ORIGINAL RESEARCH

Genetic aberrations in Chinese pancreatic cancer patients and their association with anatomic location and disease outcomes

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Abstract

Objectives: Pancreatic cancer (PC) is one of the most lethal malignancies with an increasing death rate over the years. We performed targeted sequencing and survival analyses on 90 Chinese pancreatic cancer patients, hoping to identify genomic biomarkers associated with clinical outcomes and therapeutic options.

Method: Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue specimens of 90 pancreatic cancer patients and sequenced. The associations with clinicopathological factors were analyzed.

Result: High prevalence of driver mutations in KRAS, TP53, CDKN2A, SMAD4, and ARID1A genes were found. Most mutated genes in PC belonged to cell cycle and DNA damage repair pathways. Tumors that arise from the pancreas’ body and tail (BT tumors) displayed a higher ratio of mutated KRAS and TP53 than those that arise from the pancreas’ head and neck (HN tumors), who showed less diverse KRAS subtypes. Patients with a KRAS p.G12R mutated tumor tended to have a prolonged disease-free survival (DFS) and overall survival (OS) than other KRAS subtypes. Those with an altered ARID1A gene and more than two mutated driver genes tended to have a shorter DFS and OS.

Conclusion: HN and BT tumors of the pancreas displayed different mutational profiles, which had prognostic significances and indicated different potential therapeutic options.

KEYWORDS
body and tail of pancreas, disease-free survival, DNA Damage Repair pathway, head and neck of pancreas, KRAS subtype, overall survival, pancreatic cancer

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1 | INTRODUCTION

Pancreatic cancer (PC) is a malignancy that confers a high mortality rate. The death toll due to PC is increasing over the years, and PC is predicted to be the second leading cause of cancer-related death by 2030. New strategies for late-stage PC treatment are being evaluated, including new chemotherapeutic regimens, immunotherapies, and small-molecular inhibitors that target the oncogenic pathways, emphasizing the need to improve the understanding of cancer genome and transcriptome alterations. Current data produced by massive parallel sequencing is overwhelming. They either identify crucial driver genes of the disease or subclassify PC into different molecular categories associated with patient survival.

Molecular testings have been adopted to inform the optimal therapeutic strategies for many cancers. However, in pancreatic cancer, only germline BRCA1/2 and PALB2 mutation, microsatellite instability, and NTRK fusions have gained general acceptance as actionable alterations by experts in the field.

Two years have passed since the publication of Pishvaian et al. on the molecular profile of 640 pancreatic cancer cases, where they reported that 50% of the patients harbored actionable genomic alterations. Given the rapid accumulation of knowledge on novel targets and the presence of new therapeutic compounds, some previously undruggable mutations might now become targeted. Thereby, we assume that it might still be informative to probe the known cancer-related genes in a Chinese pancreatic cancer cohort to explore the clinicopathological significance and identify possibly druggable cases according to their mutational profile.

2 | MATERIALS AND METHODS

2.1 | Patients and sample collection

The research was approved by the Institutional Review Board of Peking Union Medical College Hospital. Written informed consent to participate was obtained from each patient. The flow chart of patient enrollment was shown in Figure S1. Totally 90 pancreatic cancer patients were recruited via the pathology department’s electronic record, and experienced pathologists reviewed the Hematoxylin-Eosin (H&E) stained slides. Representative formalin-fixed paraffin-embedded (FFPE) tissue blocks containing cancer regions and non-cancer regions were retrieved from the pathology archives of the department. Resected specimens were chosen. Five-micrometer-thick FFPE sections made from the representative tissue blocks were then submitted for analyses by a 425-gene target-capture next-generation sequencing panel.

2.2 | Clinical data

Demographic and clinical information, including sex, age, clinical presentation, resectability, evidence of distant metastasis, and relevant family histories, were retrieved from the hospital information system. Of note, tumors located to the right of the superior mesenteric vein (SMV) was considered to have arisen from the pancreas’ head and neck (HN), while that located to the left of the SMV was considered to have arisen from the pancreas’ body and tail (BT). Follow-ups were conducted via either telephone or in the clinic. Patients were excluded from clinicopathological analyses if they met any of the following criteria: sample with no detectable mutations or low quality, sample without tumor primary site information, and sample without disease-free survival (DFS) and overall survival (OS) information.

