies; to achieve these results the collaboration among international research teams is a necessity, especially in pancreatic cancer research. The recent work published in Nature (25) could be an important step toward the definition of new therapeutic groups. Conversely to previous papers the Authors do not report direct evidence
of subgroup association with chemotherapy regimens but the overlap of the new classification with Collisson and Moffitt (5,22) allows a bona fide extension of their findings to the new subgroups; moreover the switch towards the contribution of the non tumor tissue to the patients survival is an interesting change of view that follow the increasing focus of cancer research on the stromal compartment. The identification of an “immunogenic” type of PDAC could potentially open PDAC therapy to the application of immunomodulating agents aimed at increasing the immune response and reduce or avoid the tumor immune-surveillance escape.

The amount of information deriving from more and more complex genetic studies certainly is a glimmer of hope to find novel therapies in pancreatic cancer, nonetheless whether these new classifications will be useful as a predictor of therapeutic response and in the process of therapeutic decision still remain to be tested in clinical trials.

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References


