



Pancreatic adenocarcinomas without *KRAS*, *TP53*, *CDKN2A* and *SMAD4* mutations and *CDKN2A/CDKN2B* copy number alterations: a review of the genomic landscape to unveil therapeutic avenues

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Background: Pancreatic adenocarcinoma is one of the cancers with the worst prognosis. The current treatment paradigm based on combination chemotherapy has improved survival over the last decade, but the disease is still fatal in most cases. New therapies exploiting the increasing understanding of the molecular pathology of the disease are needed. Although the disease presents with few recurrent molecular alterations, these represent opportunities for targeted treatments to be developed. However, a minority of cases are devoid of these common alterations. A description of the molecular landscape of this sub-set of pancreatic adenocarcinoma could uncover other molecular lesions present in them that could serve as therapeutic targets.

Methods: The sub-set of pancreatic cancers without the common alterations in *KRAS*, *TP53*, *CDKN2A* and *SMAD4* has been examined from published and publicly available pancreatic cancer cohorts for determination of their clinical and molecular characteristics. The cBioportal platform was used for this evaluation and the OncoKB knowledgebase was used for determination of the functional significance of discovered mutations.

Results: About 5% to 10% of pancreatic adenocarcinomas present without the usual molecular alterations that characterize the disease. These cases tend to be genomically stable and have low prevalence of microsatellite or chromosome instability. Molecular alterations that are observed in pancreatic cancers in lower frequencies than the four most prevalent alterations, such as DNA Damage Response and epigenetic modifier mutations, are still observed in the sub-set without the common alterations and may be pathogenically relevant.

Conclusions: Despite the absence of most frequent pancreatic cancer alterations in a sub-set of pancreatic adenocarcinomas, this sub-set possesses other alterations in frequencies similar to the rest of pancreatic cancers. Putative targeting of alterations present is discussed and can serve as the basis for targeted therapies development.

Keywords: Pancreatic adenocarcinoma; molecular pathology; mutations; copy number alterations; genomics

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Introduction

Pancreatic cancer is a gastrointestinal malignancy with rising incidence and with a 5-year survival rate of less than 10% (1). The median survival of metastatic disease is about 6 months. Prevalence is highest in the developed world compared with low-income countries and is only slightly higher in men compared to women (2). In 2019, pancreatic adenocarcinoma accounted for 45,750 deaths in the United States of America (1). Despite representing only the thirteenth most prevalent cancer worldwide, accounting for 458,000 cases, pancreatic cancer is the seventh cancer in the mortality list (3). Pancreatic cancers usually present in advanced stage due to early dissemination and lack of symptoms when in earlier stages. Moreover, despite improvements in chemotherapy treatments and management in recent years with the introduction of more effective chemotherapy regimens, resistance to radiotherapy and chemotherapy contributes to increased mortality (4-7). Thus, there is a pressing need for improved systemic therapies based on the clarification of pancreatic molecular carcinogenesis that has been the result of genomic cancer research over the last decades (8).

The molecular pathology of pancreatic adenocarcinomas is characterized by few prevalent mutations associated with a low overall tumor mutation burden (TMB). Only 4.6% of pancreatic adenocarcinomas of TCGA cohort have a TMB above 80 (9). Few cancer-associated genes display

recurrent mutations in pancreatic cancers. Oncogene *KRAS* mutations are the most prevalent, encountered in up to 90% of pancreatic cancers, followed by mutations in tumor suppressor *TP53*, encountered in up to three fourths of pancreatic cancers. Two additional tumor suppressor genes, *CDKN2A*, encoding for cell cycle inhibitor p16 and the gene encoding for transforming growth factor beta (TGF β) pathway signal transducer *SMAD4*, are mutated in about 20% of cases in pancreatic cancers (9,10). Copy number alterations are also less frequent in pancreatic cancers compared with other carcinomas. The most frequently copy altered locus, showing deep deletions, is at chromosome 9p21.3, where *CDKN2A* is located. The same locus also encodes for the p53 positive regulator p14^{ARF}, which is transcribed by the same sequence with an alternative reading frame. The locus is deleted in 10% to 25% of pancreatic cancer cases. Besides these five recurrent gene alterations, no other oncogene or tumor suppressor is commonly altered in pancreatic cancers. A minority of pancreatic cancers harbor no molecular alterations in any of these genes. The current investigation examines the molecular abnormalities present in these cases characterized by absence of typical pancreatic cancer alterations, for discovery of less common lesions present in these cases that may be associated with an alternative cancer pathogenesis and may serve as targets for novel therapies. Cases of pancreatic cancer with absence of the common pathogenic mutations may be inherently resistant to therapies targeting these mutations, should such treatments be developed, and will require alternative therapies based on putative alternative molecular targets.

Highlight box

Key findings

- Pancreatic cancers without the common alterations in *KRAS*, *TP53*, *CDKN2A* and *SMAD4* represent a genomically stable minority of cases, some having alterations in other oncogenes and tumor suppressors of pathogenic interest.

What is known and what is new?

- Although pancreatic adenocarcinoma is a cancer with few molecular alterations, recurrent mutations in oncogenes and tumor suppressors are present. We report here that some of the pancreatic cancers that present without any of the common molecular alterations, still possess other mutations such as in DNA Damage Response genes or in epigenetic modifiers.

What is the implication and what should change now?

- Opportunities for treatment of pancreatic cancers without the common molecular alterations of the disease may include targeting the DNA damage response and the epigenetic machinery with drugs already being used or in development.