2.3 | DNA extraction and quantification, library preparation

FFPE samples were de-paraffinized with xylene, and DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer’s protocols. Purified DNA was qualified by Nanodrop2000 (Thermo Fisher Scientific) and quantified by Qubit 2.0 using the dsDNA HS Assay Kit (Life Technologies) according to the manufacturer’s recommendations. Sequencing libraries were prepared using the KAPA Hyper Prep kit (KAPA Biosystems) with an optimized manufacturer’s protocol and sequenced as previously described. The tumor was sequenced to an average depth of 600–700X, while corresponding normal tissue was sequenced to at least 30X of depth on an Illumina Hiseq 4000 platform (Illumina).
2.4 Data processing

Sequencing data were processed as previously described.9 In brief, the data were first demultiplexed and subjected to FASTQ file quality control to remove low-quality data or N bases. Qualified reads were mapped to the reference human genome hg19 using Burrows–Wheeler Aligner and Genome Analysis Toolkit (GATK 3.4.0) was employed to apply the local realignment around indels and base quality score recalibration. Picard was used to remove PCR duplicates. VarScan2 was employed for the detection of single-nucleotide variations (SNVs) and insertion/deletion mutations. ADTEx was used to identify copy number variations (CNVs) with a reference human DNA sample NA18535 as the control. The cut-off of log2 ratio was set at ±0.6 for copy number changes (corresponding to 1.5-fold copy number gain and 0.65-fold copy number loss). The classification of germline mutations was based on the guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (referred to as the ACMG guidelines thereafter), where Class 4 and 5 mutations were referred to as deleterious mutations thereafter.10 Somatic mutations and therapeutic implications were annotated according to the OncoKB database, where Tier I and II mutations were referred to as actionable and potentially actionable mutations, respectively, in the following text.11

2.5 Statistics

The associations between gene alterations (mutations, CNV, chromosome gain, and loss) and clinicopathological factors were tested using Fisher’s exact test. Fisher’s exact test was used for pathway analysis as well. The correlation of gene alterations and tumor locations were tested using Fisher’s exact test. Survival analyses, including DFS and OS, were performed using the Kaplan–Meier method. Univariate and multivariate analyses, if appropriate, were conducted using the Cox proportional hazards model.

| Feature                  | Categories | Count | Overall survival (median) | Log-rank p | Progression-free survival (median) | Log-rank P |
|--------------------------|------------|-------|---------------------------|------------|-----------------------------------|------------|
| Age (years)              | <=65       | 67    | 27.0                      | 0.632      | 18.0                              | 0.907      |
|                          | >65        | 23    | 24.0                      |            | 24.0                              |            |
| Sex                      | Male       | 50    | 27.0                      | 0.282      | 18.0                              | 0.173      |
|                          | Female     | 40    | 31.0                      |            | 16.0                              |            |
| Histological subtype     | Ductal     | 83    | 27.0                      | 0.428      | 18.0                              | 0.674      |
|                          | Other      | 7     | NA                        |            | 12.0                              |            |
|                          | I          | 5     | 26.0                      | 0.257      | 24.0                              | 0.763      |
| Stage                    | II         | 75    | 16.0                      |            | 18.0                              |            |
|                          | III        | 2     | NA                        |            | 12.0                              |            |
|                          | IV         | 8     | 15.0                      |            | 12.0                              |            |
| Site                     | Head + Neck| 45    | 24.0                      | 0.762      | 14.0                              | 0.432      |
|                          | Body + Tail| 45    | 31.0                      |            | 18.0                              |            |
| Differentiation          | Well       | 11    | 24.0                      | 0.912      | 24.0                              | 0.163      |
|                          | Moderate   | 44    | 18.0                      |            | 18.0                              |            |
|                          | Poor       | 35    | 38.0                      |            | 12.0                              |            |
| DDR status               | Other      | 53    | 24.0                      | 0.406      | 18.0                              | 0.802      |
|                          | Deleterious| 16    | 38.0                      |            | 18.0                              |            |
| KRAS status              | Other      | 14    | 24.0                      | 0.965      | 14.0                              | 0.307      |
|                          | Deleterious| 55    | 31.0                      |            | 18.0                              |            |
| TP53 status              | Other      | 27    | NA                        | 0.188      | 12.0                              | 0.592      |
|                          | Deleterious| 42    | 24.0                      |            | 18.0                              |            |
| ARID1A status            | Other      | 60    | 27.0                      | 0.049      | 18.0                              | 0.059      |
|                          | Deleterious| 9     | 16.0                      |            | 12.0                              |            |

