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Original Article

Genomic alterations in tumor tissue and ctDNA from Chinese pancreatic cancer patients

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Abstract: Though the genomic feature of pancreatic cancer has been comprehensively studied in western patients, the genetic feature of Chinese patients is poorly clarified. In this study, a total of 225 pancreatic cancer patients were enrolled, mainly pancreatic ductal adenocarcinoma (PDAC, 97.33%). 140 patients (62.22%) provided sufficient tumor tissues for genomic analysis, and the rest (37.78%) were provided serum instead. Utilizing target next-generation sequencing (NGS), we analyzed genomic alterations of 618 selected genes. Corresponding data in the TCGA database were also analyzed here. In total, 26 (11.61%) patients had pathogenic or likely pathogenic germline variants, mainly (84.62%) involved genes in the DNA damage repair (DDR) pathway. The mean and median counts of somatic alterations per sample were 6.28 and 5, respectively. The most frequently mutated genes in our cohort were *KRAS*, *TP53*, *CDKN2A*, *SMAD4*, *FBXW7* and *ARID1A*, revealing a significantly different prevalence of genes including *KRAS*, *CDKN2A*, *ARID1A*, *NOTCH1*, *ARID1B* than the corresponding data in the TCGA database. 39.11% of patients were identified with actionable alteration and the ratio was not significantly different between tissue and serum samples. 22.67% of patients harbored DDR gene alterations, which were associated with a higher tumor mutation burden. We also found that all the DDR alterations were not correlated with the overall survival and immune and stroma score, but the changes in NK cells and follicular T cells were identified in samples with DDR changes according to TCGA database. In summary, we identified a distinct genomic feature of Chinese pancreatic cancer patients by comparing with the data in TCGA database, and suggested the role for genetic testing using tissue or ctDNA samples in decision-making process. DDR alterations were associated with a higher tumor mutation burden and the significantly higher counts of NK cells in DDR altered samples may raise the attention in future related drugs development.

Keywords: Pancreatic cancer, genetic alterations, DNA damage repair, germline, ctDNA

Introduction

Pancreatic cancer (PC) is one of most lethal malignancies globally, accounting for nearly 4.5% of cancer-related deaths in 2018 [1]. As reported by the Chinese national cancer center, the estimated newly-diagnosed and death cases of pancreatic cancer in China in 2015 were 4,292,000 and 2,814,000, respectively, which was regarded as the sixth most common lethal carcinoma in China [2]. Though the treatments and survivals of cancer have been continuously improving in the past decade, the 5-year survival rate of pancreatic cancer patients in China is only around 7%, lower than the USA's corresponding data (approximately 10%) [3]. To date, surgical resection remains the only chance for pancreatic cancer patients to cure; for patients with advanced or metastatic disease, systemic chemotherapy was the primary treatment, but the overall survival was still less than a year (6.8 to 11.1 months).

The genomic feature of western pancreatic cancer patients has been comprehensively studied [4]. Notably, up to 25% of advanced pancreatic cancer patients in western countries were found to have actionable genomic alterations [5]. Precision medicine-related studies in western pancreatic cancer patients, especially the Know Your Tumor (KYT) program, have demonstrated precision medicine based on personal genomic alterations had significantly improved both the patient's progression-free survival and the overall survival [6, 7]. Among them, DNA damage repair (DDR) alterations, especially *BRCA1/2*, were the actionable alterations with the strongest evidence, as PARP inhibitor olaparib has been approved by the U.S. food and drug administration (FDA) as the maintenance therapy in metastatic pancreatic cancer patients with this deficiency [8]. Meanwhile, though many previous have suggested the positive correlation between alterations and immune checkpoint inhibitors (ICIs), whether this prediction role suits for PC patients remains vague, especially considering the specific tumor microenvironment of PC and previous poor performance of ICIs in it [9, 10]. Since this, the genomic landscape and potentially actionable alterations especially DDR pathways in Chinese pancreatic cancer patients have not been much clarified.

Meanwhile, genetic testing on the tumor tissue is the gold standard for valid genomic classification in multiple solid tumors. However, as nearly 50-60% of pancreatic cancer patients have advanced or metastatic disease at diagnosis, it is hard to achieve enough or validly-achieved tumor tissue for genetic profiling [11]. Circulating tumor DNA (ctDNA) has been comprehensively studied as an emerging source for early disease detection, prognosis and response prediction in pancreatic cancer [12, 13]. Though it is still full of controversy to determine whether ctDNA could substitute tumor tissue for genetic profiling, a recently-published study has found the utilization of ctDNA could significantly reduce two-thirds of screen duration but doubled the enrollment rate than the tissue in advanced gastrointestinal cancer, highlighting the value of ctDNA in precision medicine [14].

We designed this study to determine the genomic feature, especially DDR deficiency, in the tumor tissue and ctDNA sample from Chinese pancreatic cancer patients. A comparison of genomic features between tumor tissue and ctDNA samples would provide evidence for the application value of ctDNA in the future.

Materials and methods

Samples source and ethic data

A total of 225 pancreatic cancer patients were enrolled in our cohort from November 2017 to April 2020. The study was approved by Shanghai Pulmonary Hospital Ethics Committee and the study was performed in accordance with the Declaration of Helsinki. All sample collection wasn't during the cycle of chemotherapy, target therapy and/or immunotherapy. 140 patients (62.22%) provided sufficient tumor tissues for genomic analysis, and the rest (37.78%) who didn't have valid tumor tissues provided serum for ctDNA collection instead. Among patients who provided tumor tissue samples, 16 of them provided additional matched serum sample for genetic testing. Utilizing target next-generation sequencing (NGS), we analyzed genomic alterations of 618 selected genes.

DNA isolation

The FFPE tissues and peripheral blood mononuclear cells were collected to extract DNA using DNeasy Blood & Tissue Kit (Qiagen, Inc.) under the manufacturer's instructions. Cell-free DNA (cfDNA) was extracted from serum using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Inc.) following the protocol of the manufacturer. The purified gDNA and cfDNA were quantified using the Qubit 3.0 Fluorometer (Life Technologies, Inc.) and StepOnePlus System (Life Technologies, Inc.).

Target next-generation sequencing

For the matched germline and tumor samples, 100 ng of DNA was sheared with a Covaris E210 system (Covaris, Inc.) to get 200 bp fragments. We performed next generation sequencing of tumor and gDNA matched germline DNA using Accel-NGS 2S DNA Library Kit (Swift Biosciences, Inc.) for library preparation and xGen Lockdown Probes kit (IDT, Inc.). The custom xGen Lockdown probe was synthesized by IDT, Inc. for the exons and selected intronic regions of 618 genes (Supplementary Table 1). The prepared library was quantified using the Qubit 3.0 Fluorometer (Life Technologies, Inc.),

and quality and fragment size were measured using Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Samples underwent paired-end sequencing on an Illumina Nextseq CN500 platform (Illumina Inc.) with a 150-bp read length. Mean coverage beyond 1250×, 3700× and 220× were achieved for tumor gDNA, blood cfDNA and peripheral blood mononuclear cells gDNA, respectively.

Data processing

Raw sequencing data were aligned to the reference human genome (UCSC hg19) through Burrows-Wheeler Aligner and producing a binary alignment/map (BAM) file. After the duplicate removal and local realignment, the Genome Analysis Toolkit (GATK) was used for single nucleotide variation (SNV), short insertions/deletions (indels) calling. Variants were annotated using the ANNOVAR software tool. Variants identified in gDNA from buffy coat fraction aliquots with allele fraction (AF) beyond 25% were determined as germline variants, and variants were further removing with frequency ≥1% in ExAC (http://exac.broadinstitute.org), 1000 Genomes (http://www.1000genomes.org) or ESP6500 databases (https://evs.gs.washington.edu/EVS). The interpretation of germline variants followed the standards and guidelines of American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) and independently reviewed by two genetic consultants [15].

Somatic variants with AF beyond 1% in tumor tissue and 0.5% in ctDNA were generated from each tumor gDNA by removing the germline variants and further annotated according to the Catalog of Somatic Mutations in Cancer (COSMIC) database. The functional classification of each somatic mutation was followed the interpretation and reporting standards and guidelines recommended by the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists (ASCO/CAP) [16].

The tumor mutation burden of each sample was calculated according to a published and widely applied method [17]. Genomic alterations data of western pancreatic patients from The Cancer Genome Atlas database (TCGA) was downloaded from cBioPortal (http://www.cbioportal.org).

Tumor-infiltrating immune cells analysis

Tumor-infiltrating immune cells counts were analyzed based on RNA-seq data from the PC samples in TCGA by using a CIBERSORT R package [18].

Statistical analysis

Differential mutations analysis was performed using Chi-Square test or Fisher exact test under a dominant model. Two-sided *P* values less than 0.05 were considered to be statistically significant. All analyses were performed using SPSS 25.0 software.