Methods

Four publicly available genomic series of pancreatic adenocarcinoma patients, published from The Cancer Genome Atlas (TCGA), the MSK-IMPACT study, the pancreatic cancer sub-cohort of a pan-cancer study from China, and the pancreatic cancer cohort from the American Association for Cancer Research (AACR) project GENIE (public v.12), were interrogated for identification and characterization of patients without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* (9,11-13). Analysis of the series of interest were performed in cBioportal for cancer genomics, a genomic studies platform that is freely accessible and allows for interrogation of genomic alterations in any gene of interest, included in the original studies (14). TCGA employs a whole exome sequencing

platform, while the three other studies are based on targeted next generation sequencing (NGS) genomic panels (11-13). TCGA uses the Picard pipeline, which stores data of every sequenced sample in BAM (Binary Alignment and Map) files (9). Significantly mutated genes were identified using the MutSig algorithm, version MutSig2CV (15). Analysis of copy number alterations (CNAs) in TCGA is performed with the GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm, in which a score of 2 or above denotes putative amplification of a gene (16). Chromosomal instability of each sample is measured by the Aneuploidy Score (AS), defined as the sum of the number of chromosome arms in each patient sample that displays copy number alterations (gains or losses) (17). A chromosome arm is considered copy number altered based on the length of alterations, as calculated by the ABSOLUTE algorithm from Affymetrix 6.0 SNP arrays (18). The definition of a somatic copy number alteration was set at more than 80% of the length of the arm. Alterations in 20% to 80% of a given arm length were considered inadequate to call, while chromosomal arms with somatic copy number alterations in less than 20% of the arm length were considered not altered. Another measure of chromosomal instability, the Fraction Genome Altered (FGA) was defined as the fraction of log₂ copy number gain or loss (>0.2) divided by the total length of the profiled genome. TCGA also includes mRNA expression analysis. The RSEM algorithm was used for normalization of mRNA expression (19).

The MSK-IMPACT study used a targeted hybridization capture-based NGS platform that included 341 genes and in a more updated version, a total 410 genes were included (7). The platform detects all mutations in protein coding regions of these genes, CNAs, selected mutations in promoter regions and selected structural rearrangements (20). The assay targeted 4,976 exons with a mean coverage of 700 [standard deviation (SD): 182]. Single nucleotide variants and insertions/deletions were called using the MuTect and SomaticIndelDetector tools in the Genome Analysis ToolKit (GATK) programming framework (21).

The pan-cancer study from China from which the Chinese pancreatic cancer group was derived, used a targeted NGS panel of 579 cancer-associated genes from Origimed corporation, Shanghai, China (12). Variation calling was performed with methods similar to the ones used in the MSK-IMPACT study.

AACR project GENIE included data from eight contributing institutions (5 from USA, 1 institution each from Canada, France and the Netherlands) and the targeted

NGS panels used in each institution had a variable number of genes covered (13).

The functional implications of concomitant mutations in included samples were evaluated with the help of OncoKB (22). OncoKB knowledgebase is a database of cancer-related genes and classifies these genes as oncogenes or tumor suppressors.

Statistical analysis

Statistical comparisons of categorical data were carried out using Fisher's exact test or the χ^2 test and comparisons of continuous parameters were compared with the *t*-test. The Mann-Whitney U test was used to compare median values. All statistical comparisons were considered significant if $P < 0.05$.

Results

Prevalence of common pancreatic adenocarcinoma molecular alterations and of cases without common alterations

Mutations in oncogene *KRAS* and tumor suppressor *TP53* are the most frequent molecular alterations in pancreatic adenocarcinomas, followed by mutations in *SMAD4* and *CDKN2A* genes and deletions of the 9p21.3 locus. Three extensive pancreatic cancer genomic series using targeted gene panels (Pancreatic cohort of the project GENIE, pancreatic cohort of the MSK-IMPACT series and pancreatic cohort of the Chinese Origimed study) show a consistent prevalence of *KRAS* mutations between 86.6% and 90.6% (Figure 1). The pancreatic study from TCGA, employing a whole exome platform, shows a lower mutation rate at 65.4%. Mutations in *TP53* have also more consistent frequency in the three former series with prevalence between 69.5% and 75.5%, while TCGA shows again a lower prevalence at 59.8%. The four studies display a more homogeneous frequency of *SMAD4* and *CDKN2A* mutations around 20% and between 15% and 20%, respectively. The prevalence of deletions of the *CDKN2A/CDKN2B* locus varies between the series and is the lowest in the Chinese series at 3.5% and the highest in TCGA at 28% (Figure 1). The prevalence of cases without any of the above mutations or deletions is similar in the 3 series, excepting TCGA (5.4% in the Chinese series, 5% in MSK-IMPACT and 8.3% in project GENIE). TCGA, due to lower prevalence of *KRAS* and *TP53* mutations displays a

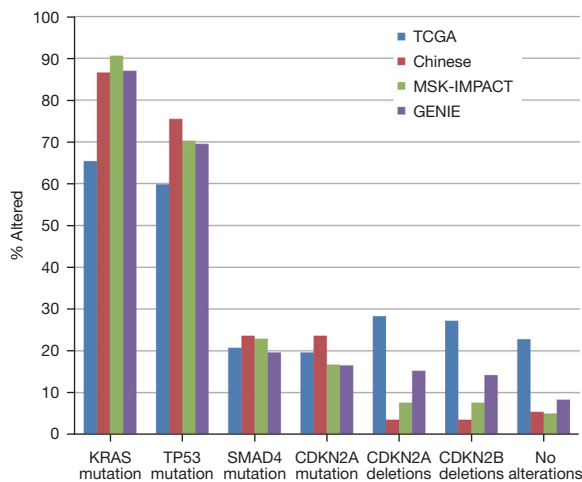


Figure 1 Prevalence of the common pancreatic cancer molecular alterations and of cases without these common alterations in four genomic series (TCGA, the MSK-IMPACT study, the pancreatic cancer sub-cohort of pan-cancer study from China, and the pancreatic cancer cohort from AACR project GENIE). TCGA, The Cancer Genome Atlas; MSK-IMPACT, Memorial Sloan Kettering- Integrated Mutation Profiling of Actionable Cancer Targets; GENIE, Genomic Evidence Neoplasia Information Exchange.

higher rate of cases without any of the common pancreatic cancer alterations at 22.8%.