Note: DDR: DNA Damage Repair; note that only 69 cases entered mutational analyses and 54 cases entered survival analyses.
3 | RESULTS

3.1 | Patient overview

The clinicopathological characteristics of 90 pancreatic cancer patients were presented in Table 1. The median age of patients was 59 years, ranging from 36 to 80 years. The majority of the patients had pancreatic ductal adenocarcinomas (PDAC, 92.2%, 83 out of 90). The cohort comprised slightly more males (55.6%, 50 out of 90) than females (44.4%, 40 out of 90). Most of the patients were at stage 2 upon diagnosis (83.3%, 75 out of 90). Regarding the primary tumor location, the number of patients with BT cancers was equivalent to those with HN cancers. About 42.2% (38 out of 90) of pancreatic cancer patients present with gastrointestinal symptoms. All cases were resectable or borderline resectable when assessed preoperatively, according to the consensus statement of Abdominal Radiology and the American Pancreatic Association. All but three cases (3.33%) had macroscopic residual tumor after surgery.

3.2 | Somatic mutations in pancreatic cancer

Sequencing was successful for specimens from 69 (76.7%) patients. Detailed genetic information for each patient was disclosed in Table S1. Frequently mutated genes included KRAS, TP53, CDKN2A, SMAD4, and ARID1A. The prevalence of mutational events in our cohort was 81% for KRAS, 62% for TP53, 19% for CDKN2A, 17% for SMAD4, and 14% for ARID1A (Figure 1, top bars). All KRAS mutations identified were missense mutations affecting hotspots. And all TP53 mutations identified were predicted to be deleterious. For CDKN2A, all but one mutation were classified as oncogenic. The remaining mutation of unknown significance was an in-frame deletion CDKN2A c.266_286del, which resulted in the deletion of codons 89 to 96, where no deleterious

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** Distribution of genetic variations associated with pancreatic cancer. Distribution of individual gene Mutations (Top) and copy number variations (middle) in the cohort as assessed by target sequencing. Affected pathways in pancreatic cancer were shown on the bottom. Clinicopathological information was provided as bars on the top. Each column represents one patient.
non-truncating mutation was identified. Of note, the mutual exclusivity of mutations was insignificant in pancreatic cancer, except for KRAS and BRAF genes. The median tumor mutation burden was six for the cohort.

Copy number calling was successful in 69 (76.7%) cases. The cohort displayed gains of MCL1 (14%, 10 out of 69), and losses of CEBPA (16%, 11 out of 69), PTEN (10%, 7 out of 69), AMER1 (9%, 6 out of 69), and CDKN2A (7%, 5 out of 69) (Figure 1, lower bars). On chromosome level, pancreatic cancer displayed copy number changes of 13q, 4p, 4q, 20q, 10p. Interestingly, we identified 14% (10 out of 65) of patients who carried a chromosome 20q gain, which is frequently observed in pancreatic cancer in the western population13 (Figure 1, middle bars).

Pathway analyses revealed that the DNA damage repair (DDR) pathway was the most frequently altered pathway in pancreatic cancer, having excluded mutations curated in the Catalog of Somatic Mutations in Cancer (COSMIC). ARID1A was the most significantly mutated gene in the DDR pathway. With 11 mutations distributing in 10 cases, it affected 14% of all valid cases. Twenty-two percent of the patients had an altered cell cycling (CC) pathway, and CDKN2A was the most frequently mutated gene in the CC pathway, comprising 26% of all patients (Figure S2A).

3.3 | Deleterious germline mutations in Chinese pancreatic cancer patients

Using corresponding normal tissue as control, we were able to distinguish between germline and somatic mutations. Of the 69 patients, 13 (14.44%) harbored a deleterious germline mutation. Table 2 detailed all patients with deleterious mutations. Among the 13 mutated genes identified, nine (69.23%) were associated with DNA damage response. Two BRCA2 germline mutations, BRCA2 p.F1460fs and BRCA2 p.N2134fs, were detected in two patients, respectively. The patient with a BRCA2 p.F1460fs mutation was a 47-year-old male. A 60-year-old female patient was found to carry a germline BRCA1 p.R794X mutation. One 54-year-old male patient had a germline mutation in the BRIP1 (BRCA1 interacting protein C-terminal helicase 1) gene, which is known to increase the risk of ovarian, breast, and colon cancers.14 A deleterious germline mutation in the PALB2 (Partner and Localizer of BRCA2) gene, which accounts for 3–4% of familial pancreatic cancer cases, was identified in a 53-year-old female patient.15 Other pathogenic germline mutations included mutations in POLE and RAD51C, which were also identified in different cohorts.16,17 Germline mutations affecting other pathways included an LZTR1 p.Y726X, a PKHD1 c.2592 + 1 G > T, and a recurrent UGT1A1 p.Y486D mutations, which appeared in two patients.

