GENOMICS OF PANCREATIC TUMOURS
- WHAT WE NOW KNOW

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Abstract

Each year, over 2500 patients in Australia are diagnosed with pancreatic cancer. Pancreatic cancer is one of the most lethal tumour types with a five year survival of just 5%, thus there is a need to find alternative approaches to treatment. In recent years, the application of next generation sequencing has revealed the complex genomic landscape of pancreatic cancer, uncovering the mutation processes that occur during tumour development and has begun to identify new or repurposed therapeutic opportunities for pancreatic cancer patients. The identification of targets for therapy is a crucial goal of the large next generation sequencing studies as we move into an era of targeted or personalised medicine, where drugs will be selected based on the characteristics of a patient’s tumour. Due to the large degree of heterogeneity in pancreatic cancer, a personalised approach to treatment seems particularly warranted. This review will summarise some of the key findings from genome sequencing of pancreatic cancer, describing the major driver genes and perturbed pathways, and highlighting some of the new potential and promising therapeutic opportunities that have been uncovered.

Cancer is a genetic disease caused by mutations that accumulate within the DNA sequence of cells. Cells that are normally functioning have repair mechanisms that detect and repair DNA mutations. If however, mutations occur in key regions within the genome, they can disrupt the failsafe repair and checkpoint regulatory system and may enable the mutated cells to grow uncontrollably, resulting in cancer. Mutations affecting genes that confer regulatory or growth advantages are positively selected in tissues to promote tumorigenesis and are referred as ‘driver’ mutations.1 The dysregulated cellular system also permits other mutations that do not contribute to tumorigenesis to escape repair. These are referred to as passenger mutations as they are carried along in the clonal expansion of cancer cells.

In recent years, next generation sequencing has made it feasible to generate genome wide catalogues of the DNA mutations present in individual cancers. Two large sequencing initiatives were established to use this technology to sequence thousands of tumour samples from many different cancer types - The Cancer Genome Atlas (www.cancergenome.nih.gov) and the International Cancer Genome Consortium (www.icgc.org).2 The main goals of these consortia include cataloguing of commonly mutated genes and disrupted cellular mechanisms that might be ‘drivers’ of cancer, and identification of actionable markers for therapy in the hope of improving therapy selection and patient outcome. Such genomic studies have revealed the molecular basis underlying pancreatic cancer, identified molecular subtypes and discovered potential therapeutic opportunity in repurposing treatments.

Need for molecular profiling of pancreatic cancer

The term ‘pancreatic cancer’ describes several tumour types histologically classified on the tissue structures from which they arise. Individual or up to hundreds of samples from the most common pancreatic tumour types have been subjected to exome or whole genome sequencing, however there remain rare subtypes for which no genomic data has been generated. For the tumour types with genomic data, the studies have confirmed and identified key driver genes which are frequently mutated within each tumour subgroup (table 1). The genomic findings have reinforced that pancreatic cancer is a heterogeneous disease comprised of many distinct tumour subgroups.3 In addition, the degree of heterogeneity between tumours from the same tumour subgroup is high, as tumours of the same subgroup may harbour a different repertoire of
mutations and few genes are mutated at high frequency within sample cohorts. Instead, many recurrently mutated genes are present only at low frequency. This large number of genes mutated at a low frequency impacts on the ability to robustly identify driver mutations, genes or pathway events. One solution is to increase the number of samples studied to increase the power of detection, however this may also increase the confounding passenger mutation signal. Another is that improvements are being made to next generation sequencing analysis methods which enable integrated multiple data types to pinpoint key tumour promoting pathways.

**Frequent somatically mutated genes**

There are four genes which are frequently mutated in PDAC (mutated in >30% of samples), and many more genes which are less frequently seen but still significantly mutated (mutated at a higher frequency than by chance alone) which affect common pathways and thus are likely to be driving the disease (figure 1). The most frequently mutated gene is the KRAS oncogene. The KRAS protein is involved in RAS signalling and cellular growth through the MAPK and PIK3CA pathways and is mutated in >90% of PDAC cases.\(^4\)\(^-\)\(^7\) Mutations in the KRAS gene are clustered in hotspots and result in activation changes at codon 12 (92% of KRAS mutated cases) and less frequently at codon 13 and 61 (~8% of KRAS mutated cases).\(^5\)\(^-\)\(^8\) The type of KRAS mutation may have clinical significance, as patients with codon 61 mutations have been shown to have a more favourable outcome to patients with other KRAS mutations.\(^8\) The remaining three genes mutated at a high frequency are tumour suppressor genes and frequently harbour mutations combined with a loss of heterozygosity in PDAC. TP53 is an important regulator of cell response to DNA damage and is mutated in >70% of patients. CDKN2A is a cell cycle regulation gene and SMAD4 is involved in TGF-β signalling. Both are mutated in >30% of samples.\(^4\)\(^-\)\(^7\)

