Editorial

# Mutational and transcriptome based sub-classification of pancreatic cancer: are we there yet?

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With a 5-year survival rate of ~8% (1) and limited therapeutic options, pancreatic cancer takes an enormous economic, financial and emotional toll on the patients and the caregivers alike. Resistance to standard cytotoxic chemotherapies, which have been the game changer in many diseases including breast cancer, makes treatment of pancreatic cancer an enormous challenge. Efforts at targeted therapies in this disease have met with failure, possibly due to incomplete understanding of its pathogenesis (2). Moreover, unique stromal biology poses additional challenge in design and delivery of standard as well as targeted therapies. To change the course of this difficult disease, a detailed understanding of the pathogenesis, mutational landscape, stromal biology, immune microenvironment and recurrence mechanisms is needed. In such a background, the genomic analysis done by Bailey *et al.* (3) provides an important insight into the molecular biology and taxonomy of this tumor and has huge translational importance.

The study by Bailey *et al.* needs to be reviewed in light of previous efforts by multiple groups to better understand the mutational landscape of pancreatic cancer. The first large scale effort to define the mutational landscape of pancreatic cancer was made by Jones *et al.* (4). In that study the authors sequenced the protein coding exons and used SNP microarrays to define the mutations and copy-number alterations in all protein coding genes. In the discovery phase of their study, the authors focused on pancreatic adenocarcinoma specimen from 24 patients. While most of the study tissue was obtained from resected pancreatic cancer, 7 of the 24 samples used in this study were obtained from pancreatic cancer metastases. In this study the cell lines or patient derived xenografts were used to address the issue of contamination by non-neoplastic cells, thus facilitating the detection of the mutations. Comparison of the tumor DNA sequence to the normal DNA from the same patient was done to ensure if the observed mutations were somatic. The authors observed on an average 48 mutations in each pancreatic cancer specimen. The mutations identified in this study were observed to be part of 12 core set of pathways and processes. While the pathway components which were altered in any individual tumor varied widely, most of these pathways were affected in each tumor. The variety of the pathway components affected in different patients and the diversity of the mutation in a specific gene partially explained the heterogeneity of the tumors.

To further characterize the pancreatic cancer genome, Biankin *et al.* (5) performed hybrid-selection-based capture and sequencing of whole exome from pancreatic tumors and matched it with normal DNA derived from 142 consecutive patients with primary operable, untreated PDAC who underwent pancreatectomy. Authors enriched for tumor epithelial content by performing full face frozen sectioning and macrodissection. The cellularity of the samples was further estimated through deep mutant KRAS sequencing and SNP array-based cellularity estimate using a novel algorithm and this was used to predict the relative sensitivity of mutation detection for a given sample before sequencing. In this study the average number of mutations detected per patient was 26 (range, 1–116). Between this study and that by Jones *et al.*, there was only 48% overlap in the mutations that were observed in more than one patient and 19% overlap in all the observed mutations. Considering

Jones *et al*. and Biankin *et al*. together, *KRAS* (96% of all patients), *TP53* (41% of all patients), *SMAD4* (20% of all patients) are some of the most common mutations and all other mutations in pancreatic cancer are present in <10% of the patients (mostly <5% of patients). Biankin *et al*. also used data from two independent sleeping beauty transposon mutagenesis screens in *KRAS* transgenic mouse models of PDAC and from an *in vitro* short hairpin RNA screen to infer functional consequence for the individual genomic events and pathways identified in their study, thus separating driver from passenger mutations.

Recently Waddell *et al*. (6) went one step further and performed whole-genome sequencing which allowed, besides analysis of mutational landscape, analysis of structural rearrangements as well. This is important as somatic structural rearrangement of chromosome is capable of gene disruption, gene inactivation and formation of novel oncogenic gene products by gene fusion. Whole genome sequencing was performed on 100 primary PDAC samples with an epithelial cellularity of ≥40%. The authors discovered on an average 119 structural variants per individual. Combining structural variation events with deleterious point mutations increased the prevalence of inactivation events for *TP53* to 74%, *SMAD4* to 31% and *CDKN2A* to 35%. Two additional genes, *KDM6A* and *PREX2* were found to have mutations and structural variants at a rate of 18% and 10% respectively. The structural rearrangement data was used to classify the tumors into 4 major subtypes: (I) stable subtype (20% of patients in this study) where the genome contained ≤50 structural variation events; (II) locally rearranged subtype (30% of patients in this study) which demonstrated significant focal event on one or two chromosomes; (III) scattered subtype (36% of patients in this study) which demonstrated moderate range (>50 but ≤200 structural events); and (IV) the unstable subtype (14% of all tumors in this study). Interestingly, the authors observed that there was a relationship between the unstable subtype and mutations in the genes associated with *BRCA* pathway as well as a recently described mutational signature associated with mutations in *BRCA1* and *BRCA2*. The authors observed that patients with unstable subtype preferentially had remarkable response to platinum based therapy, thus underscoring the predictive ability of the genomic medicine.