3.4 | Actionable mutations in pancreatic cancer

Five cases carried actionable mutations. These included two cases carrying a germline BRCA2 mutation each, one carrying a germline BRCA1 mutation, one carrying a germline PALB2 mutation, and one carrying a somatic BRCA1 mutation. No NTRK fusion was identified. Mismatch repair genes were negative for all cases, except for a missense MLH1 mutation of uncertain significance, which was in accordance with their microsatellite status.

Apart from the 51 KRAS hotspot mutations and 12 CDKN2A loss-of-function mutations, most potentially actionable mutations fell within the DDR and PI3K pathways.

Mutations associated with FDA-approved therapies in other cancer types, which were potentially actionable,

| Patient ID | Sex | Age | Gene | AA change | Location |
|------------|-----|-----|------|-----------|----------|
| P02        | Female | 80  | LZTR1 | p.Y726X  | BT1      |
| P11        | Female | 66  | FANCC | p.K369fs | HN2      |
| P14        | Male   | 47  | BRCA2 | p.F1460fs |          |
| P16        | Male   | 54  | BRIP1 | c.2492_2492+3delGGTA |     |
| P17        | Male   | 48  | PKHD1 | c.G2592+1T | HN      |
| P29        | Male   | 76  | UGT1A1| p.Y486D  | BT       |
| P36        | Male   | 59  | UGT1A1| p.Y486D  | BT       |
| P37        | Female | 53  | PALB2 | p.S1169fs | HN      |
| P45        | Male   | 71  | FANCD2| p.R794X  | HN       |
| P58        | Male   | 59  | POLE  | c.A4779-2G | BT      |
| P59        | Female | 50  | RAD51C| p.V169fs | BT       |
| P77        | Female | 60  | BRCA1 | p.R794X  | BT       |
| P78        | Female | 70  | BRCA2 | p.N2134fs| HN       |
included three \textit{PIK3CA} mutations (p.K111E, p.G118D, and p.E545K), two \textit{BRAF} mutations (p.G466R and p.D594G, respectively), one \textit{ERBB2} p.H878Y mutation, a \textit{MAP2K1} p.P124S mutation, and an \textit{AKT1} mutation (p.E17K).

Fifteen potentially actionable somatic mutations in DDR genes were identified, including 10 \textit{ARID1A} mutations, two \textit{ATM} mutations, an \textit{ATR} mutation, a \textit{FANCI} mutation, and a \textit{RECQL4} mutation. Of note, one patient harboring an \textit{ARID1A} p.369fs mutation also had a concomitant \textit{ARID1A} p.R1335X mutation and is, therefore, speculated to have bi-allelic deactivation of the \textit{ARID1A} gene. Two patients had concurrent \textit{ARID1A}/\textit{ATM} and \textit{ARID1A}/\textit{RECQL4} mutations, respectively. None of the patients had concurrent germline and somatic deleterious DDR pathway mutations.

3.5 The correlations of mutational signatures and tumor anatomic location in pancreatic cancer

To determine whether the mutational signature was correlated with tumor location, we assorted tumor samples into two groups: HN and BT tumors. As shown in Figure 2, the frequency of \textit{KRAS} and \textit{TP53} mutations showed a significant difference between HN and BT tumors. BT tumors had a higher ratio of mutations in \textit{KRAS} and \textit{TP53} than HN tumors (Figure 2A). The pancreatic cancer cohort from TCGA also showed an increased proportion of \textit{KRAS} and \textit{TP53} in the BT group compared to HN; however, without significant difference (Figure 2B).

Pancreatic tumors from HN and BT shared a similar \textit{KRAS} subtype distribution pattern, predominately \textit{KRAS} p.G12D, followed by \textit{KRAS} p.G12V and \textit{KRAS} p.G12R (Figure 2C, upper). This \textit{KRAS} subtype distribution pattern was also observed in the TCGA cohort (Figure 2B, lower). On the contrary, HN tumors had a greater \textit{KRAS} subtype diversity than BT tumors. \textit{KRAS} subtype p.G12C, p.G12A, and p.G13D were observed in tumors from HN but not in tumors from BT (Figure 2B).