Characteristics and molecular lesions of pancreatic cancers without the common molecular alterations

The clinical and molecular characteristics of pancreatic cancer cases without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* were evaluated in the four cohorts. The 42 patients without these alterations in the TCGA cohort were younger (61.9% younger than 65 years-old versus 52.2% younger than 65 years-old in the whole pancreatic cohort), but this difference was not statistically significant (Fisher's exact test $P=0.3$). Similarly, neither the mean age nor the percentage of patients younger than 65 years-old in the Chinese series or the GENIE cohort disclosed any differences between the 2 groups (Table 1). Early (stage I) patients constituted 25% of cases in the group without common alterations in TCGA (versus 11.5% in the whole cohort, Fisher's exact test $P=0.04$) (Table 1). In contrast, no differences in the prevalence of localized versus metastatic disease were observed between the 2 groups in the MSK-IMPACT series and in the Chinese

cohort (Fisher's exact test $P=0.33$ and 0.37 , respectively). Genes with mutations in the 42 patients of TCGA cohort encountered in more than one case included *ATM* and *GNAS* in 3 cases each (8.1%) (Table 2) and *DROSHA*, *RNF213*, *KAT6A* and *LRRK2* in 2 cases each (5.4%). CNAs (all amplifications) in the 42 cases encountered in more than one sample were at genes in loci 12p13.33 (*RAD52* and *CCND2*), 18q11.2, 8p11.23 and 9p13.3, all observed in 2 samples. Besides the cases with *ATM* mutations no other mutations in DNA damage response (DDR) genes, such as *BRCA1*, *BRCA2*, *PLB2*, *BRIPI*, or *RAD51* homologues were present in the 42 cases. The only CNA in DDR-associated genes was an amplification in *BRIPI* observed in a single case. In addition, no alterations in mismatch repair (MMR) genes *MSH2*, *MSH6*, *MLH1* and *PMS2* or in the proofreading polymerases *POLE* and *POLD1* were observed (Table 3). The mean mutation count in the 33 cases of the 42 with available data was 19.6 and no cases had more than 115 mutations (Table 4). Twenty-five of the 33 cases (75.8%) had a low AS of 4 or less.

Pancreatic cancers without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* from the MSK-IMPACT cohort ($n=19$) showed also mutations in *GNAS* gene in 3 cases (15.8%) and 2 cases (10.5%) had mutations in *BCOR*. Several other cancer associated genes assayed in the targeted panel employed in this study, including *TGFBR2*, *BAP1*, *AXIN1*, *MDM2*, *RNF43*, *NF1*, *BARD1*, *PARP1*, *NFE2L2* and *ARAF*, had mutations in one case each. The only CNA seen in more than one case was an amplification at locus 12p13, harboring genes *RAD52* and *CCND2* observed in 2 cases (10.5%). No cases with mutations in DDR-associated genes such as *BRCA1*, *BRCA2*, *PLB2*, *BRIPI*, and *RAD51* or mutations in MMR associated genes *MSH2*, *MSH6*, *MLH1* and *PMS2* and the proofreading polymerases *POLE* and *POLD1* were present among the 19 cancers without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations (Table 2). All 19 cases had low mutation counts of 7 or less and 14 of 19 cases had a low FGA below 0.1 (Table 4).

In the Chinese Origene series, which also employed a targeted genomic panel, 25 patients had pancreatic cancers without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B*. Three genes had mutations in more than one case, including mutations in *ATM* in 3 cases (12%), in *BCOR* in 2 cases (8%) and in *ITK*, also in 2 cases. Among DDR associated genes one patient (4%) had a *BRIPI* mutation. One case each had a mutation and amplification of *LZTR1* gene, encoding for a component of an E3 ligase complex involved in *KRAS* degradation. All patients with

Table 1 Characteristics of all pancreatic cancers and pancreatic cancers without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* mutations from TCGA, MSK-IMPACT pancreatic adenocarcinoma cases, the Chinese Origene series and the pancreatic cases of the GENIE project

Characteristic	TCGA		MSK-IMPACT		Chinese Origene		Project GENIE	
	All (n=184)	Without alterations (n=42)	All (n=384)	Without alterations (n=19)	All (n=461)	Without alterations (n=25)	All (n=5,423)	Without alterations (n=448)
Age mean ± SD, years	64.8±11	64.5±9.9	NA	NA	60.1±9.8	59.3±11	63.9±10.5	61.3±13.7
Age (years), n (%)								
≤65	96 (52.2)	26 (61.9)	NA	NA	323 (70.1)	17 (68.0)	2,260 (53.2)	220 (57.1)
>65	88 (47.8)	16 (38.1)	–	–	138 (29.9)	8 (32.0)	1,988 (46.8)	165 (42.9)
NA	–	–	–	–	–	–	1,175	63
Sex, n (%)								
Male	101 (54.9)	22 (52.4)	205 (53.5)	13 (68.4)	278 (60.3)	16 (64.0)	2,792 (53.2)	239 (54.2)
Female	83 (45.1)	20 (47.6)	178 (46.5)	6 (31.6)	183 (39.7)	9 (36.0)	2,457 (46.8)	202 (45.8)
NA	–	2	–	–	–	–	13	7
Stage, n (%)								
I	21 (11.4)	10 (25.0)	231 (60.3)	9 (stages I–III) (47.4)	100 (23.5)	6 (27.3)	NA	NA
II	151 (82.1)	29 (72.5)	–	–	119 (28.0)	10 (45.4)	–	–
III	5 (2.7)	1 (2.5)	–	–	43 (10.1)	0	–	–
IV	5 (2.7)	0	152 (39.7)	10 (52.6)	163 (38.4)	6 (27.3)	–	–
NA	2	2	–	–	36	3	–	–