Results

Patient characteristics

225 Chinese patients with pancreatic cancer were enrolled in this study with a median age of 63 (range, 35 to 93 years). Enrolled patients were mainly pancreatic ductal adenocarcinoma (PDAC, n=219, 97.33%), and the rest were pancreatic neuroendocrine carcinoma (PNEC, n=6). The clinical characteristics of all cases were listed in **Table 1**.

Pathogenic or likely pathogenic germline variants

26 patients (11.61%) carried pathogenic or likely pathogenic (P/LP) germline variants, mainly involving genes in DDR, RTK/MAPK and pancreatitis pathway (**Table 2**). 9.82% (22/225) of patients had deleterious germline variants in the DDR pathway, including 8 (3.56%) with *BRCA2* variants, 4 (1.78%) with *ATM*, and one (0.44%) of each with *BRCA1*, *BRIP1*, *FANCC*, *FANCG*, *FANCI*, *MSH6*, *MUTYH*, *PALB2*, *RAD50* and *RAD51D*, respectively. The median age at diagnosis of carriers of P/LP germline variants, DDR genes P/LP and BRCA1/2 P/LP were all 57 years old, which was significantly younger than the patients without any P/LP germline variants in our cohort (median 63 years old, P<0.05). Two rare and likely pathogenic germline variants, *FGFR4* D127H and *ERBB4* E542K, were identified in a specific individual, respectively, which had not been reported in PDAC before. Another two (0.88%) carriers of chronic pancreatitis related *SPINK1* c.194+2T>C variant were identified. No carrier of P/LP germline variant in other previous-reported genes related to PDAC

Table 1. Clinical characteristics of 225 patients with pancreatic cancer

Variable		N (%)
Total		225
Age	Median (range)	63 (35-93)
Gender	Male	26 (11.56%)
	Female	199 (88.44%)
Histologic type	PDAC	219 (97.33%)
	PNEC	6 (2.67%)
AJCC Prognostic Groups	I	15 (6.67%)
	II	66 (29.33%)
	III	22 (9.78%)
	IV	115 (51.11%)
	NE	7 (3.11%)
Distant metastasis status	M0	110 (48.89%)
	M1	115 (51.11%)
Sample Type	Serum	85 (37.78%)
	Tumor	140 (62.22%)

NE: none evaluation.

Predisposition, including *CDKN2A*, *TP53*, *MLH1* and *MSH2*, was identified in our cohort.

Somatic genomic alterations in Chinese pancreatic cancer patients

After removing the germline variants, 1,405 somatic variants were identified in our cohort. The mean and median counts of somatic alterations per sample were 6.28 and 5, respectively. The most frequently mutated genes in the patients were *KRAS* (64.60%), *TP53* (55.75%), *CDKN2A* (17.26%), *SMAD4* (17.26%), *FBXW7* (11%), and *ARID1A* (10.18%), respectively (**Figure 1A**). The most frequent mutated signaling pathway included RAS/RAF/MAPK (78.22%), cell cycle (63.56%), Epigenetic_modifiers/Chromatin_remodelers (37.78%) and DNA damage repair (DDR, 36.00%) and others (**Figure 1B**).

Differences of genetic alterations in pancreatic cancer patients between Chinese cohort and TCGA cohort

To determine the potential differences of genomic features between Chinese and Western pancreatic cancer patients, we compared the gene alterations data of the selected 618 genes between our cohort and western cohort (published by TCGA). The prevalence of alterations in *KRAS* was significantly higher in the TCGA database (78.57% vs. 90.67%, P< 0.001), while genes including *CDKN2A* (22.86% vs. 14.67%), *ARID1A* (11.43% vs. 5.33%), *NOTCH1* (6.43% vs. 1.33%) and *ARID1B* (5.71% vs. 0.67%) were significantly more mutated in the tissues sample in our cohort (**Figure 2A**). Similarly, *KRAS* alterations were more prevalent in the TCGA database when comparing with the genetic alterations in the serum samples in our cohort (90.67% vs. 43.53%), along with a higher incidence of alterations in *TP53* (69.33% vs. 45.88%) and *SMAD4* gene (24.67% vs. 10.59%) (**Figure 2B**). The prevalence of specific *KRAS* alterations was similar between Chinese and TCGA cohort, both with dominant variants in Gly12Asp and Gly12Val (Supplementary Figure 1A, 1B). Notably, a higher prevalence of Gly12Arg was identified in the TCGA cohort (19.9% vs. 9.5%, P<0.05) (Supplementary Figure 1C). There were only two patients (0.89%) identified with coexistence of *KRAS* alterations (one had Gly12Asp with Gly13Asp, and another had Ala146Val with Gly12Asp), which was also rare in the TCGA cohort (2.67%, P=0.2228).

Differences of genetic alterations between the tumor tissue and serum samples

According to the OncoKB Levels of Evidence V2 (12/20/2019), 88 patients (39.11%) with 27 actionable genes were identified in our cohort (**Table 3**). The frequency of samples with actionable alterations for tissue and blood was 43.57% and 30.59% (P=0.063), respectively. The actionable genes mainly confer sensitivity to PARP inhibitor (*BRCA*1/2, *ATM* and other homologous recombination repair genes), and meanwhile, rarely actionable alterations in *EGFR*, *FGFR* and *BRAF* may confer sensitivity in related RTK inhibitors. 82 patients with sufficient tumor tissues had microsatellite instability test, though none of them was microsatellite instability-high (MSI-H). Though eight patents (3.56%) were identified as having alterations in mismatch repair (MMR) genes, only one *MLH1* variant (0.44%) was annotated as deleterious. The rare incidence of deficiency MMR in Chinese cohort was in concordance with the findings in the TCGA dataset, as only two patients harbored alterations in MMR genes

Table 2. Pathogenic or likely pathogenic germline variants in 225 patients with pancreatic cancer

Sample ID	Age at diagnosis	Gene	Transcript ID	Mutation Type	Exon	Coding sequence change	Protein change	Heterozygous/Homozygous
CH165	44	*ATM*	NM_000051	Stopgain	38	c.5697C>A	p.Cys1899*	Heterozygous
CH017	62	*ATM*		Frameshift deletion	3	c.172delG	p.Asp58fs	Heterozygous
CH039	67	*ATM*		Stopgain	37	c.5644C>T	p.Arg1882*	Heterozygous
CH150	68	*ATM*		Splice	15	c.2377-2A>G	-	Heterozygous
CH220	60	*BRCA1*	NM_007294	Stopgain	9	c.607G>T	p.Glu203*	Heterozygous
CH038	45	*BRCA2*	NM_000059	Stopgain	11	c.4363G>T	p.Glu1455*	Heterozygous
CH213	45	*BRCA2*		Stopgain	11	c.5344C>T	p.Gln1782*	Heterozygous
CH202	51	*BRCA2*		Nonsynonymous	15	c.7522G>A	p.Gly2508Ser	Heterozygous
CH063	52	*BRCA2*		Stopgain	11	c.5682C>G	p.Tyr1894*	Heterozygous
CH118	57	*BRCA2*		Stopgain	11	c.3631G>T	p.Glu1211*	Heterozygous
CH143	57	*BRCA2*		Frameshift deletion	11	c.4380_4381delTT	p.Ser1461fs	Heterozygous
CH146	65	*BRCA2*		Stopgain	11	c.2484T>G	p.Tyr828*	Heterozygous
CH148	74	*BRCA2*		Frameshift deletion	11	c.5164_5165delAG	p.Ser1722fs	Heterozygous
CH078	78	*BRIP1*	NM_032043	Frameshift deletion	11	c.1609delC	p.Leu537fs	Heterozygous
CH200	53	*ERBB4*	NM_005235	Nonsynonymous	14	c.1624G>A	p.Glu542Lys	Heterozygous
CH065	50	*FANCC*	NM_000136	Splice	13	c.1330-1G>A	-	Heterozygous
CH061	51	*FANCG*	NM_004629	Stopgain	5	c.572T>G	p.Leu191*	Heterozygous
CH134	47	*FANCI*	NM_001113378	Frameshift deletion	34	c.3622dupC	p.Leu1208fs	Heterozygous
CH025	62	*FGFR4*	NM_213647	Nonsynonymous	4	c.379G>C	p.Asp127His	Heterozygous
CH012	52	*MSH6*	NM_000179	Frameshift deletion	4	c.1804_1805delTC	p.Ser602fs	Heterozygous
CH140	40	*MUTYH*	NM_012222	Nonsynonymous	9	c.704A>G	p.Asn235Ser	Heterozygous
CH044	65	*PALB2*	NM_024675	Frameshift deletion	4	c.472delC	p.Gln158fs	Heterozygous
CH054	68	*RAD50*	NM_005732	Stopgain	23	c.3592C>T	p.Arg1198*	Heterozygous
CH181	77	*RAD51D*	NM_002878	Stopgain	9	c.898C>T	p.Arg300*	Heterozygous
CH047	35	*SPINK1*	NM_003122	Splice	4	c.194+2T>C	-	Heterozygous
CH102	69	*SPINK1*		Splice	4	c.194+2T>C	-	Heterozygous