Regarding somatic mutations in the DDR pathway, a significantly higher rate in the BT tumors was identified (Figure S2B). When focusing on the ARID1A gene, although the rate of ARID1A mutation in BT tumors was three times that of the HN tumors, the difference was statistically insignificant. Differences in the pathological and molecular features of HN and BT tumors were detailed in Table 3.
Survival analysis

On Kaplan–Meier analysis, KRAS subtypes were not associated with significant differences in DFS and OS. Compared to cases with a KRAS p.G12D mutation, those carrying a KRAS p.G12R mutation had a better median DFS and OS, which both were 38 months (Figure 3A&B). Meanwhile, patients with a KRAS p.G12R mutation also showed better DFS and OS than patients with a wild-type KRAS gene or other KRAS subtypes (Figure 3C&D).

It has been reported that pancreatic cancer patients with mutated driver genes suffered from a poorer OS.18 In our cohort, each driver gene mutation alone, or combined, including KRAS, CDKN2A, TP53, and SMAD4, were not associated with DFS or OS (Data not shown). Interestingly, mutations in ARID1A, a candidate driver gene in pancreatic carcinogenesis, were associated with inferior DFS and OS. Patients with mutated ARID1A showed a median DFS and OS of 12 and 16 months, respectively, while those with a wild-type ARID1A had a median DFS and OS of 24 months (Figure 4A&B). However, no significant difference in DFS and OS was observed in the TCGA patient cohort (Figure S3A&B).

Then, we investigated the number of mutated genes among driver genes (KRAS, CDKN2A, TP53, and ARID1A). The OS of patients with 0–1 mutated driver genes was significantly longer than that of patients with 2–4 mutated driver genes (median OS, 38.0 vs. 18.0 months). No significant difference was found in DFS between these two groups (Figure 3C&D). On the contrary, in the TCGA pancreatic cohort, we observed a significant difference in DFS but not in OS between patients with 0–1 mutated 2–4 mutated driver genes (Figure S3C&D).

The impact of three (4.35%) macroscopic residual tumors on DFS and OS was also evaluated by Kaplan–Meier Test. While these cases did demonstrate inferior DFS and OS, the statistics were insignificant.

Regretfully, none of the above factors were significantly associated with OS or DFS in multivariate analyses (Table S2).

4 DISCUSSION

In this study, we investigated the clinicopathological characteristics and mutation profile of 90 Chinese pancreatic cancers. While consensus targeting therapeutic options is only available for a small fraction of the cases based on current
In this cohort, we learned that 83% of the cases carried a KRAS mutation, which was relatively lower than the Western population, as reported in TCGA (90%-96%). In a preprint paper under consideration in Cancer Cell International (https://www.researchsquare.com/article/rs-60530/v1), which adopted a Chinese PDAC cohort consisted of 195 patients. A KRAS mutation was found in 83.6% of their cases. No significant difference in the prevalence of KRAS mutations was found in these two cohorts. Therefore, it is reasonable to assume that the relatively low KRAS mutation rate might have reflected difference between races. KRAS activating mutations used to be considered undruggable, probably because of the lack of “pouch” formation in the variants, making it difficult to bind to. Strategies to target a KRAS mutation is either by covalent inhibition or inhibiting its downstream signals. Both yielded promising results. Covalent inhibitors are difficult to find and require different agents for different variants. Currently, only KRAS p.G12C has been successfully targeted. However, KRAS p.G12C mutation only occurred in a small fraction of pancreatic cancer, as evident in our cohort and the TCGA cohorts. More patients may be immediately benefited from inhibiting the downstream elements, such as MEK. Recent studies have partially revealed the underlying mechanism of rapid resistance acquisition to

FIGURE 3 The correlation of KRAS subtype with disease-free survival and overall survival in pancreatic cancer. Comparison of KRAS p.G12D and p.G12R in disease-free survival (A) and overall survival (B). Comparison of KRAS WT, p.G12R, and other KRAS subtypes in disease-free survival (C) and overall survival (D)
MEK inhibitors, which was associated with autophagy dependency. The addition of chloroquine, capable of inhibiting autophagy in this context, shed light on the solution to resistance development. In previous retrospective studies, KRAS p.G12D was found a poor prognostic factor for OS across different cancer types. In our cohort, KRAS p.G12D carriers showed shorter DFS and OS than those with a p.G12R, which were not different from the previous reports.