**Table 1: Frequently mutated genes in pancreatic cancer tumour subgroups detected by sequencing.**

<table>
<thead>
<tr>
<th>Pancreatic tumour type</th>
<th>Driver gene</th>
<th>Approximate proportion of mutated samples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal adenocarcinoma</td>
<td>KRAS</td>
<td>&gt;90</td>
<td>5-8</td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td>74-86</td>
<td>5,7</td>
</tr>
<tr>
<td></td>
<td>SMAD4</td>
<td>36-43</td>
<td>5,7,8</td>
</tr>
<tr>
<td></td>
<td>CDKN2A</td>
<td>30-41</td>
<td>5,7,8</td>
</tr>
<tr>
<td>Intraductal papillary-mucinous neoplasm (IPMN)</td>
<td>KRAS</td>
<td>62-74</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>GNAS</td>
<td>40-61</td>
<td>9,10</td>
</tr>
<tr>
<td>Acinar cell carcinoma</td>
<td>TP53</td>
<td>13-31</td>
<td>11</td>
</tr>
<tr>
<td>Pancreatic neuroendocrine tumours</td>
<td>MEN1</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>DAXX/ATRX</td>
<td>43</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MTOR pathway genes</td>
<td>15</td>
<td>12</td>
</tr>
</tbody>
</table>

**Genome studies**

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent tumour type which accounts for approximately 90% of all pancreatic tumours,\(^13\) and has been the most comprehensively studied by genome sequencing. Genome studies were initially performed by amplicon exon sequencing of small numbers of PDAC cell lines and patient derived xenograft mouse models (n=24),\(^5\) or cell lines (n=15).\(^4\) Subsequently, exome sequencing was performed on large cohorts of patient tumour samples (n=99),\(^6\) and micro-dissected tumours (n=109).\(^6\) These exome studies identified point mutations and small indels, but had limited ability to detect chromosome structural rearrangement, an alternative mechanism of gene mutation which can result in inactivation of tumour suppressors by gene breakage or activation of oncogenes by amplification of dysregulation. The genome wide view of large rearrangements can be detected by whole genome sequencing, therefore the International Cancer Genome Consortium pancreatic project recently employed whole genome sequencing to survey the complete repertoire of somatic mutations in PDAC.\(^7\) To optimally determine the drivers of PDAC, a recent meta-analysis of available genome data was conducted (Bailey et al, in press Nature). Together, these studies have iteratively identified the genes and pathways which are recurrently mutated in PDAC.
In addition to these four key driver genes, there are many genes mutated at low frequency which are also likely drivers of disease. These include the ARID1A gene, which is involved in chromatin modelling and was identified as recurrently mutated in different samples, and detected in multiple studies.\(^5,6\) Other genes have also been detected by more than one study including TGFB2,\(^4,6\) MLL3 and SF3B\(^1,5,6\) and ROBO\(^2,6,7\) while many more genes have been identified as significantly mutated in a small percentage of samples. This so called ‘long tail’ of uncommon, but recurrently mutated genes, highlights the between patient tumour heterogeneity and indicates that more samples will need to be characterised to identify all drivers of pancreatic cancer.

**Frequently perturbed pathways**

The large numbers of genes that are recurrently mutated suggest that PDACs are highly heterogeneous with multiple pathways affected by mutation. The first study which systematically sequenced the exons of thousands of genes in pancreatic cancer described 12 core signalling pathways as driving PDAC,\(^5\) and included pathways whose dysregulation has been described as a hallmark of many other cancer types.\(^14,15\) The PDAC core signalling pathways have subsequently been refined as more genome data has been generated. The axon guidance pathway, particularly SLIT/ROBO signalling, was later identified as frequently mutated,\(^6\) and subsequently shown to be frequently methylated in PDAC.\(^16\) The chromatin modelling SWI/SNF pathway was also identified in 42% of cases and frequently involves mutations in ARID1A which occur in 15% of cases and has been associated with a poor outcome.\(^8\) A summary of the genes and pathways perturbed in PDAC is shown in figure 1.