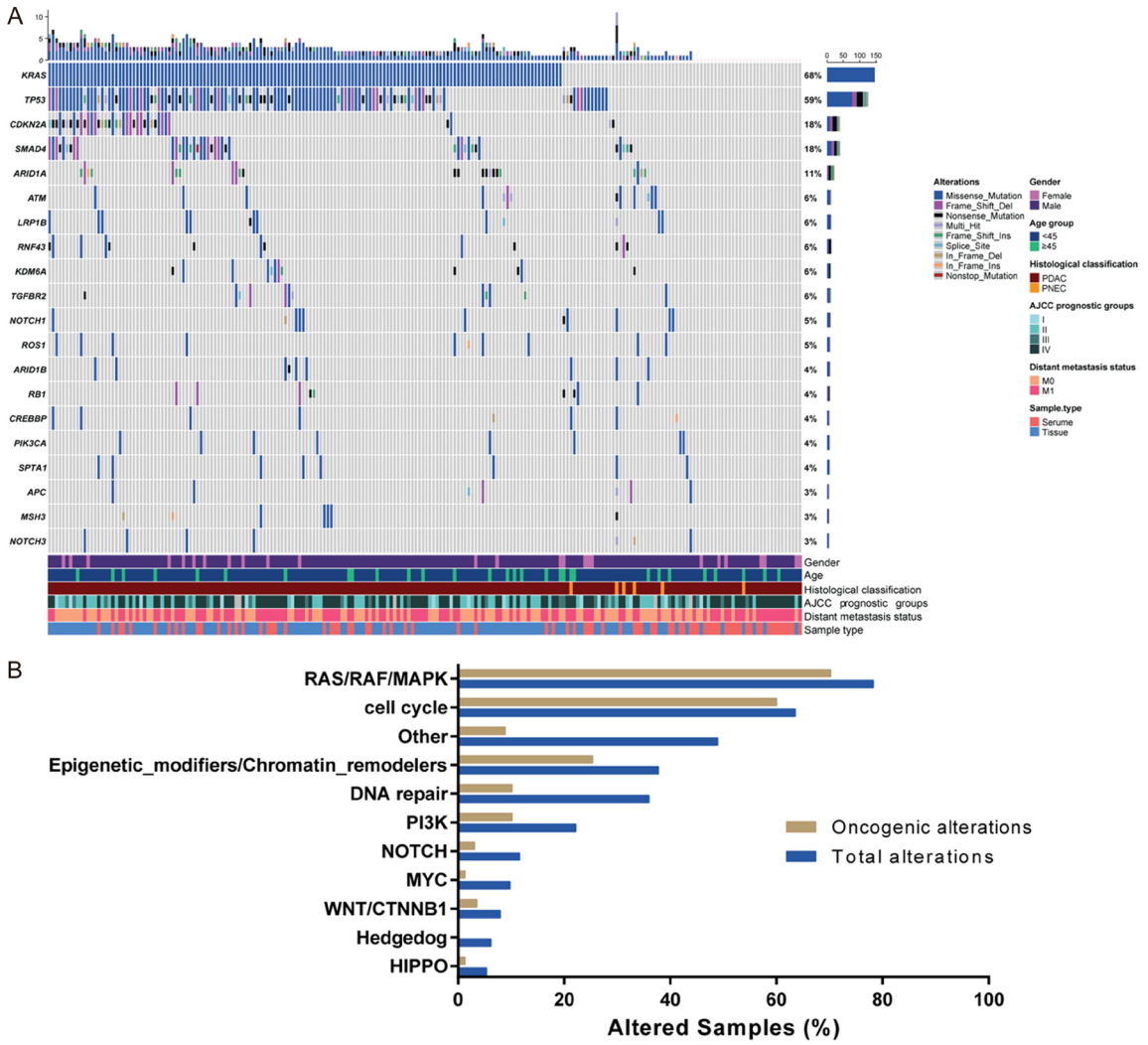


Figure 1. Genetic alterations in 225 Chinese pancreatic cancer patients. A. OncoPrint of the most frequently mutated genes; B. The prevalence of oncogenic and total alterations in specific signal pathway.

and only one deleterious *MSH6* alteration (0.67%) was identified. The median tumor mutation burden (TMB) for tumor tissue and serum samples was 6.39 and 9.33 mutations/Mb, respectively. 36 (25.71%) of tumor tissue samples had a TMB value beyond 10 mutations/Mb, and 11 (12.94%) of serum tissue samples had a blood TMB value beyond 16 mutations/Mb.

To access the concordance of genetic alterations in tissue and serum samples, 15 patients who had tumor tissue testing provided additional matched serum samples. A total of 152 alterations were identified in all the tumor and serum samples (mean =5.06 alterations, median =4 alterations). Though the median concordance for all identified genetic alterations was only 12.12%, the concordance for *KRAS* and *TP53* alterations was 60% and 66.67%, respectively (**Figure 3**). As for the four most prevalent genes (*KRAS*, *TP53*, *SMAD4* and *CDKN2A*) in PDAC, the mean testing sensitivity was 31.55% (range, 0-50.00%), specificity was 87.69% (range, 72.73%-100.00%), and accuracy was 74.41% (64.29%-86.67%) for ctDNA testing.

DDR alterations significantly correlate with high TMB value in PC samples

A total of 51 patients (22.67%) harbored at least one somatic alteration in the selected 37 DNA damage repair genes. The distribution of

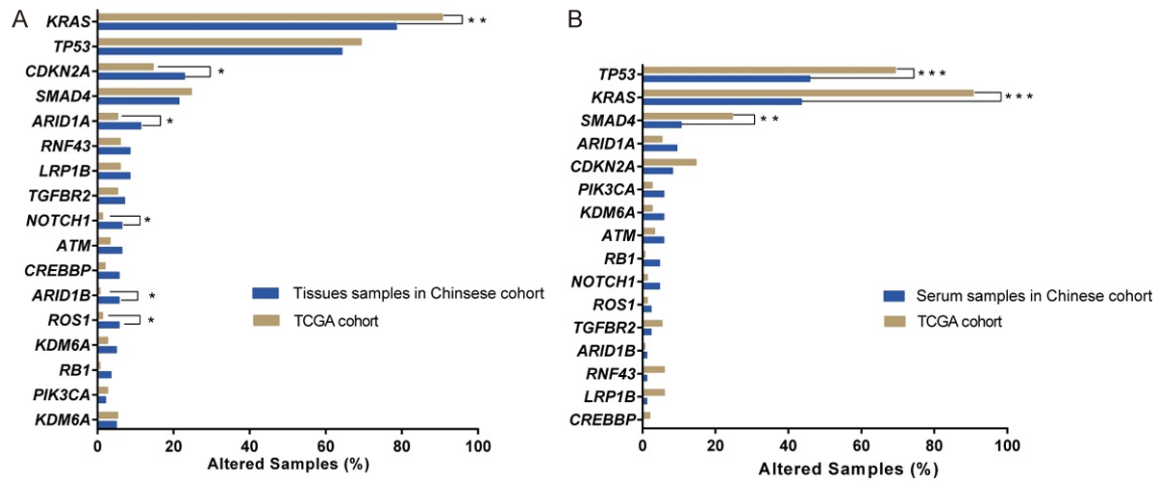


Figure 2. Comparison of the prevalence of mutated genes between the tumor tissue (A) and serum (B) ctDNA samples in Chinese cohort with the TCGA cohort.

specific genes was exhibited in **Figure 4A**, **4B**. The most frequently mutated DDR genes were in Checkpoint (8.67%), Fanconi anemia (8.23%) and homologous recombination repair pathway (5.63%). Meanwhile, the most prevalent genes with deleterious variants were *ATM* (n=7, 31.82%), *PALB2* (n=3, 13.64%) and *BRCA2* (n=3, 13.64%) (**Figure 4C**). There was neither hotspot nor clustered alterations in the *BRCA2* and *ATM* (**Figure 4D**, **4E**). The TMB value was significantly higher in the samples with DDR alterations, regardless of the specific alteration's clinical significance. The median TMB value for samples with potentially deleterious DDR alteration (DelDDRmt), DDR alteration with uncertain significance (DDRmt) and without DDR alteration (DDRmt) was 6.72, 10.27 and 5.23 mutations/Mb, respectively ($P<0.05$, **Figure 4F**). The median TMB value for samples with any DDR alteration (DDRmt (total)) and without DDR alteration (DDRmt) was 9.01 and 5.23 mutations/Mb, respectively ($P<0.001$, **Figure 4G**). Similarly, in the TCGA cohort, samples with deleterious DDR alteration had significantly higher TMB value than samples without any DDR alteration (1.7 vs. 1.1 mutations/Mb, $P<0.001$, **Figure 4H**, **4I**).