Numbers in parentheses are percentages and are rounded to the first decimal digit. NA, not available; TCGA, The Cancer Genome Atlas; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; GENIE, Genomic Evidence Neoplasia Information Exchange.

without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* and data available had a low TMB (Table 2). This series provided data on the purity of the samples analyzed. The purity of tumor specimens without common pancreatic gene alterations (mean 34%, Standard deviation: 17.07%) was not different from the purity of the specimens in the entire cohort (mean 35.56%, Standard deviation: 16.75%, *t*-test *P*=0.6).

The pancreatic cohort of project GENIE includes 448 patients without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B*. Most common mutations present in these cases, observed in more than 2% of patients include mutations in *ARID1A* (6.3%), *GNAS* (4.3%), *BRAF* (3.6%), *PRBM1* (3.1%), *BRCA2* (2.9%), *ATM* (2.8%), *PIK3CA* (2.7%), *BCOR* (2.5%) and *NF1* (2.2%). Overall, none of the genes mutated with a prevalence of 3% or higher in at least 2 of the 4 series examined was significantly more frequently mutated in cases without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* compared with

the corresponding whole series (Table 2). Table 3 shows molecular alterations of interest that are potentially enriched in pancreatic cancers without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* and for which there exist targeted therapies. Alterations including fusions involving *NRG1*, encoding for the EGFR family ligand Neuregulin 1 and fusions involving one of the neurotrophic receptor tyrosine kinase family members, *NTRK1*, *NTRK2* and *NTRK3* are observed in about 5% cases.

Transcription factors of acinar cell differentiation such as *NR5A2*, *BHLHA15* and *RBPJL*, together with genes associated with endocrine pancreatic differentiation, such as *INS*, *NEUROD1*, *NKX2-2*, and *MAFA* were up-regulated in cases without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* compared with counterparts bearing alterations in one or more of these genes (Figure 2). In addition some cases without alterations present up-regulation of immune checkpoint genes *CTLA4* and *PDCD1*, encoding for PD-1.

Table 2 Mutations (besides *KRAS*, *TP53*, *CDKN2A*, and *SMAD4*) with a prevalence of 3% or higher in at least 2 of the 4 series and corresponding frequency in cases without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B*

Mutation	TCGA		MSK-IMPACT		Chinese Origene		Project GENIE	
	All (n=184), n (%)	Without alterations (n=42), n (%)	All (n=384), n (%)	Without alterations (n=19), n (%)	All (n=461), n (%)	Without alterations (n=25), n (%)	All (n=5,423), n (%)	Without alterations (n=448), n (%)
<i>ARID1A</i>	9 (4.9)	1 (2.7)	36 (9.4)	0	55 (11.9)	0	427 (8.9)	23 (6.3)
<i>GNAS</i>	7 (3.9)	3 (8.1)	13 (3.4)	3 (15.8)	4 (0.9)	0	142 (2.6)	19 (4.3)
<i>ATM</i>	8 (4.5)	3 (8.1)	11 (2.9)	0	21 (4.6)	3 (12.0)	227 (4.4)	12 (2.8)
<i>PIK3CA</i>	5 (2.8)	0	13 (3.4)	0	12 (2.6)	0	163 (3.0)	12 (2.7)
<i>BRCA2</i>	2 (1.1)	0	13 (3.4)	0	8 (1.7)	0	212 (4.3)	11 (2.9)
<i>RNF43</i>	11 (6.1)	0	27 (7.0)	1 (5.3)	30 (6.5)	1 (4.0)	297 (6.3)	6 (1.7)
<i>KMT2D</i>	7 (3.9)	1 (2.7)	15 (3.9)	1 (5.3)	21 (4.6)	0	291 (6.3)	6 (1.8)
<i>TGFBR2</i>	8 (4.5)	1 (2.7)	13 (3.4)	1 (5.3)	22 (4.8)	0	127 (3.6)	5 (1.1)
<i>KDM6A</i>	7 (3.9)	0	13 (3.4)	0	18 (3.9)	1 (4.0)	181 (3.9)	3 (0.9)
<i>KMT2C</i>	7 (3.9)	0	12 (3.1)	1 (5.3)	12 (2.6)	0	123 (3.7)	6 (2.7)

Numbers in parentheses are percentages and are rounded to the first decimal digit. TCGA, The Cancer Genome Atlas; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; GENIE, Genomic Evidence Neoplasia Information Exchange.

Prognosis of pancreatic cancers without the common molecular alterations

Survival data were available in 2 of the 4 series analyzed, the one from TCGA and the MSK-IMPACT cohort. The overall survival (OS) of patients without the common pancreatic cancer molecular alterations was better than the OS of the whole TCGA pancreatic cancer cohort (Log Rank $P=0.03$, Figure 3). In contrast, no OS difference between the patients without common alterations and the entire pancreatic group was present in the pancreatic cancer cohort of MSK-IMPACT which had a lower percentage of patients without alterations (Log Rank $P=0.45$, not shown).