Most genes with a germline mutation detected in our cohort belonged to the DDR pathway. The prevalence of BRCA2 mutation was 2.90% (2 out of 69) in our cohort, assimilating the study results in the western population (1.95%, 59 out of 3060). The prevalence of other altered DDR genes, including BRCA1, BRIPl, RAD51C, and FANCC, were slightly higher than in the literature, which was probably incidental in the context of a relatively small denominator. The similarities in the profile of germline DDR gene mutations suggest the generalizability of relating studies in the western populations to Chinese patients.

Recently, Poly (ADP-ribose) polymerase (PARP) inhibitors have emerged as a treatment option for solid tumors with DDR gene deficiencies. Olaparib, a PARP inhibitor, has been approved by the FDA in pancreatic cancer patients with BRCA1/2 or PALB2 mutations. In the present study,
while only four patients had a germline BRCA1/2 or PALB2 mutation, another 7.24% (5 out of 69) patients had a deleterious germline mutation in other DDR genes, and 22 (31.88%) cases had at least one deleterious DDR gene mutation, germline, and somatic combined. Our data suggested the urgent need to extend PARP inhibitor trials to include patients with other germline and somatic DDR gene mutations.

Compared to TCGA data, this Chinese cohort exhibited relatively lower SMAD4 frequency yet higher ARID1A frequency, suggesting ARID1A may be a more potent driver in Chinese than SMAD4. ARID1A gene has been reported to participate in many aspects of carcinogenesis. It is involved in DNA damage response and has been demonstrated to confer sensitivity to combined radiotherapy and PARP inhibitor therapy.\textsuperscript{26} It is also considered to have a role in epigenetic modulation in tumor biology, as evidenced by the synthetic lethality of EZH2 inhibition in ARID1A-mutated tumors.\textsuperscript{27} Furthermore, ARID1A deficiency sensitized PC to PI3K/AKT inhibition in vitro.\textsuperscript{28} In the present study, we reported a statistically insignificant yet clinically important predilection of ARID1A somatic mutations in BT tumors. With 19.4% (7 out of 36) BT cases having at least one deleterious somatic ARID1A mutation, our data provided essential information to facilitate clinical trial allocation that aims at this very promising target. Altered ARID1A was also found to be associated with significantly shorter DFS and OS in the cohort, in concordance with the result of an earlier study enrolling 109 micro-dissected pancreatic ductal adenocarcinoma cases.\textsuperscript{29} Worse survival was also reported in patients with ARID1A gene mutations in a cohort of 22 PDAC patients treated with neoadjuvant chemoradiation therapy.\textsuperscript{30} This study also analyzed the prognostic value of ARID1A mutations and KRAS p.G12R mutation, along with other clinicopathological factors (Table S2). In univariate analysis, ARID1A mutations tend to be associated with DFS and OS. However, the association became insignificant in the subsequent multivariate analysis. The result might be due to the presence of confounding factors and small sample size.

The present study has limitations. The sample size is limited compared to existing studies, although a relatively small sample size had enabled the possibility of analyzing the mutation profile case by case. Also, to ensure the availability of sufficient tissue and intact clinicopathological information for subsequent analyses, we have discarded quite a few cases in the library; thereby, the cohort is neither randomized nor consecutive. However, the point of this preliminary report has been that the druggability of PC should never be overlooked.

In summary, in the present study, we confirmed the importance of sequencing tumor-associated genes in pancreatic cancer to inform therapeutic options and clinical trial allocation. The relationship between mutational features and pancreatic tumor locations was established. Also, we showed the correlation of the altered driver gene ARID1A and KRAS subtype with DFS and overall OS.

In conclusion, pancreatic tumors with different anatomic locations showed a difference in mutation profiles, which could divert future treatment options for HN and BT tumor patients.

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CONFLICTS OF INTEREST
Authors RL and RL were employed by the company Geneseeq Technology Inc. Author YS was employed by the company Nanjing Geneseeq Technology Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

AUTHOR CONTRIBUTIONS
JL analyzed the data and developed the introduction and discussion section of the manuscript. RL and RY analyzed and visualized the data and develop the method and result section of the manuscript. XL and ZZ followed the patients. JS and HW revised the manuscript; JG provided clinical data and HW revised the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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