DDR and tumor features

To evaluate if DDR alteration correlated with the prognosis in PDAC patients, we compared the overall survival (OS) and disease-free survival (DFS) between patients with and without DDR alterations in TCGA database. Interestingly, changes in DDR, including variants in DNA (**Figure 5A**) and downregulation in mRNA level (**Figure 5B**), were all associated with increasing lymph node metastasis in PC patients. However, instead of DDR alterations, only downregulation in DDR mRNA levels was significantly associated with a worse OS (**Figure 5C**, **5D**). The unique features in enriched signaling pathways of DNA alteration and expression independently were found: more metabolism-related features, including multiple calcium and ion regulation pathways were identified in samples with DDR alterations (**Figure 5E**); otherwise, more immune regulated pathways were found in samples with downregulation in DDR mRNA (**Figure 5F**). DDR alterations were not significantly correlated with stromal score and immune score (**Figure 5G**); instead, downregulation in DDR expression was associated with a significantly lower stromal and immune score (**Figure 5H**). Finally, the tumor immune microenvironment is similar among patients with or without DDR alterations and downregulation. More activated NK cells were identified in DDR altered samples, but higher level of follicular helper T cells and lower-level resting NK cells were only found in samples with downregulation in DDR genes (**Figure 5I**, **5J**).

Discussion

To date, the genomic landscape and potentially actionable alterations in Chinese pancreatic

Table 3. Actionable Alterations identified in our cohort

Level of evidence based on OncoKB (12/20/2019)	Altered genes	Mutational type	No of patients (%)	No of tissue samples (%)	No of blood samples (%)	Related drugs
			88 (39.11%)	61 (43.57%)	26 (30.59%)	
1	*BRCA1*	Germline	1 (0.44%)	0	1 (1.18%)	Olaparib
1	*BRCA2*	Germline	8 (3.56%)	3 (5.71%)	5 (5.88%)	Olaparib
3	*EGFR*	19 exon del or L858R	3 (3.56%)	3 (5.71%)	0	Erlotinib, Afatinib, Gefitinib, Osimertinib, Dacomitinib
3	*FGFR2*	Fusions	1 (0.44%)	1 (0.71%)	0	Erdafitinib, Pemigatinib
3	*ATM*	Oncogenic	12 (14.12%)	11 (7.86%)	1 (1.18%)	Olaparib
3	*BRCA2*	Oncogenic	2 (0.89%)	0	2 (2.35%)	Olaparib
3	*BRIP1*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Olaparib
3	*CHEK2*	Oncogenic	2 (0.89%)	1 (0.71%)	1 (1.18%)	Olaparib
3	*EZH2*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Tazemetostat
3	*IDH1*	Oncogenic	2 (0.89%)	1 (0.71%)	1 (1.18%)	Ivosidenib
3	*IDH2*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Enasidenib
3	*PALB2*	Oncogenic	4 (1.78%)	2 (1.43%)	2 (2.35%)	Olaparib
3	*PIK3CA*	Oncogenic	8 (3.56%)	3 (5.71%)	5 (5.88%)	Alpelisib
3	*RAD51B*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Olaparib
3	*RAD51D*	Oncogenic	2 (0.89%)	2 (1.43%)	0	Olaparib
4	*AKT1*	Oncogenic	1 (0.44%)	1 (0.71%)	0	AZD5363
4	*ARAF*	Oncogenic	2 (0.89%)	0	2 (2.35%)	Sorafenib
4	*BRAF*	Oncogenic	2 (0.89%)	1 (0.71%)	1 (1.18%)	PLX8394
4	*EGFR*	Amplification	1 (0.44%)	1 (0.71%)	0	Afatinib
4	*FGFR1*	Oncogenic	1 (0.44%)	1 (0.71%)	0	AZD4547, BGJ398, Debio1347, Erdafitinib
4	*FGFR2*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Erdafitinib, Debio1347, BGJ398, AZD4547
4	*FGFR3*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Erdafitinib, AZD4547, Debio1347, BGJ398
4	*EGFR*	L747P	4 (1.78%)	4 (2.86%)	0	Afatinib
4	*HRAS*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Tipifarnib
4	*KDM6A*	Oncogenic	7 (3.11%)	5 (3.57%)	2 (2.35%)	Tazemetostat
4	*MAP2K1*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Cobimetinib, Trametinib
4	*MTOR*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Everolimus, Temsirolimus
4	*NF1*	Oncogenic	1 (0.44%)	1 (0.71%)	0	Cobimetinib, Trametinib
4	*KRAS*	G12C	2 (0.89%)	1 (0.71%)	1 (1.18%)	AMG-510
4	*PTEN*	Oncogenic	5 (2.22%)	4 (2.86%)	1 (1.18%)	AZD8186, GSK2636771
4	*CDKN2A*	Oncogenic	37 (16.44%)	31 (22.14%)	6 (7.06%)	Palbociclib, Ribociclib, Abemaciclib

cancer patients have not been clarified. We investigated the somatic and germline alterations profiles in Chinese patients with PDAC here. Overall, 39.11% of investigated patients had at least one actionable genomic alteration that may direct further therapeutic strategy. This ratio was close to the previous published results in the western cohorts [5, 19]. In this study, we also found a unique genetic feature of Chinese PC patients compared with Caucasian patients (**Figure 2B**). A lower incidence of *KRAS* alterations, and a significantly different prevalence of genes, involving *CDKN2A*, *ARID1A*, *NOTCH1* and *ARID1B*, may represent the different pathogenesis and tumor microenvironment between eastern and western PC patients.

Previous studies found that approximately 10-20% of western PDAC patients have hereditary disease; however, the frequency of predisposing genes alteration in Chinese PDAC patients is still poorly clarified [20]. In this study, we identified 11.61% of Chinese PDAC patients had P/LP germline variants, and this ratio was relatively higher than what was found in western cohorts which utilized suggested genetic criteria with limited genes. For instance, researchers in Mayo Clinic identified that 8.2% of 3030 PDAC patients carried deleterious germline variants in 21 selected candidate PDAC predisposition genes [20]. Expanding the sequenced genes, more genes related to PDAC susceptibility may be identified. In 638 patients with familial pancreatic cancer, Roberts

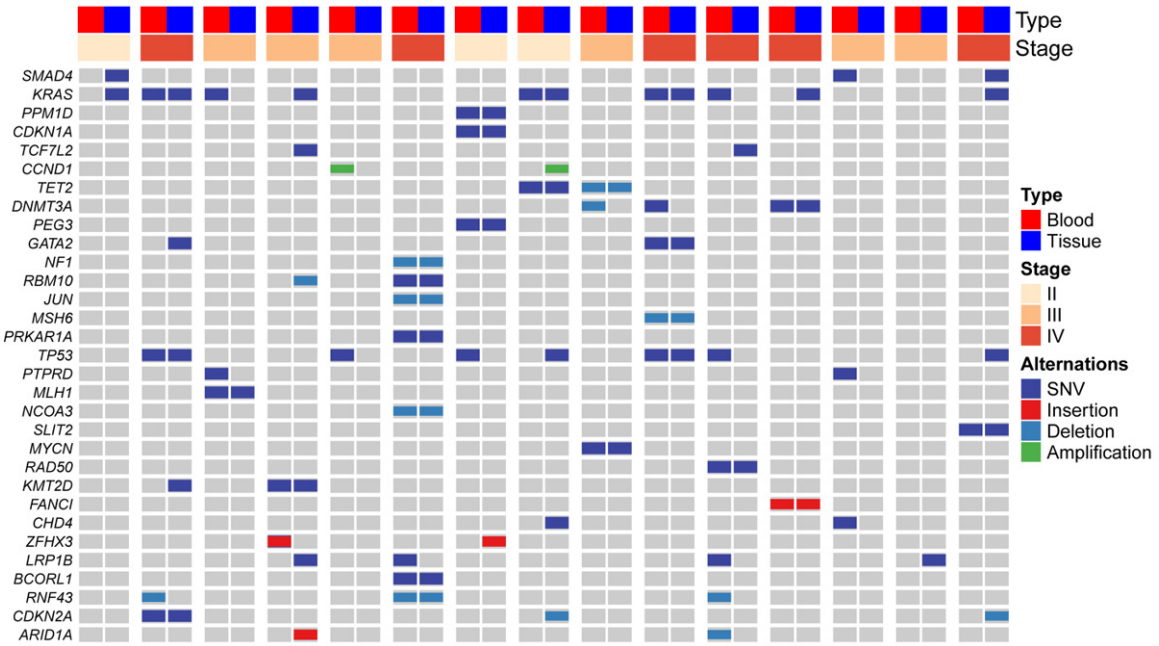


Figure 3. The concordance of genetic alterations between matched tumor tissue and serum ctDNA in 15 PDAC patients.

and colleagues identified novel candidate PDAC predisposition genes, including *POLN*, *POLQ* and *ASXL1,* by using whole exome sequencing [21]. We also identified deleterious germline alterations in genes that were out of guidelines' recommendation and rarely reported before, including *ERBB4* and *FGFR4,* and interestingly, all these two variants were in the RAS/RAF/MAPK pathway and activated the receptor tyrosine kinases [22, 23]. Furthermore, among those patients identified with germline alterations, we identified 36.36% of them carried germline alterations in *BRCA1/2,* which were the most prevalent predisposing and targetable genes in PDAC. This was similar to the frequency identified in the POLO trial, which found 5.9% of 2167 metastatic pancreatic cancer patients had deleterious germline *BRCA1/2* alterations [24]. Meanwhile, we also found two of our patients (0.44%) had chronic pancreatitis-related *SPINK1* c.194+2T>C whose function in the development of PC is still controversial, and our result is the also the first report on its frequency in Chinese PDAC patients [25].