Discussion

Mutations in oncogene *KRAS* are present in most pancreatic adenocarcinomas and are considered hallmarks of the disease (23). Pancreatic cancer has the highest prevalence of mutations in *KRAS* among cancers with such mutations, which include colorectal and lung carcinomas. Most commonly *KRAS* mutations in pancreatic cancer occur at codon 12, with a minority of about 6.5% and 1.2% respectively, occurring at codons 61 and 13 (23). However, these mutations alone may not be sufficient for development of the disease and additional alterations are

required for transformation (24). In a mouse model, *KRAS* activation alone leads only to pancreatic carcinoma in situ, whereas addition of mutant p53 activation leads to early invasive pancreatic carcinogenesis (25). Mutations in *TP53* gene encoding for p53, promote pancreatic carcinogenesis through neutralization of the apoptotic and metastasis suppressive functions of p53 (26). Besides *KRAS* and *TP53* mutations, two other commonly mutated genes in pancreatic adenocarcinomas, *CDKN2A* and *SMAD4*, lead to deregulation of the cell cycle and the activation of the TGF β /SMAD pathway, respectively (27). Additionally, the locus of *CDKN2A* at chromosome 9p is altered by deep deletions in pancreatic cancer, which also affect the overlapping *CDKN2B* gene, encoding for the p53 positive regulator p14^{ARF}. Among the common molecular alterations of pancreatic cancer, only the *KRAS* G12C mutations, present in 1% to 2% of cases are currently targeted with available kinase inhibitors. A phase 1/2 trial has shown that inhibitor sotorasib produced a confirmed objective response in 21% of pretreated metastatic pancreatic cancer patients with the specific *KRAS* mutation (28). Inhibitors for the more common *KRAS* mutation G12D are also in development (29). Other molecular alterations occur sporadically in pancreatic cancer, with a prevalence in general of less than 8%. The current review of the landscape of pancreatic cancers without the common alterations in

Table 3 Mutations and fusions of therapeutic interest in the four pancreatic series and corresponding frequency in cases without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B*

Alterations	TCGA		MSK-IMPACT		Chinese Origene		Project GENIE	
	All (n=184), Without alterations n (%)	(n=42), n (%)	All (n=384), Without alterations n (%)	(n=19), n (%)	All (n=461), Without alterations n (%)	(n=25), n (%)	All (n=5,423), Without alterations n (%)	(n=448), n (%)
<i>MSH2</i> mutations	1 (0.6)	0	1 (0.3)	0	2 (0.4)	0	43 (0.9)	1 (0.3)
<i>MSH6</i> mutations	1 (0.6)	0	4 (1.0)	0	3 (0.7)	0	69 (1.4)	2 (0.5)
<i>MLH1</i> mutations	1 (0.6)	0	3 (0.8)	0	5 (1.1)	0	44 (0.8)	3 (0.7)
<i>PMS2</i> mutations	1 (0.6)	0	0	0	4 (0.9)	0	28 (0.6)	0
<i>POLE</i> mutations	2 (1.1)	0	2 (0.5)	0	3 (0.7)	0	70 (1.5)	2 (0.6)
<i>POLD1</i> mutations	1 (0.6)	0	2 (0.5)	0	3 (0.7)	0	55 (1.3)	2 (0.7)
<i>BRAF</i> mutations	2 (1.1)	0	3 (0.8)	0	13 (2.8)	0	83 (1.5)	2 (0.5)
<i>BRAF</i> fusion	1 (0.5)	1 (2.4)	5 (1.3)	1 (5.3)	NA	NA	14 (0.3)	16 (3.5)
<i>ERBB2</i> mutations	2 (1.1)	0	3 (0.8)	0	3 (0.7)	0	68 (1.3)	6 (1.5)
<i>ERBB2</i> amplifications	9 (4.9)	0	6 (1.6)	0	4 (0.9)	0	41 (0.7)	2 (0.5)
<i>FGFR2</i> mutations	0	0	2 (0.5)	0	1 (0.2)	0	28 (0.5)	1 (0.3)
<i>FGFR2</i> amplifications	1 (0.5)	0	0	0	0	0	4 (0.07)	0
<i>FGFR1</i> amplifications	7 (3.8)	2 (4.8)	6 (1.6)	0	5 (1.1)	0	57 (1.4)	0
<i>FGFR2</i> fusions	0	0	2 (0.5)	1 (5.3)	NA	NA	9 (0.2)	5 (1.2)
<i>FGFR3</i> fusions	0	0	0	0	NA	NA	3 (0.1)	0
<i>ALK</i> fusions	0	0	1 (0.3)	0	NA	NA	9 (0.2)	1 (0.3)
<i>RET</i> fusions	0	0	0	0	NA	NA	1 (0.02)	0
<i>MET</i> fusions	0	0	0	0	NA	NA	3 (0.1)	1 (0.3)
<i>ERBB4</i> fusions	0	0	0	0	NA	NA	1 (0.02)	0
<i>NRG1</i> fusions	2 (1.1)	1 (2.4)	NA	NA	NA	NA	11 (0.8)	5 (4.4)
<i>NTRK1</i> fusions	1 (0.5)	1 (2.4)	0	0	NA	NA	6 (0.1)	1 (0.3)
<i>NTRK2</i> fusions	0	0	0	0	NA	NA	2 (0.04)	0
<i>NTRK3</i> fusions	1 (0.5)	0	2 (0.5)	2 (10.5)	NA	NA	6 (0.1)	3 (0.9)

Numbers in parentheses are percentages and are rounded to the first decimal digit. NA, not available; TCGA, The Cancer Genome Atlas; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; GENIE, Genomic Evidence Neoplasia Information Exchange.

