Whole Genome Sequencing Informs Therapeutic Selection for Pancreatic Cancer

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BACKGROUND
Pancreatic ductal adenocarcinoma (PDAC) is the 4th most common cause of cancer death in Western societies and is projected to be the second leading cause within a decade. Recent exome and copy number variation (CNV) analyses of PDACs have revealed a complex mutational landscape, which may explain why therapeutic development has been challenging. Apart from point mutation and CNV, somatic structural rearrangement of chromosomes represents a common class of mutation that causes gene disruption, activation and formation of novel oncogenic gene products. Many of these events actively drive carcinogenesis and in some instances generate therapeutic targets.

METHODS
We performed deep whole genome sequencing (WGS) and CNV analysis of 100 PDACs (75 primary tumours and 25 patient-derived cell lines) recruited through the Australian Pancreatic Cancer Genome Initiative (APGI) as part of the International Cancer Genome Initiative (ICGC). Point mutation and SV events are analysed and PDACs are classified based on SV profiles with potential clinical relevance. We then use a combination of classification, mutational signature and gene mutations to define candidate biomarkers of therapeutic responsiveness for platinum-based chemotherapy by correlating the genotype to patient outcomes and patient-derived xenograft (PDx) therapeutic testing data.

RESULTS
In total, 11,868 somatic structural variants (SV) were detected at an average of 119 per individual (range 15 – 558). The majority of SVs were intra-chromosomal events, with inter-chromosomal translocations much less prevalent. Recurrent gene fusions were not detected. Apart from point mutations and small insertion and deletions, chromosomal rearrangements leading to gene disruption was common, including those known to be important in pancreatic cancer (TP53, SMAD4, CDK2NA, ARID1A, ROBO2) and novel candidates (KDM6A, REV3L and PALB2).

Subtyping PDAC using Structural Rearrangement
Genome-wide patterns of SV classified PDAC into 4 subtypes:

- **Stable**: Localised events (≤ 50 SV events).
- **Locally rearranged** (28%): >50 SV events predominantly localised to 1 or 2 chromosomes. A third of these tumours harboured focal amplifications, many of which contained druggable oncogenes (ERBB2, MET, FGFR1, CDK6, PIK3R3 and PIK3CA), but at low individual frequency (1-2%). The remaining two-thirds contained complex genomic events such as breakage-fusion-bridge repair or chromothripsis.
- **Scattered (40%)**: 50 to 200 events across the genome.
- **Unstable** (14%): ≥ 200 events globally distributed across the genome. This scale of genomic instability suggested defects in DNA maintenance, which potentially defines sensitivity to DNA damaging agents.

Genomic Markers of Defective DNA Maintenance
We mapped the relationship between the unstable subtype, mutations in BRCA pathway genes and a mutational signature associated with deleterious mutations in BRCA1 or BRCA2 in breast, ovarian and pancreatic cancer.

- The majority of unstable tumours (10 of 14) fell within the top quintile of the BRCA mutational signature when ranked by prevalence per Mb (Figure 2). In addition, the top quintile of the BRCA signature was associated with deleterious mutations of BRCA1 (n = 2; both somatic), BRCA2 (n = 7; 4 germline and 3 somatic) and PALB2 (n = 2; both germline). All deleterious BRCA1 and BRCA2 mutations had evidence of inactivation of the second allele. In contrast, tumours containing a somatic heterozygous silent mutation of BRCA2, a heterozygous intrinsic SV and 2 unclassified heterozygous missense mutations in BRCA1 (predicted benign or possibly damaging by PolyPhen2) were not associated with a high-ranking BRCA mutational signature or an unstable genome. In addition, we also identified non-BRCA mediated genomic instability such as bi-allelic inactivation of RPA1 and REV3L.

Genotypes of Platinum Responsiveness
We then correlated these candidate biomarkers of platinum responsiveness to patient palliative chemotherapy responses (observational cohort), and complemented with PDX therapeutic testing data. Overall, 8 patients received a platinum based therapy and 7 PDXs were treated with gemcitabine and cisplatin. Four of 5 patients with unstable genomes and/or a high BRCA mutational signature burden (designated as "on-genotype") had exceptional responses to platinum based therapy (Figure 5a), whilst 3 patients who did not have any of these characteristics ("off-genotype") did not respond. These observations were supported by PDX studies where 2 of 3 "on-genotype" PDX responded to cisplatin (Figure 5b), compared to none of 4 PDXs in the "off-genotype" group (Figures 3 and 4). Combining patient and PDX response data, "on-genotype" tumours were associated with response to platinum-based therapy (P = 0.0070 Fisher’s exact test).

CONCLUSIONS
This study provides the most comprehensive description of the genomic events that characterise PDAC to date and demonstrates that SV is a prominent mechanism of genomic damage in this disease. This approach may provide new opportunities in the development of biomarkers of therapeutic responsiveness to platinum-based therapies and PARP inhibitors for a number of cancer types including pancreas, breast, ovary and prostate.