Recently, ctDNA has gained wide attention to overcome the difficulty in real-time collection of biopsy tumor tissue and genetic heterogeneity [26]. It has been proved that ctDNA can be utilized as a biomarker for dynamic monitoring of therapies and prognosis evaluation for both the localized and metastatic PC patients [27, 28]. There was no significant difference in the actionable alteration's identification between tissue and ctDNA samples in our cohort, although we found a relatively poor concordance (12.12%) in the genomic findings between 15 matched tissue-blood samples. Noticeably, Oliver and colleagues reported a 90.3% concordance of alterations in 54 selected genes in 17 advanced pancreatobiliary carcinomas patients all with metastatic disease [29]. The significant differences in concordance may attribute to the differences in detection method, candidate genes involved, disease stage and/or metastasis status. For example, analysis of *KRAS* alteration using digital droplet PCR and NGS, the concordance between matched tissue and ctDNA was 39%-77.3% [30, 31], which was close to our *KRAS* alteration result (60%). As limited by the sample sizes in all past studies, further studies with expanding sample sizes with more comprehensive clinical features should be taken to fully understand the accuracy of ctDNA in genotyping than tumor tissue in pancreatic cancer.

With decades of stagnation in the development of novel and effective treatments in PC,

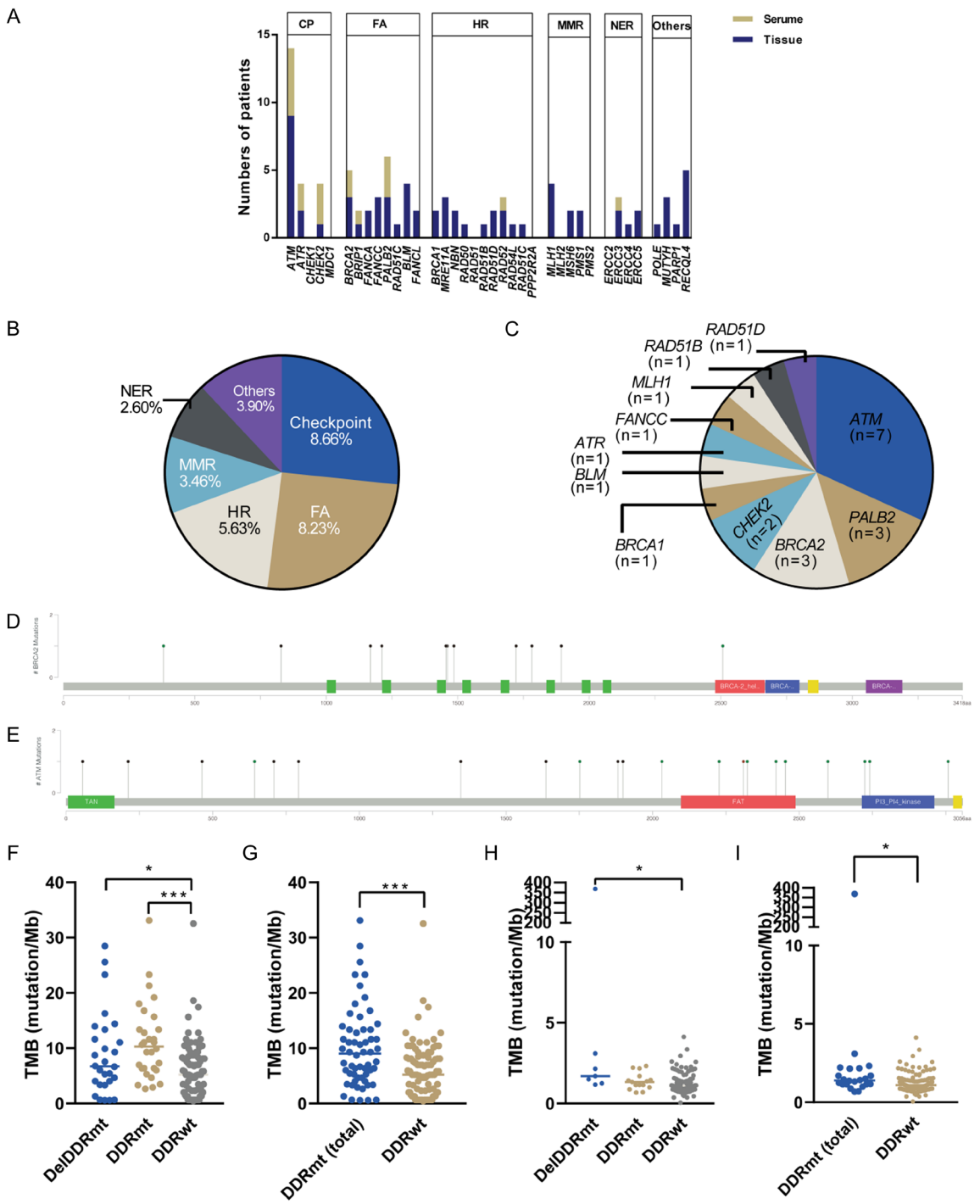
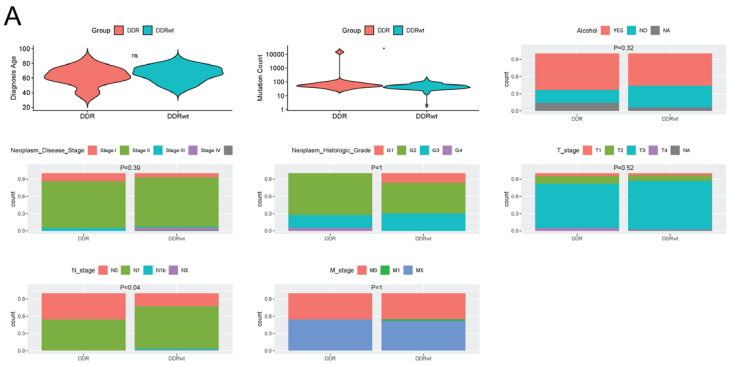
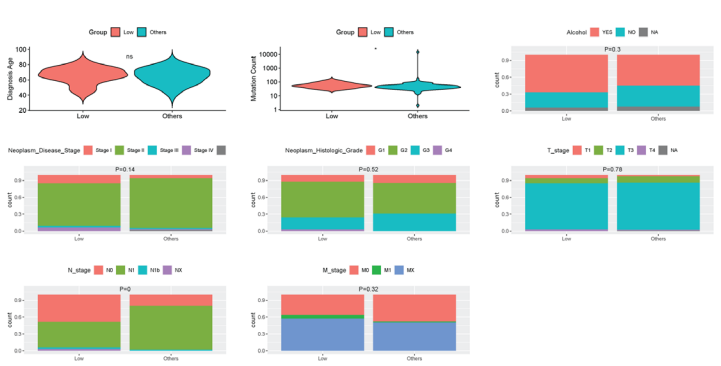


Figure 4. Somatic DNA damage repair (DDR) alteration in Chinese cohort. (A) The prevalence of DDR alterations from tissue (blue) and serum (yellow) samples in specific DDR pathway. (B) The combined prevalence of somatic alterations in different DDR pathways. (C) Number of patients with specific deleterious DDR alterations. The distribution of alterations in *BRCA2* (D) and *ATM* (E). The difference of the TMB value among samples with deleterious DDR alteration (delDDRmt), DDR alteration with uncertain significance (DDRmt) and samples without DDR alteration (DDRwt) in our cohort (F) and in the TCGA cohort (H). The difference of the TMB value between samples with any DDR alteration (DDRmt (total)) and samples witout DDR alteration (DDRwt) in our cohort (G) and in the TCGA cohort (I). *P<0.05; ***P<0.001.

it has been changed since the approval of PARPi for the maintenance therapy of germline *BRCA1/2*-altered patients. Meanwhile, subsequent trials including know your tumor and


A
Group DDR DDRwt
Diagnosis Age
ns
DDR
DDRwt
Group DDR DDRwt
Mutation Count
Alcohol YES NO NA
P=0.32
Neoplasm_Disease_Stage Stage I Stage II Stage III Stage IV
P=0.39
Neoplasm_Histologic_Grade G1 G2 G3 G4
P=1
T_stage T1 T2 T3 T4 NA
P=0.52
N_stage N0 N1 N1b NX
P=0.04
M_stage M0 M1 MX
P=1
count