KRAS, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* examines whether any of the less frequent molecular alterations is prevalent in these cases. Such alterations could dominantly contribute to the molecular pathogenesis, substituting for the common alterations in these cases. Four extensive pancreatic cancer genomic series formed the basis of this evaluation. Three of the series display similar prevalence of pancreatic cancers without common alterations between 5% and 8.3%, while TCGA shows higher prevalence of such cases (22.8%). This discrepancy is most probably due to the different sequencing platforms used. Targeted gene

panels used in the three former studies allow for deeper coverage of the included genes while the whole exome sequencing platform of TCGA employs a lower coverage of each gene and could thus miss altered cases. Another recently published report of pancreatic cancer patients who had genomic analysis with a targeted NGS panel from Caris Life Sciences (Phoenix, AZ) showed that 5.6% of 2,483 patients were *KRAS* and *TP53* wild type (30).

Mutated genes with an overall prevalence between 5% and 10% in pancreatic series (for example *ATM*, *BRCA2*, *GNAS*, and *ARID1A*) are present in cases without *KRAS*,

Table 4 TMB and FGA of all cases and of the cases without alterations in *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* in the four series

Characteristic	TCGA		MSK-IMPACT		Chinese Origene		Project GENIE	
	All (n=184), n (%)	Without alterations (n=42), n (%)	All (n=384), n (%)	Without alterations (n=19), n (%)	All (n=461), n (%)	Without alterations (n=25), n (%)	All (n=5,423), n (%)	Without alterations (n=448), n (%)
TMB								
Low	171 (98.3)	32 (97.0)	249 (64.8)	16 (84.2)	229 (51.1)	12 (100.0)	2,736 (53.0)	138 (73.8)
Intermediate	2 (1.1)	1 (3.0)	129 (33.6)	3 (15.8)	193 (43.1)	0	1,999 (38.8)	36 (19.3)
High	1 (0.6)	0	6 (1.6)	0	26 (5.8)	0	425 (8.2)	13 (6.9)
NA	10	9			13	13	263	261
FGA								
<0.08	89 (48.6)	34 (82.9)	242 (63.0)	12 (63.2)	NA	NA	1,588 (57.2)	121 (75.2)
0.08–0.35	77 (42.1)	3 (7.3)	111 (28.9)	4 (21.0)			882 (31.8)	29 (18.0)
>0.35	17 (9.3)	4 (9.8)	31 (8.1)	3 (15.8)			304 (11.0)	11 (6.8)
NA	1	1					2,469	287

TMB low: <100 for TCGA and ≤ 5 for others; TMB intermediate: 100–200 for TCGA and 5–10 for others; TMB high: >200 for TCGA and >10 for others. Numbers in parentheses are percentages and are rounded to the first decimal digit. NA, not available; TMB, tumor mutation burden; FGA, Fraction Genome Altered; TCGA, The Cancer Genome Atlas; MSK-IMPACT, Memorial Sloan Kettering- Integrated Mutation Profiling of Actionable Cancer Targets; GENIE, Genomic Evidence Neoplasia Information Exchange.

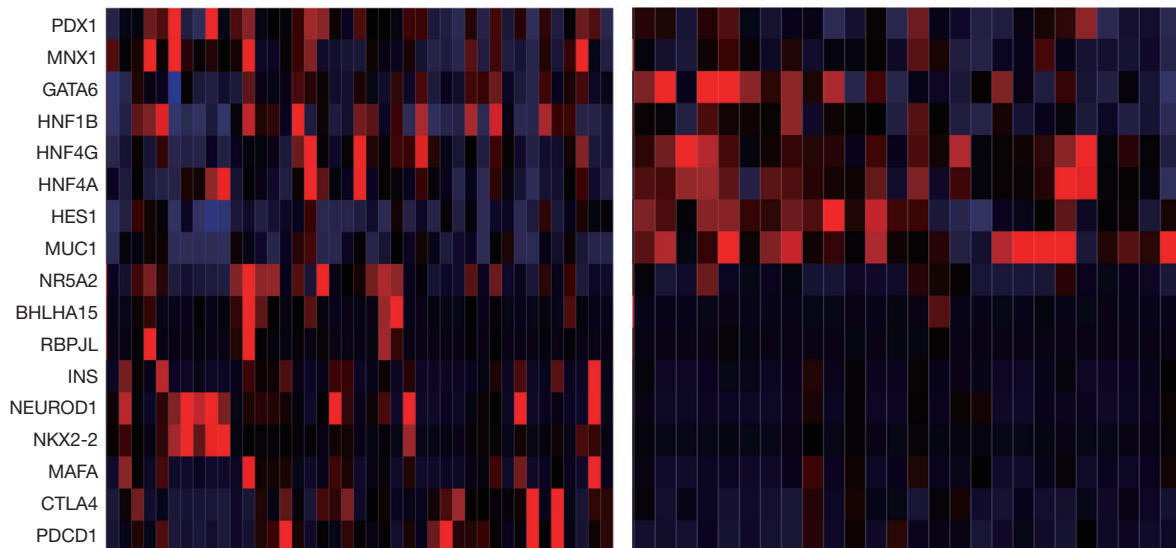


Figure 2 mRNA expression of key genes in pancreatic cancer cases without common pancreatic cancer molecular alterations (left grid) and of cases with one or more such alterations (right grid). Data are from TCGA. TCGA, The Cancer Genome Atlas.