In the study by Bailey *et al*., authors building upon their previous work (6) performed high coverage whole genome and deep exome sequencing of treatment naive resected pancreatic cancer. To date, this is the largest study evaluating the whole exome data in patients with pancreatic cancer. A total of 382 samples from Australian Pancreatic Cancer Genome Initiative (APGI) were pooled with a previously published exome data (n=74) to provide a total sample size of 456 pancreatic ductal adenocarcinomas. A total of 32 significantly mutated genes were identified which aggregated in 10 molecular pathways. In the pool of 456 pancreatic cancer samples, the significant mutations affected *KRAS* pathway in 92%, G1/S checkpoint machinery in 78%; TGF-β signaling in 46%, histone modification pathways in 24%, and SWItch/Sucrose Non Fermentable complex in 14% of all samples. The BRCA pathway was affected in 17%, WNT signaling was affected in 5% and RNA processing genes were affected in 16% of the patients. The authors also confirmed previous findings that DNA deamination, ectopic APOBEC activity, BRCA-deficiency and mismatch repair were the predominant mechanisms of the mutations.

Another unique feature of this study is that the authors used a combination of transcriptome and microarray analysis of these tumors to resolve pancreatic cancer into 4 subtypes and correlated these with histopathology and prognosis. This analysis identified 26 coordinately expressed gene programs, out of which 10 classified the pancreatic tumors into 4 subtypes. The analysis clustered tumors into squamous, pancreatic progenitor (PP), aberrantly differentiated endocrine exocrine (ADEX) and immunogenic subtypes with uniquely associated histopathology. Squamous subtype was associated with high TP63ΔN expression and mutations in *TP53* and *KDM6A*. In this subtype, hypermethylation and downregulation of genes that govern pancreatic endodermal cell-fate determination led to complete loss of endodermal identity. PP subtype was associated with overexpression of transcriptional networks which are important for the pancreatic endoderm cell-fate determination like PDX1, MNX1, FOXA2 and HES1. IPMN-associated PDAC and TGFBR2 inactivating mutations clustered in this subtype. ADEX subtype is characterized by expression of transcriptional networks that are important in later stages of pancreatic development and differentiation, including those that are important for both exocrine and endocrine lineages. Importantly, many of the human-derived cell lines were found to be of this subtype. The immunogenic subtype was the unique subtype defined for the first time by this study. While this subgroup shared many of the transcriptional networks with the PP subtype, it was unique in terms of upregulated expression of genes associated with immune cells. The authors correlated these

 

subtypes with prognosis and observed that the patients with squamous subtype had the worst prognosis. Even though up to 35 IPMN associated cancer clustered with PP subtype, PP subtype did not have better prognosis than other non-squamous subtypes. This is intriguing as IPMN associated pancreatic cancer tends to have better prognosis than the classical pancreatic adenocarcinoma. Furthermore, it is peculiar that the only mutation-subtype correlation observed in this study was that of squamous subtype associating with mutation in *TP53* and *KDM6A* and *TGFBR2* inactivating mutations clustering with PP subtype.

The study by Bailey *et al*. is not the first effort at sub-classifying pancreatic cancer. Few earlier studies have developed classification systems to better define the pathogenesis and not only help with prognostication but also with prediction of response to various therapies. Collisson *et al*. (7) studied the transcription profile of resected PDAC samples after microdissection and pooled that with a previously published database. Based on the expression profile the authors identified 62-gene signature, which was designated as PDAssigner, and this gene signature was used to classify the pancreatic cancer into three subtypes: classical, quasi mesenchymal and exocrine-like. Classical subtype had high expression of adhesion associated and epithelial genes and was highly enriched in GATA6 gene signature. Quasi mesenchymal subtype had high expression of mesenchymal genes, whereas the exocrine-like subtype showed relatively high expression of tumor-cell derived digestive enzyme genes. This gene signature not only prognosticated patients, where patients with classical subtype had the best prognosis, it also helped predict response to therapies. The response of pancreatic cancer cell lines to gemcitabine and erlotinib was evaluated and correlated with the subtype (defined based on the 62 gene signature) and it was observed that the cell lines with classical subtype were more sensitive to erlotinib whereas the quasi mesenchymal subtype was more sensitive to gemcitabine. Intriguingly, the authors also observed that the classification could predict the oncogenic *KRAS* addiction where the classical subtype pancreatic cancer cell lines were more dependent on *KRAS* then the quasi mesenchymal subtype. Interestingly, Bailey *et al*. compared their classification system with that of Collison *et al*. and used the 62 gene signature to re-classify their patient data, and observed that the quasi mesenchymal, classical, Exocrine-like subtype corresponded to Squamous, Pancreatic Progenitor and ADEX subtypes respectively. Of note, in the study by Collison *et al*., the Quasi-mesenchymal subtype had worse prognosis when compared to the other subtypes, similar to its corresponding squamous subtype in Bailey *et al*. Collison *et al*. do not define an immunogenic subtype, possibly because they only focused on the cancer cell transcriptome analysis and excluded the transcriptional networks of surrounding stroma and immune cells, a strategy which led to elucidation of immunogenic subtype in the study by Bailey *et al*.

