Background: Genomic profiling has identified KRAS mutations in 88-90% of PC. KRAS WT tumors report a molecularly heterogeneous group that may harbor targetable alterations. We studied KRAS WT PC using NexSeq sequencing (NGS) and whole transcriptome sequencing (WTS) in a large cohort of pancreatic tumors to characterize the molecular landscape of this unique group and to assess the prevalence of targetable alterations.

Methods:
- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NexSeq (592 genes) (Illumina, Inc., San Diego, CA).
- All variants were detected with greater than 99% confidence based on allele frequency and amplification coverage, with an average sequencing depth of coverage of more than 500 and an analytic sensitivity of 5%.
- A combination of multiple test platforms including NGS, IHC and fragment analysis was used to determine MSI-H/MMMR status.
- Tumor mutational burden (TMB) was estimated from 592 genes (1-4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found in a tumor that had not previously been described as germline alterations.
- IHC was performed on FFPE sections of glass slides. PD-L1 testing was performed using the SP142 (Ventana, Tucson, AZ) anti-PD-L1 clone.
- Gene fusion detection using the Illumina NovaSeq platform (Illumina, Inc., San Diego, CA) and Agilent SureSelect Human All Exon V7 bait panel (Agilent Technologies, Santa Clara, CA).
- Microenvironment Cell Population-counter (MCP-counter) was used for quantification of the abundance of immune and stromal cell population using transcriptomic data. (Becht et al. Genome Biology 2016, 17:238)
- Chi-square and Wilcoxon were used for comparative analyses and Benjamini-Hochberg was used to correct for multiple comparison.

Results:

Table 1: patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>KRAS MT (N=60)</th>
<th>KRAS WT (N=1020)</th>
<th>Total (N=1080)</th>
<th>KRAS WT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>60%</td>
<td>90%</td>
<td>30%</td>
<td>60%</td>
</tr>
<tr>
<td>Stage</td>
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</tr>
<tr>
<td>Grade</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Tumor type</td>
<td>1.6%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

Methods:

- Based on our findings, comprehensive profiling of PC at the DNA and RNA level is recommended to provide patients with therapeutic opportunities beyond standard treatments.
- TMB and MSI tend to be higher in KRAS WT tumors; microenvironment inferred from WTS using MCP counter suggest more activated innate immunity with a lower fibroblast abundance, suggesting unique immune treatment strategy design.

Conclusions:

- KRAS WT PC is significantly more enriched with targetable alterations (e.g., BRAF, ALK, ROS1, NRG1, MSI-H) as compared to KRAS MT tumors, suggesting potential benefit of using targeted therapies.
- The use of WTS in combination with NGS identifies activated molecular pathways in the majority of KRAS WT tumors.
- Based on our findings, comprehensive profiling of PC at the DNA and RNA level is recommended to provide patients with therapeutic opportunities beyond standard treatments.
- TMB and MSI tend to be higher in KRAS WT tumors; microenvironment inferred from WTS using MCP counter suggest more activated innate immunity with a lower fibroblast abundance, suggesting unique immune treatment strategy design.

Reference


Figure 1: Notable alterations in the 144 KRAS-wild type pancreatic tumors

Figure 2: Oncoprint of the 144 KRAS-WT pancreatic tumors. Colored squares: alteration (mutation, fusion, copy number amplification, IHC overexpression, CISH amplification). Gray squares: no alteration detected. Blank: test not done or indeterminate results.

Figure 3: Immune characterization of KRAS-WT vs. KRAS MT tumors. Top: PD1L, MSI/MMR and TMB; Middle and Bottom: MCP counter calculated NK and fibroblasts in the tumor microenvironment.