TP53, *SMAD4*, *CDKN2A* and *CDKN2B* alterations. *ATM* is a pancreatic cancer predisposition gene, with individuals carrying germline pathogenic mutations in the gene having over 6 times the risk of non-carriers to develop pancreatic cancer (31). Results from the four series examined here

show that a small number of cases without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations possess *ATM* mutations. Thus, targeting these mutations therapeutically remains an option in this subset of pancreatic cancers should effective therapies become available. Preclinical studies

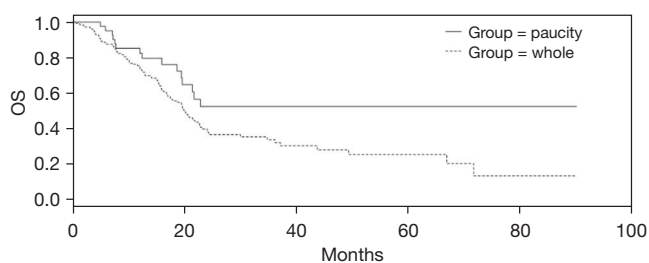


Figure 3 OS of patients without common pancreatic cancer molecular alterations (group paucity: continuous line) and the entire pancreatic cancer cohort of TCGA (group whole: interrupted line). Log Rank $P=0.03$. OS, overall survival; TCGA, The Cancer Genome Atlas.

suggest that although cancer cells with *ATM* mutations are resistant to PARP inhibitors induced apoptosis, undergoing cell cycle arrest, they are sensitive to a combination of PARP inhibitors with ATR inhibitors (32). The combination of olaparib with ATR inhibitor ceralasertib (AZD6738) led to cell death of *ATM* deficient cells within 1 to 2 cell divisions, while olaparib monotherapy required multiple replications (33). Besides ATR, another related kinase with a major role in DNA double strand break repair is DNA-PK, which, when recruited in break sites promotes an error prone repair through non-homologous end joining (34). In pancreatic cancer cells and human xenografts with *ATM* mutations, combinations of PARP inhibitors with ATR and DNA-PK inhibitors were effective in inhibiting growth and in preventing selection of chemotherapy resistant clones (35,36).

The other comparatively frequently mutated DDR associated gene in pancreatic cancer, *BRCA2* showed no mutations in pancreatic cancers without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations in 3 of the 4 examined series, but in the project GENIE pancreatic cohort who had higher numbers, 2.9% of cases without such alterations had *BRCA2* mutations. This suggests that *BRCA2* mutations must be excluded in these cases similar to the rest of pancreatic cancers, in view of the therapeutic option for treatment with PARP inhibitors and the sensitivity to platinum based chemotherapy (37-39).

GNAS encodes for the $G\alpha_s$ sub-unit of a heterotrimeric G protein which signals through the cAMP/protein kinase A (PKA) pathway and the Wnt/beta-catenin pathway (40). *GNAS* has been identified in the original TCGA report as one of the oncogenes often encountered in pancreatic cancers with wild type *KRAS* (5). *GNAS* mutations

at codon R201 are common in intraductal papillary mucinous neoplasms (IPMN) of the pancreas, especially the intestinal and colloid subtypes (41). These neoplasms may also possess *KRAS* mutations, which are not mutually exclusive with *GNAS* mutations. IPMN derived invasive carcinomas [also called intraductal papillary mucinous carcinomas (IPMC)] possess also *GNAS* mutations, and are concordant with the precursor lesions for *GNAS* and *KRAS* mutations, while accumulating further mutations in *TP53*, *SMAD4* and *CDKN2A* (42,43). The intestinal and colloid subtypes of IPMC that possess more frequently *GNAS* mutations have a better prognosis than the usual pancreatic adenocarcinomas which harbor *GNAS* mutations rarely (44). In contrast the tubular subtype of IPMC display a lower prevalence of *GNAS* mutations and a higher prevalence of *KRAS* mutations and behave similarly to pancreatic adenocarcinomas of the usual histology (45). The *GNAS*/PKA pathway inhibits NOTCH signaling and may decrease cancer cell invasion and metastasis, which is consistent with a better prognosis of cancers with *GNAS* mutations (46). From a therapeutic point of view, the obvious implication is that, if inhibitors of the pathway become available, their development in established invasive cancers, which possess additional alterations, should proceed with caution. A more straightforward therapeutic candidate for pancreatic cancers with *GNAS* mutations and colloid histology is immunotherapy with checkpoint inhibitors, as these cancers may be more commonly deficient in MMR or MSI-H than pancreatic cancers with usual histology (47). However, in the pancreatic cancer cases without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations from the four series examined here, most have a low TMB and mutations in MMR associated genes or proofreading polymerases were rare.

ARID1A (AT-rich Interaction Domain-containing protein 1A), encoding for a subunit of chromatin modifier complex SWI/SNF, is mutated in 5% to 10% of pancreatic adenocarcinomas and although only mutated in 1 case (2.7%) without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations from the TCGA cohort it displays a prevalence of 6.3% in the same group of the project GENIE cohort. ARID1A functions to maintain differentiation of pancreatic ductal cells through expression of transcription factor SOX9 and activation of the mTOR pathway, as measured by phosphorylation of protein S6 (48). Loss of arid1a in mouse pancreas leads to acinar to ductal metaplasia and, when associated with *KRAS* and *TP53* mutations, to development of cancer with short

latency and poor differentiation (49). This loss promotes epithelial-mesenchymal transition in human pancreatic cells and sensitizes human pancreatic cancer xenografts to treatment with HSP90 inhibitor NVP-AUY922, by destabilizing client protein vimentin, which is upregulated in ARID1A null cells (50). Decreased activity of ARID1A is required for development of pre-cancerous lesions in the pancreas, including pancreatic intraepithelial neoplasia, intraductal tubulopapillary neoplasm and IPMN (51). ARID1A as part of the SWI/SNF complex participates in DNA repair, interacting with kinase ATR and promoting ATR activation in double strand breaks and induction of the G2/M DNA damage checkpoint (46). Cancer cells with ARID1A deficiency are sensitive to PARP inhibitors olaparib, veliparib, rucaparib and BMN673 (52). Moreover, a case report of a metastatic pancreatic cancer patient with a pathogenic *ARID1A* mutation (and concomitant *KRAS* and *TP53* mutations in this case) showed a response lasting for over a year (53). Pancreatic cancer patients with SWI/SNF mutations, such as *ARID1A*, *ARID1B*, *SMARCA4* and *SMARCB1*, are reported to benefit from immunotherapy with checkpoint inhibitors, independently from their MSI status, a finding awaiting prospective validation (54).