B
Group Low Others
Diagnosis Age
ns
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Group Low Others
Mutation Count
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P=0.14
Neoplasm_Histologic_Grade G1 G2 G3 G4
P=0.52
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P=0
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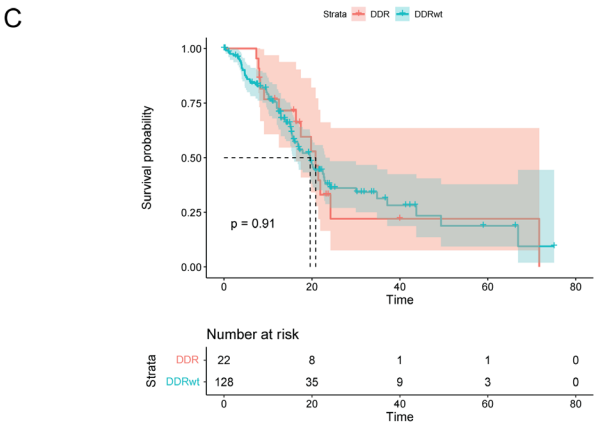
count


C
Strata DDR DDRwt
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.91
0
20
40
60
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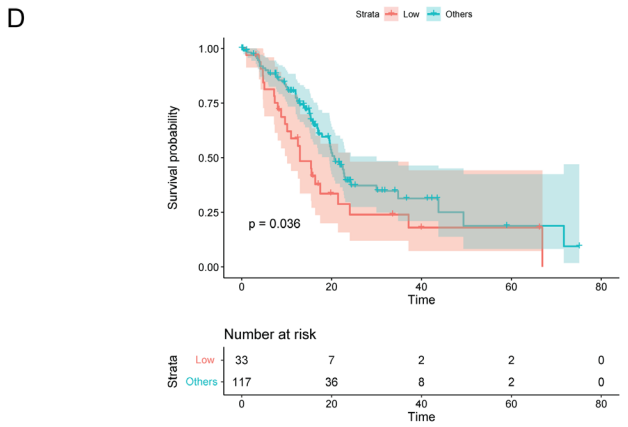
Time
Number at risk
Strata
DDR 22 8 1 1 0
DDRwt 128 35 9 3 0
0
20
40
60
80
Time


D
Strata Low Others
Survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.036
0
20
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Time
Number at risk
Strata
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Others 117 36 8 2 0
0
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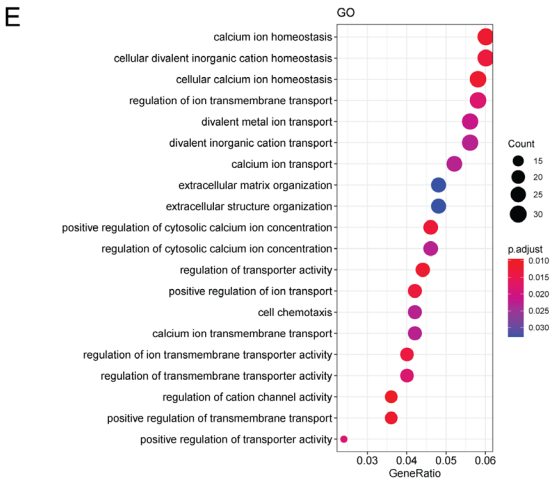
Time


E
GO
calcium ion homeostasis
cellular divalent inorganic cation homeostasis
cellular calcium ion homeostasis
regulation of ion transmembrane transport
divalent metal ion transport
divalent inorganic cation transport
calcium ion transport
extracellular matrix organization
extracellular structure organization
positive regulation of cytosolic calcium ion concentration
regulation of cytosolic calcium ion concentration
regulation of transporter activity
positive regulation of ion transport
cell chemotaxis
calcium ion transmembrane transport
regulation of ion transmembrane transporter activity
regulation of transmembrane transporter activity
regulation of cation channel activity
positive regulation of transmembrane transport
positive regulation of transporter activity
0.03
0.04
0.05
0.06
GeneRatio
Count
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20
25
30
p.adjust
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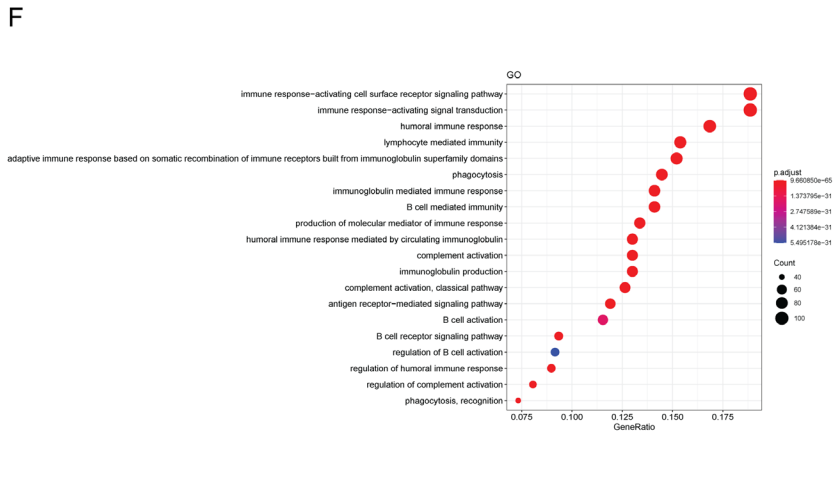
0.015
0.020
0.025
0.030


F
GO
immune response−activating cell surface receptor signaling pathway
immune response−activating signal transduction
humoral immune response
lymphocyte mediated immunity
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains
phagocytosis
immunoglobulin mediated immune response
B cell mediated immunity
production of molecular mediator of immune response
humoral immune response mediated by circulating immunoglobulin
complement activation
immunoglobulin production
complement activation, classical pathway
antigen receptor−mediated signaling pathway
B cell activation
B cell receptor signaling pathway
regulation of B cell activation
regulation of humoral immune response
regulation of complement activation
phagocytosis, recognition
0.075
0.100
0.125
0.150
0.175
GeneRatio
p.adjust
9.660850e−65
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5.495178e−31
Count
40
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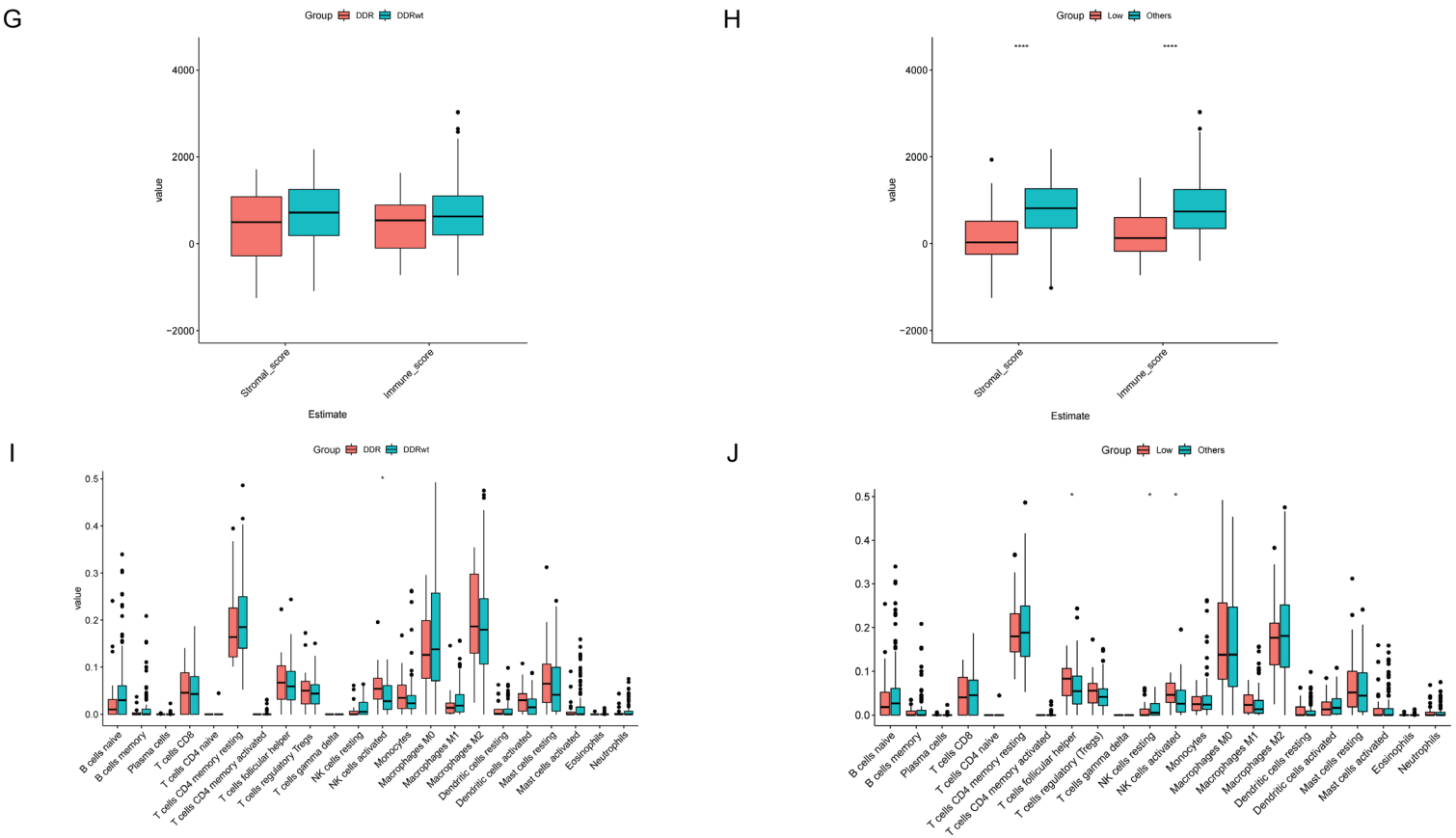