The transcriptome analysis by Bailey *et al*. was done on whole tumor samples and no effort was made to identify which gene expression data was contributed by tumor cells *vs*. the tumor microenvironment. This approach offers a distinct advantage as it is a departure from old strategy where the focus was on the cancer cell and tumor microenvironment was ignored. This strategy led to the identification of the immunogenic subtype, which has not been observed in previous analyses. However, this strategy is not without its shortcomings, and is especially highlighted by the study by Moffitt *et al*. In the study by Moffitt *et al*. (8), the authors used non-negative matrix factorization (NMF) to digitally dissect the transcriptome of each sample into stroma, tumor and normal tissue and then the molecular signature of each component was characterized. The authors observed that the gene expression of the stromal component in various tumors aggregated into two distinct subtypes (I) normal and (II) activated stroma and patients with the latter had worse outcomes. The stromal origin of this signature was clear from the observation that cancer cell lines did not express it and that metastatic samples express it only at low levels. Furthermore, cancer associated fibroblasts overexpressed this stromal signature. When focusing on the transcriptome of the tumor component, Moffitt *et al*. observed that the gene expression aggregated into two distinct profiles, namely classical and basal-like. This classification correlated with the prognosis where the basal-like tumors had worse median survival as compared to that of classical subtype. The authors found an association of their classification system of tumor transcriptome with the KRAS mutation subtype as well as with the response to adjuvant therapy. However, as pointed out in a critique of this study (9) , this strategy of digitally subtracting the transcriptome of normal tissue to understand the contribution from the tumor and stromal component is also fraught with challenges. Studies suggest that pancreatic cancer arises from acinar cells (10) and thus, normal acinar cell gene expression, while still being present in cancer cells, may be excluded by this approach.

These studies, including the one from Bailey *et al*., have

 

raised a very important concern. Are we using valid models to study pancreatic cancer? In the study by Moffitt *et al*., all the cell lines evaluated by the authors corresponded to basal subtype and no cell line represented the classical subtype. In a collection of 19 human derived and 15 mouse derived pancreatic cancer cell lines, Collisson *et al*. did not find any cell line which represented the exocrine-like subtype. Similarly, Bailey *et al*., observed that several cell lines represented the ADEX subtype of PDAC. Cell lines are the most commonly used model of pancreatic cancer for studying the pathophysiology as well as for studying therapeutic evaluation. That all the subtypes of pancreatic cancer are not represented in the cell line repertoire is concerning. Furthermore, 80% of the patients with pancreatic cancer present with locally advanced unresectable or metastatic disease. In the absence of adequate tissue available from surgical resection from these patients, most of the studies include only patients with resected pancreatic cancer. E.g., in the studies by Biankin *et al*. and by Waddell *et al*. primarily resected pancreatic cancer samples were included and in the study by Jones *et al*., only a minority of the samples were from metastatic lesions. It is not implausible that patients who present with advanced un-resectable and metastatic disease have a different set of mutations which reflect either emergence of novel mutations during later stage of the disease or even a different disease altogether. Efforts at proper representation of this advanced stage of disease is important.

What kind of future does precision medicine hold for pancreatic cancer? Genomic medicine has transformed the treatment of many cancers. Discovery of ALK rearrangements in lung cancer has opened doors for a highly effective ALK inhibitor therapy for a small but significant proportion of patients with this disease. Development of immunotherapy has led to durable responses in a small but significant proportion of patients with melanoma. Imatinib for patients with Gastrointestinal Stromal tumors is another example of success of genomic medicine. Hope is that better understanding of the mutational and transcriptional landscape of pancreatic cancer will lead to development of novel therapies and improved outcomes for these unfortunate patients. However, till date, patients with pancreatic cancer have only few actionable mutations as most of the mutations do not have targeting drugs. As a starting point it is imperative that most, if not all, of the patients with pancreatic cancer get their tumor sequenced. This will lead to the generation of a database with mutation-outcome correlation as well as to data regarding impact of mutational status on response to current therapies. Next, there is a need for smaller trials with patients inducted based on specific biomarker level or mutational profile. E.g., patients with squamous subtype or with certain mutations will be enrolled in small trials of standard of care, with and without targeted therapies. This data will also help in the selection of first line of therapy. E.g., mutation in DNA repair pathway or unstable subtype of Waddell *et al*. may suggest better response to platinum based regimens. Also, cell lines, organoids or patient derived xenografts need to be generated from patient specimens and their ability to predict response in clinical situations has to be evaluated. Lastly, the genomic revolution is not an end in itself, but is means to an end. There is still a place for decades old strategy of evaluating the biological significance of various mutations, proteins and pathways identified through genomic, proteomic and transcriptomic analyses respectively. Personalized medicine will be an important culmination of this era of easily accessible and less expensive '-omics' analyses. And the extensive work done by Bailey *et al*. brings us one step closer to achieving this hallowed goal.

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