BCOR (BCL6 transcription corepressor), another epigenetic modifier and a member of Polycomb Repressive Complex 1 (PRC1), is mutated in a small percentage of pancreatic cancers without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations. Mutations in BCOR and related PRC1 subunit BCORL1 are involved in leukemogenesis, leading to dysfunction of PRC1 and oncogenic signaling through derepression of normally suppressed genes (55). Although this molecular defect is not currently targeted directly, data from lung cancer suggest that BCOR mutations as well as mutations in two other epigenetic modifiers, *KMT2C* and *KDM5C*, may be predictive of response to immune checkpoint inhibitors (56).

Fusions involving *NRG1*, the gene encoding for the HER3 ligand Neuregulin 1, are observed in a subset of cases without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations. These and a few other fusions are enriched in cases without the common pancreatic cancer molecular alterations compared with pancreatic cancers presenting with these alterations. *NRG1* fusions were previously reported in pancreatic cancer patients without *KRAS* mutations and are present in other cancers, including lung, ovarian and endometrial cancers (57,58). The partner in these fusions varies but the *NRG1* part consistently contains the EGF-like domain of the protein, which enables

HER3 binding and oncogenic signaling downstream through the *KRAS*/*BRAF*/*MEK* and the *PI3K*/*AKT* pathways (59). Thus, *NRG1* fusion containing pancreatic cancers possess an alternative way of activating *KRAS* (60). Targeting with anti-HER3 monoclonal antibodies in development may offer a viable therapeutic option in this subset of molecularly defined pancreatic cancers (61-63). Other rare fusions that are present in pancreatic cancers with or without common alterations present targeted therapeutic opportunities (63).

Studies based on genomic profiles of pancreatic adenocarcinomas have shown that, similar to other cancers, the disease is heterogeneous and can be classified into four sub-types: squamous, pancreatic progenitor, aberrant differentiation endocrine-exocrine (ADEX) and immunogenic (64). The squamous sub-type is characterized by mutations in *TP53* and *KDM6A* and down-regulation of endodermal cell fate determiner genes *PDX1*, *MNX1*, *GATA6* and *HNF1B* (64). This sub-type corresponds to the quasi-mesenchymal sub-type of another genomic classification proposed by Collisson *et al.* (65). In contrast, the pancreatic progenitor sub-type, corresponding to the classical sub-type of Collisson *et al.*, shows up-regulation of *PDX1*, *MNX1* and *HNF1B* as well as of *HNF4G*, *HNF4A*, *HNF1A*, *FOXA2*, *FOXA3* and *HES1* (64). The ADEX subtype, corresponding to the exocrine-like sub-type of Collisson *et al.*, is characterized by both up-regulation of exocrine transcription factors such as *NR5A2*, *BHLH1A15* and *RBPJL* and up-regulation of endocrine differentiation markers such as *INS*, *NEUROD1*, *NKX2-2* and *MAFA*. Several cases in the group of pancreatic cancers without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations in TCGA cohort show up-regulation of these exocrine and endocrine defining genes, suggesting that they align with the ADEX sub-type. In addition, a few cases have immune checkpoint proteins up-regulation suggesting that they belong to the immunogenic sub-type. Genomic classifications have yet to be validated as predictive markers of therapies and their clinical value remains to be determined (66). However, they may imply different pathogenesis of sub-types, which could facilitate successful rational targeted therapies development. Thus, membership of cases without common alterations in the ADEX or immunogenic sub-type may allow their inclusion in therapeutic trials aiming at these groups.

OS of the group of cancers without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations in TCGA cohort is better than OS of counterparts with common

alterations (Log Rank $P=0.03$, *Figure 3*). In contrast, the OS comparison between the two groups in the MSK-IMPACT series, disclosed no differences (Log Rank $P=0.4$, not shown). This inconsistency may be due to differences in the stage of included cases as the group without *KRAS*, *TP53*, *SMAD4*, *CDKN2A* and *CDKN2B* alterations in TCGA contained a significantly higher percentage of stage I patients, while the two groups in the MSK-IMPACT cohort which showed no survival difference, had no significant different stages distribution. Alternatively, absence of common alterations may have different prognostic implications according to the stage of the disease. Indeed, absence of the most common *KRAS* G12D mutations were not prognostic in a cohort of pancreatic cancer patients across stages, but were associated with a better survival compared with patients that had mutated cancers in localized resectable disease (67).

Conclusions

In conclusion, the sub-set of pancreatic cancers without the common molecular alterations characterizing the disease has a prevalence that is somewhat variable between series but is between 5% and 10% in most series. Although their prognosis stage by stage may not be distinct from cases with common alterations, these cases are a group still possessing molecular lesions of therapeutic interest. These alterations could serve as therapeutic targets, as additional molecularly based therapies are introduced in the clinic.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at <https://cco.amegroups.com/article/view/10.21037/cco-22-108/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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