Figure 5. Correlation between tumor DNA damage repair (DDR) changes and tumor feature in PC samples from the TCGA database. The difference of multiple clinical features, including age at diagnosis, mutation count, alcohol use, neoplasm disease stage, histologic stage, Tumor (T) stage, lymph node metastasis (N) and distant metastasis (M) in samples with or without DDR alteration (A) and in samples with or without downregulation in DDR genes (B). The difference of overall survival among samples with or without DDR alteration (C) and in samples with or without downregulation in DDR genes (D). The difference in enriched signaling pathway in samples with or without DDR alteration (E) and in samples with or without downregulation in DDR genes (F). The stromal and immune score in samples with or without DDR alteration (G) and in samples with or without downregulation in DDR genes (H). The infiltrated immune cells status in samples with or without DDR alteration (I) and in samples with or without downregulation in DDR genes (J). *P<0.05; ***P<0.001.

Profound, have proved that except *BRCA1/2*, other genes involved in DDR are also biomarkers for benefit from PARPi treatment [32]. In this study, we identified 22.67% of patients harbored somatic DDR alterations near the findings in the western cohorts (19.1%) [33]. Similar to our findings, previous studies did not find a significant correlation between DDR alterations and the prognosis in PDAC [34]. With prolonging of overall survival by the development of platinum-based regimen-FOLFIRINOX, this trend would be changed due to except from PARPi, it is of note that DDR alteration may correlate with the better response to platinum-based therapy in PDAC [35]. Based on the retrospective study of platinum-based therapies treated PDAC patients, it was found the median progression-free survival (PFS) and/or OS were superior in those with DDR alterations than wild-type patients [36, 37]. Supporting results were also identified in Know your tumor trial, as PDAC patients with homologous recombination genes alterations, the major type of DDR, had a trend of better survival if they were treated with platinum-based therapy (median OS: 1.13 vs. 0.76 years, P=0.1535). Though FOLFIRINOX is the only approved first-line platinum-based therapy for metastatic PC patients, nearly 75% of patients are ineligible for this regimen because of the adverse effects. To select patients with more benefit from platinum-based therapy, especially FOLFIRINOX, the DDR status may contribute to the decision-making.

Furthermore, immune checkpoint inhibitors (ICIs) have made revolutionary improvements in multi-kinds of cancers; nevertheless, they have a poor response rate beyond 10% in PDAC patients except for dMMR/MSI-H positive tumor. The incidence of dMMR/MSI-H was rare in PDAC (0.8% of 833 patients) and not found in our cohort [38]. Meanwhile, patients with DDR alterations, regardless of their specific clinical significance, may have improved response rate to the combined therapy of PARP inhibitor with ICIs, which had shown promising effects in other types of cancer [39]. Whether this correlation suited for PC patients remained unclear, but our findings may provide a clue suggesting the invalid role for DDR changes in predicting the efficacy of ICIs in PC patients. Though TMB value which was another ICIs-response biomarker in pan-cancer was significantly associated with DDR alteration in both our and TCGA cohort, we found relatively lower immune score in patients with changes in DDR genes. Moreover, unlike other cancers, samples with DDR alterations were not presented a higher immune infiltrated immune cell, especially CD8 positive T cells in PC patients, which highlighted the unique immune-suppressed microenvironment of PC. This was also supported by a recently finding that there was no significantly association between MMR/homologous recombination deficiency (HRD) status and CD8 positive T cell infiltrate in PC samples from COMPASS trial [40]. Furthermore, we found a significantly higher counts of activated NK cells in DDR changed samples, shed a light in the application of developing therapies based on NK cells, including NK cell-recruiting protein-conjugated antibody and allogeneic NK cell immunotherapy, which had already showed promising antitumor effects in treating PDAC [41, 42]. Previous studies mainly focused the genetic alterations, and although deleterious alterations in DDR were mainly nonsense or truncating which may mediate mRNA decay, there is no direct correlation between alteration and change of mRNA level [43]. We're the first to identify the expression level of DDR and tumor characteristics in PC. Instead of alterations, we only found the association between PC patients' survival and changes in DDR expression level, suggesting the different function of these two change types. As there was no public data about the genomic feature of PC patients who took ICI therapies, and the limited sample size of our cohort, the potential relationship between DDR and ICI efficacy needs additional studies.

In summary, in this genetic analysis of 225 Chinese PC patients, a different genomic feature was revealed by comparison with western cohort. The prevalence of germline and actionable alterations may suggest that genetic testing in tissue or ctDNA will both provide important guide for further management in Chinese PC patients. Different to other cancers, DDR changes were not associated with a more immune-prone microenvironment in PC but shed a light on therapy relating to NK cells instead.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. The selected intronic regions of 618 genes

Gene
ABL1
ABL2
ACVR1
ACVR1B
ADGRA2
AGO2
AIP
AKT1
AKT2
AKT3
ALK
ALOX12B
AMER1
ANKRD11
APC
AR
ARAF
ARFRP1
ARID1A
ARID1B
ARID2
ARID5B
ASXL1
ASXL2
ATM
ATR
ATRX
AURKA
AURKB
AXIN1
AXIN2
AXL
B2M
BABAM1
BAP1
BARD1
BAX
BBC3
BCL10
BCL11A
BCL2
BCL2L1
BCL2L11
BCL2L2
BCL6
BCOR

BCORL1
BIRC3
BIRC5
BLCAP
BLK
BLM
BMPR1A
BRAF
BRCA1
BRCA2
BRD3
BRD4
BRIP1
BTG1
BTK
BUB1
BUB1B
BUB3
CALR
CARD11
CARM1
CASP8
CBFB
CBL
CCND1
CCND2
CCND3
CCNE1
CD274
CD276
CD74
CD79A
CD79B
CDC25C
CDC42
CDC73
CDH1
CDK1
CDK12
CDK2
CDK4
CDK5
CDK6
CDK7
CDK8
CDK9
CDKN1A
CDKN1B
CDKN1C

CDKN2A
CDKN2B
CDKN2C
CEBPA
CENPA
CHD1
CHD2
CHD3
CHD4
CHEK1
CHEK2
CIC
CREBBP
CRLF2
CSDE1
CSF1R
CSF3R
CTCF
CTLA4
CTNNA1
CTNNB1
CUL3
CXCL8
CXCR4
CYLD
CYSLTR2
DAXX
DCUN1D1
DDB2
DDR2
DICER1
DIRAS3
DIS3
DIS3L2
DNAJB1
DNMT1
DNMT3A
DNMT3B
DOT1L
DROSHA
DUSP4
E2F1
E2F3
EED
EGF
EGFL7
EGFR
EIF1AX
EIF4A2

EIF4E
ELF3
EMSY
EP300
EPAS1
EPCAM
EPHA3
EPHA5
EPHA7
EPHB1
ERBB2
ERBB3
ERBB4
ERCC1
ERCC2
ERCC3
ERCC4
ERCC5
ERF
ERG
ERRFI1
ESR1
ETV1
ETV4
ETV5
ETV6
EWSR1
EXT1
EXT2
EZH1
EZH2
FAM175A
FAM46C
FAM58A
FANCA
FANCB
FANCC
FANCD2
FANCE
FANCF
FANCG
FANCI
FANCL
FANCM
FAS
FAT1
FAT4
FBXW7
FGF10

FGF14
FGF19
FGF23
FGF3
FGF4
FGF6
FGFR1
FGFR2
FGFR3
FGFR4
FH
FLCN
FLT1
FLT3
FLT4
FOLR3
FOXA1
FOXA2
FOXL2
FOXO1
FOXP1
FRS2
FUBP1
FYN
GABRA6
GALNT12
GATA1
GATA2
GATA3
GATA4
GATA6
GEN1
GID4
GLI1
GLI2
GNA11
GNA13
GNAQ
GNAS
GOPC
GPC3
GPS2
GRB2
GREM1
GRIN2A
GRM3
GSK3B
H3F3A
H3F3B