**Mutational signatures**

Cancer genomes may carry tens to thousands of somatic mutations. A handful of these mutations will have a functional consequence and act as drivers of disease, while the vast majority are passenger or bystander mutations which have little functional consequence. However, all the mutations (driver and passenger) can reveal information about the aetiology of each tumour. Genomic mutations range from single base substitutions to large chromosomal structural rearrangements and the pattern or distribution of these mutations can reveal important insights into how the tumours arose. In particular, the sequence context of single base substitutions can be used to identify the underlying mutational processes or signatures which have occurred in tumour development.\(^17,18\) For many signatures the cause is unknown, while others are associated with mutagenic exposure e.g. tobacco smoking, ultra-violet light and defective DNA damage repair.\(^17,18\)

In PDAC, at least six mutation signatures have been described (see [http://cancer.sanger.ac.uk/cosmic/signatures](http://cancer.sanger.ac.uk/cosmic/signatures)). These include two signatures that are present in most PDACs and are ubiquitously expressed in many other tumour types, one of which is associated with ‘ageing’ or deamination of 5-methylcytosine, while the cause of the other signature is not known. Other mutation signatures detected include two signatures thought to be caused by the AID/APOBEC family, however...
the precise mechanism resulting in the AID/APOBEC signature in PDAC is not known. The remaining two signatures are linked with defects in DNA damage repair. The first is termed the ‘BRCA signature’, and is associated with a defective homologous recombination DNA repair pathway.7,19,20 Tumours which contain a high proportion of mutations classified within the profile of the ‘BRCA signature’ are present in approximately 14% of PDAC samples. These tumours are also associated with unstable tumour genomes which contain a high number of rearrangements.7 In PDAC, many of the tumours with a high proportion of ‘BRCA signature’ mutations contain pathogenic germline variants or somatic mutations in key genes involved in homologous recombination, including BRCA1, BRCA2, ATM and PALB2.7 However, for some ‘BRCA signature’ high tumours, the genes or processes driving this signature have yet to be identified. The second DNA damage repair signature correlates with a mismatch repair (MMR) deficiency and has been identified in microsatellite unstable tumours which are associated with a hypermutation phenotype.17 Microsatellite instability and the underlying MMR defects have been shown to occur occasionally in PDAC (<10% cases),21 and PDAC tumours with a high mutation rate have been associated with loss of the mismatch repair gene MLH1.4 However, to better understand the prevalence of MMR in PDAC and determine what is driving the MMR signature, a systematic screen of a large cohort of PDAC is required.

Patterns of rearrangements reveal genomic subtypes

The patterns of large chromosomal rearrangements have been used to classify PDAC into four molecular subtypes,7 which are termed as ‘stable’, ‘scattered’, ‘locally rearranged’ and ‘unstable’. Similar groups have also been described in other tumour types, including oesophageal and ovarian cancer.20,22 The stable subtype contains few genomic rearrangements and comprises 20% of PDAC samples. The scattered subtype contains a modest number of chromosome rearrangements distributed throughout the genome and comprises 36% of samples. In contrast, the locally rearranged PDACs contain focal clusters of breakpoints on one or few chromosomes resulting in amplification of several oncogenes (30% of samples). The genomic location of the focal amplifications affects a variety of candidate PDAC oncogenes, each amplified in a small subset of patients, suggesting that the locally rearranged PDACs are promiscuous in their selection of oncogenes. The candidate oncogenes include the ERBB2 gene which encodes the HER2 oncoprotein, FGFR1, MET, CDK6, PIK3R3 and PIK3CA. The unstable subtype contains many structural rearrangements (>200) distributed throughout the genome.7 The unstable genomes are associated with a high number of mutations contributing to the BRCA signature and frequently contain mutations or pathogenic germline variants in genes involved in homologous recombination.