H3F3C
HDAC1
HDAC2
HDAC3
HDAC4
HDAC6
HDAC8
HGF
HIF1A
HIST1H1C
HIST1H2BD
HIST1H3A
HIST1H3B
HIST1H3C
HIST1H3D
HIST1H3E
HIST1H3F
HIST1H3G
HIST1H3H
HIST1H3I
HIST1H3J
HIST2H3C
HIST2H3D
HIST3H3
HLA-A
HLA-B
HNF1A
HOXB13
HRAS
HSD3B1
ICOSLG
ID3
IDH1
IDH2
IFNGR1
IGF1
IGF1R
IGF2
IGF2R
IKBKE
IKZF1
IL10
IL7R
INHA
INHBA
INPP4A
INPP4B
INPPL1
INSR

IRF2
IRF4
IRS1
IRS2
JAK1
JAK2
JAK3
JUN
KAT6A
KDM5A
KDM5C
KDM6A
KDR
KEAP1
KEL
KIT
KLF4
KLHL6
KMT2A
KMT2B
KMT2C
KMT2D
KMT5A
KNSTRN
KRAS
LATS1
LATS2
LMO1
LRP1B
LRRK2
LYN
LZTR1
MAGI2
MALT1
MAP2K1
MAP2K2
MAP2K4
MAP3K1
MAP3K13
MAP3K14
MAP4K1
MAPK1
MAPK3
MAPKAP1
MAX
MCL1
MDC1
MDH2
MDM2
MDM4

MED12
MEF2B
MEN1
MET
MGA
MITF
MLH1
MLH3
MPL
MRE11A
MSH2
MSH3
MSH6
MSI1
MSI2
MST1
MST1R
MTOR
MUTYH
MYC
MYCL
MYCN
MYD88
MYO1B
MYOD1
NAT1
NAT2
NBN
NCOA3
NCOR1
NEGR1
NF1
NF2
NFE2L2
NFKBIA
NKX2-1
NKX3-1
NOTCH1
NOTCH2
NOTCH3
NOTCH4
NPM1
NRAS
NRG1
NSD1
NTHL1
NTRK1
NTRK2
NTRK3
NUF2

NUP93
OPRM1
PAK1
PAK3
PAK5
PALB2
PARK2
PARP1
PARP2
PARP3
PAX5
PBRM1
PDCD1
PDCD1LG2
PDGFRA
PDGFRB
PDK1
PDPK1
PEG3
PGR
PHOX2B
PIK3C2B
PIK3C2G
PIK3C3
PIK3CA
PIK3CB
PIK3CD
PIK3CG
PIK3R1
PIK3R2
PIK3R3
PIM1
PLCG2
PLK1
PLK2
PMAIP1
PMS1
PMS2
PNRC1
POLD1
POLE
PPARG
PPM1D
PPP2R1A
PPP2R2A
PPP4R2
PPP6C
PRDM1
PRDM14
PREX2

PRKAR1A
PRKCE
PRKCG
PRKCI
PRKD1
PRKDC
PRRT2
PRSS8
PTCH1
PTCH2
PTEN
PTK2
PTP4A1
PTPN11
PTPRD
PTPRS
PTPRT
RAB35
RAC1
RAC2
RAD21
RAD50
RAD51
RAD51B
RAD51C
RAD51D
RAD52
RAD54L
RAF1
RARA
RARB
RASA1
RASSF1
RASSF8
RB1
RBM10
RECQL
RECQL4
REL
RET
RFWD2
RHBDF2
RHEB
RHOA
RICTOR
RIT1
RNF43
ROCK1
ROS1
RPS6KA1

RPS6KA4
RPS6KB1
RPS6KB2
RPTOR
RRAGC
RRAS
RRAS2
RTEL1
RUNX1
RUNX1T1
RUNX2
RXRA
RYBP
SDHA
SDHAF2
SDHB
SDHC
SDHD
SERPINB3
SERPINB4
SESN1
SESN2
SESN3
SETD2
SF3B1
SH2B3
SH2D1A
SHH
SHOC2
SHQ1
SLC16A7
SLIT2
SLX4
SMAD2
SMAD3
SMAD4
SMARCA1
SMARCA4
SMARCB1
SMARCD1
SMO
SMYD3
SNAI1
SNAI2
SNCAIP
SOCS1
SOS1
SOX10
SOX17
SOX2

SOX9
SPEN
SPINK1
SPOP
SPRED1
SPTA1
SRC
SRSF2
STAG2
STAT3
STAT4
STAT5A
STAT5B
STK11
STK19
STK40
SUFU
SUZ12
SYK
TAF1
TAP1
TAP2
TBX3
TCEB1
TCF3
TCF7L2
TEK
TERT
TET1
TET2
TFE3
TGFBR1
TGFBR2
TMEM127
TMPRSS2
TNF
TNFAIP3
TNFRSF14
TNFSF11
TOP1
TOP2A
TP53
TP53BP1
TP63
TPX2
TRAF2
TRAF7
TSC1
TSC2
TSHR

TUBB
TWIST1
U2AF1
UPF1
VEGFA
VEGFB
VHL
WEE1
WHSC1
WHSC1L1
WISP3
WNT1
WNT5A
WNT6
WRN
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XIAP
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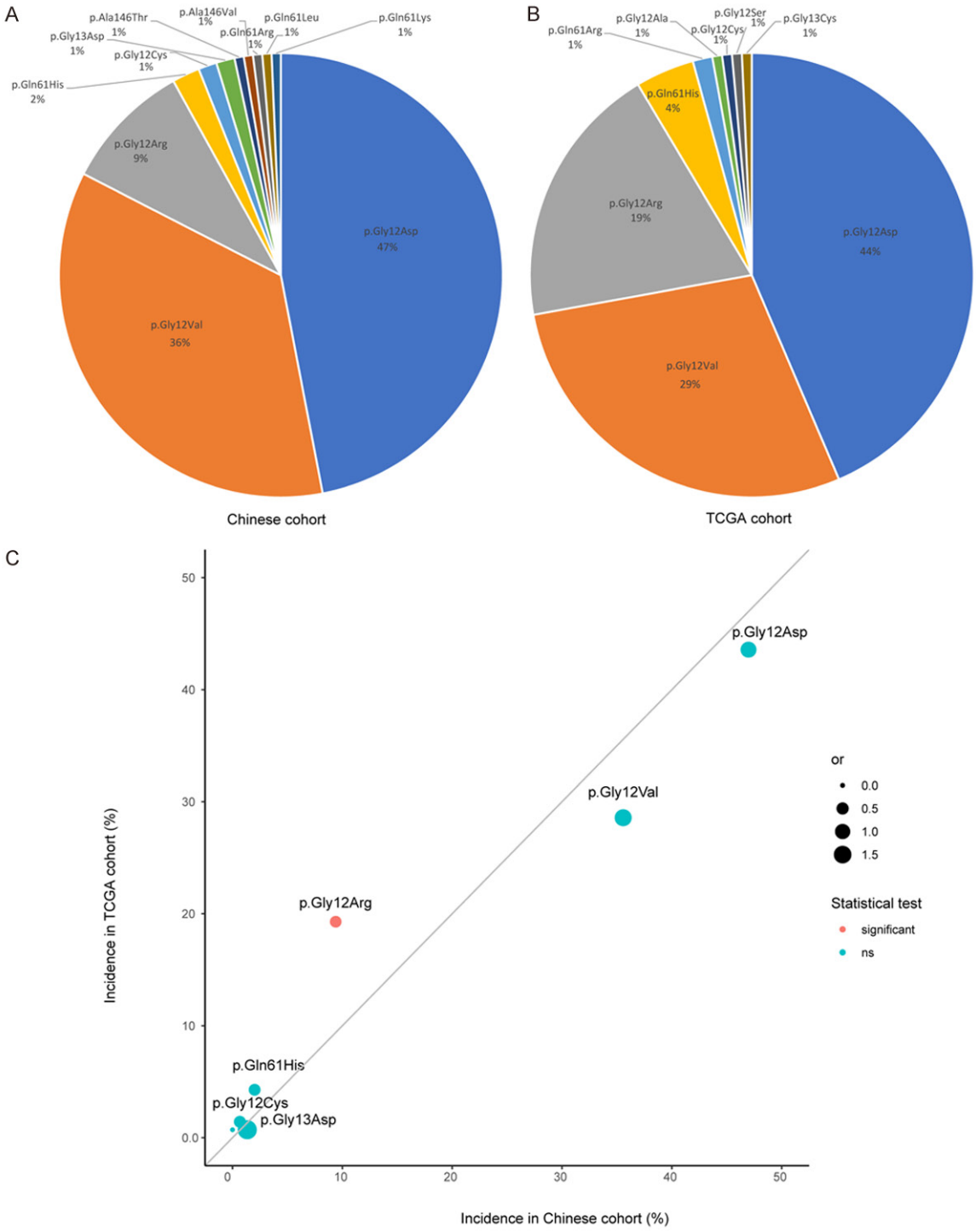
ZNF217
ZNF703



Supplementary Figure 1. Distribution of *KRAS* alterations in Chinese (A) and TCGA cohort (B). (C) Enrichment of specific *KRAS* alterations in Chinese (x axis, n=225) and TCGA cohort (y axis, n=150).