Opportunity for targeted treatment

PDAC is an aggressive disease with a five year survival of 5%. The majority of PDAC patients with advanced disease will receive gemcitabine based chemotherapies which is the standard of care, but this only provides a marginal survival advantage. Thus there remains a pressing need to improve therapy regimes. Personalised or targeted treatment, whereby drugs will be selected based on the characteristics of the tumour will become more prevalent. The large degree of heterogeneity in PDAC means it is an ideal disease for a personalised approach. In support of this there have been several reviews discussing some of the therapeutic opportunities in PDAC and a phase II trial (IMPaCT) was established to implement and test the value of precision medicine for recurrent or metastatic pancreas cancer.23-26

Targeting the DNA repair pathway

The large amount of PDAC mutation data has revealed an abundance of therapeutic opportunity. Of particular note is the potential to target defects in DNA damage repair pathways. Individual case reports of patients with advanced gemcitabine resistant disease that harbour defects in the homologous recombination pathway are showing positive responses to DNA damaging agents.17,27 Genome studies revealed that tumours with defective homologous recombination can be associated with a BRCA signature mutational profile and unstable rearrangement patterns, and comprise 14% of PDAC samples.7 Prospective patient clinical data and preclinical mouse models have demonstrated the potential utility of targeting these tumours with platinum based therapy. A challenge now is to identify those tumours with defective homologous recombination, so there is need for a test, other than whole genome sequencing, which has clinical utility to accurately and rapidly identify tumours that have a defective homologous recombination pathway.

Defects in an alternative DNA repair pathway, MMR, occur in small number of PDAC tumours. Similar to other cancer types such as colorectal,28 these tumours are associated with hypermutation. Due to the evidence suggesting that a high mutation burden may predict the likely chance of success of cancer immunotherapies,29 further work is warranted to determine whether this group of MMR defective PDAC tumours will respond to immunotherapies.

Other candidate targets for therapy

Oncogenic driver mutations are major treatment targets for molecular cancer therapies. There are several genes which are amplified at low frequency in PDAC (<5%) for which there are inhibitors developed for different tumours, including HER2, CDK4/6, FGFR and PI3-kinases, which have the potential to be utilised in PDAC treatment. HER2 amplification is one of the best characterised and occurs in 20% of breast cancers. Targeting this event with the anti-HER2 monoclonal antibody trastuzumab has revolutionised the outcome of patients with HER2 positive aggressive breast cancer.30,31 HER2 amplification has been detected in other tumours types including oesophageal, lung, bladder and gastric cancer, raising the possibility that anti-HER2 therapy can be repurposed to other tumour types. Encouragingly, in gastric cancer it has
been shown that HER2-overexpressing tumours treated with trastuzumab gain a survival advantage. In PDAC, amplifications of HER2 occur in 2% of cases, which makes it an attractive target for therapy.

Pathways which are perturbed in PDAC can also be targeted therapeutically, although identification of these pathways in each tumour can be problematic. The genome sequencing studies have been immensely useful and have identified the genes which are significantly mutated in these pathways, which may represent markers of therapy response. For example, a small proportion of PDAC samples contain mutations in RNF43, which is involved in Wnt signalling. The presence of RNF43 mutation has been shown to act as a predictive biomarker for Wnt inhibitors in PDAC cell lines. In addition, other candidate therapeutic targets which are mutated in small sets of PDAC include mutations in the splicing factor SF3B1, as SF3B1 mutant breast cancer cells are sensitive to a SF3b complex inhibitor spliceostatin A. These and other candidate targets now need to be verified experimentally for efficacy to determine if they can be used in treatment of PDAC.

Conclusion

There has been enormous progress in our understanding of the genes and pathways mutated in pancreatic cancer. These findings have informed about the mutational processes during tumour development and revealed new therapeutic opportunities. Pancreatic cancer is a heterogeneous disease and so it makes sense that this disease will benefit from a personalised approach to patient treatment. Although not specific to pancreatic cancer, a number of challenges remain for targeted therapy. These include development of suitable biomarkers of therapy selection, the within tumour heterogeneity which means some cells will contain the targeted mutation but other cells may not, and the potential of drug resistance development. However, in the near future it is anticipated that large collaborative clinical trials using many of the markers identified by genome sequencing, will commence in the treatment of PDAC.

References