

The Germline/somatic DNA Damage Repair Gene Mutations Modulate the Therapeutic Response in Chinese Patients With Advanced Pancreatic Ductal Adenocarcinoma

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Abstract

Background: PDAC is a fatal disease with molecular heterogeneity, inducing differences in biological behavior and therapeutic strategy. NGS profiles of pathogenic alterations in Chinese PDAC population are limited. We conducted a retrospective study to investigate the predictive role of DNA damage repair (DDR) mutations in precise medicine.

Methods: NGS were performed on resected tissues or peripheral blood from 195 Chinese PDAC patients. Baseline clinical or genetic characteristics, and survival status were collected. The Kaplan–Meier survival analyses were performed by the R version 3.6.1.

Results: The main driver genes were KRAS, TP53, CDKN2A, and SMAD4. Advanced patients with KRAS mutation showed worse OS than those without ($p=0.048$). DDR deficiency was identified in 30 (15.38%) of overall patients, mainly involving BRCA2 ($n=9$, 4.62%), ATM ($n=8$, 4.10%) and RAD50 genes ($n=3$, 1.54%). There was no significant improvement of OS between patients with or without DDR mutations ($p=0.88$). Treatment with platinum-based chemotherapy ($p=0.0096$) or olaparib ($p=0.018$) respectively improved the overall survival of patients with DDR mutation. No statistical correlation between tumor mutation burden (TMB) and DDR mutations was identified. Treatment of PD-1 blockades did not bring significantly improved OS to DDR-mutated patients than intact DDR group ($p=0.14$).

Conclusions: In this multi-center retrospective study, we showed the role of germline and somatic DDR mutation in predicting the efficacy of olaparib and platinum-based chemotherapy. However, DDR alteration has shown limited value in prediction of hypermutational status and the sensitivity to PD-1 blockade.

Background

Between 2009 and 2016, the five-year survival of pancreatic ductal adenocarcinoma (PDAC) fluctuated less than 9% [1]. The reasons for the high mortality of PDAC primarily include the insidious onset, fast-growing invasion, and ineffective treatment [2]. The standard of care was limited to gemcitabine in metastatic settings, novel cytotoxic agents, and cell signaling inhibitors hardly improve clinical outcomes [3]. Given the increasing incidence of PDAC, there is a major unconquered challenge to develop more effective therapies.

Therapeutic strategies targeting DNA damage repair (DDR) pathways are widely used in anti-tumor treatment [4]. For example, poly (ADP-ribose) polymerase (PARP) inhibitors used in BRCA mutated patients may lead to disruption of two redundant DDR pathways and accumulation of DNA damages [5], thus presenting the phenomenon of synthetic lethality and triggering the apoptosis or necroptosis of tumor cells [6]. Platinum is a chemotherapeutic agent to cross-link purines on DNA and cause DNA damages. Theoretically, these DNA breaks induced by PARP inhibitors or platinum cannot be effectively repaired when DDR genes are mutated. Another indirect DNA repair-related therapy is immune checkpoint inhibitor (ICIs) [7]. Compromised repair of DNA induces accumulation of cytoplasmic DNA fragments,

which may increase neoantigen load and immunogenicity. As a result, high-mutational status, such as high level of tumor mutation burden and high expression of PD-L1, may result in high sensitivity to immunotherapy, especially ICIs [8].

Tumor genetic profiling may help determine optimal treatments. Most of the current large-scale studies involved PDAC patients from western countries. The prevalence of tumor driver genes and DDR deficiency in Chinese PDACs remains unknown, and the relationship between both germline and somatic DDR mutations and the survival or the sensitivity to relevant therapy is not clear. Previous studies have demonstrated the role of germline BRCA1/2 mutations in prediction of the sensitivity to DNA damage targeting treatment [9–10]. Currently, mounting evidence showed that DDR deficiency also occurred in sporadic PDACs, such as somatic BRCA1/2, ATM, RAD51 mutations [11–12]. It was more comprehensive to take both germline and somatic mutations into consideration. Herein we conducted a study to demonstrate the mutation landscape of Chinese PDAC patients and explore the predictive role of germline and somatic DDR mutations in guiding the treatment strategies.

Methods And Material

1. Study population and patient enrollment

Patients who were pathologically confirmed PDAC and were profiled by NGS of formalin-fixed paraffin-embedded tissue and peripheral blood between January 2016 to November 2019 were eligible for our study. These patients were admitted to West China Medical Center, People's Hospital of Sichuan Province and the Cancer Hospital of Fudan University. Exclusion criteria included final pathology other than PDAC, and patients who had less-than-one-month overall survival.

The study was approved by the Institutional Review Board of West China Medical Center with a waiver of informed consent. This was mainly because of the retrospective nature of the study and the fact that most patients had died at the time of study conception.

2. Clinical Characteristics Collection

Baseline demographic, clinical and pathologic information of enrolled patients were collected and recorded at the time of diagnosis. The definition of family history are these first degree relative with a history of any solid malignancy. R1 was defined as a distance of tumor cells < 1 mm from the closest resection margin and R0 \geq 1 mm. Platinum-based chemotherapy included the use of cisplatin or oxaliplatin. Patients were followed with a CT scan of the chest, abdomen, and pelvis every 3 months for the first half year and 6 months once thereafter. Recurrence was defined as the imaging observation of distant metastases or progressing change within the surgical bed including the pancreas remnant or anastomosis sites. Limited stage referred to the resectable carcinoma that still limited to pancrea. Advanced disease was defined as local infiltrated, unresectable, or metastatic lesion during the whole

course of the disease. The date of diagnosis to the date of death or censored at the date of the last follow-up was collected for OS calculation.

3. Ngs Profiling

Gene panels used in this study were designed to describe the critical gene mutations in solid tumors. A total of three gene panels were performed retrospectively

in this study: “My-Lumin”, “Bor-Lumin” and “OK Partner” (3DMed Company). Methods and protocol of NGS profiling was described in detail as the previous article [13]. The NGS platform use ILLUMINA Nextseq500 to perform whole exome sequencing. Each of the bases in the genome is sequenced more than 500 times to deliver accurate data and an insight into unexpected DNA variation.

The three gene panels provided a comprehensive genomic profile of 390, 138, and 417 genes in one single test, respectively. The detailed genes detected in these panels were provided in **Supplemental List 1**. The alterations of SNV, InDels, fusions/rearrangements, and amplification/loss were detected in these panels. 10 main DDR-related genes identified in “Bor-Lumin” panel including ARID2, ATM, ATR, BARD1, BRIP1, CHEK1, CHEK2, MRE11A, PALB2, RAD50. Whereas in “My-Lumin” and “OK partner”, the number of detected DDR genes expanded to 50. The functional significance of variants in DDR genes was determined by self-developed variants interpretation database, updated with the latest literature online. Pathogenic mutations were defined as those variants that would affect the function of a gene, including nonsense, frameshift, and splice-site mutations. The evidence for pathogenic mutations was mainly derived from the public databases or published literature. Mutations with variants of unknown significance were excluded in this study. Germline variation referred to the heritable variation detected in blood samples whereas somatic mutation testing was done using tumor tissue.

In addition, “OK Partner” also presented biomarkers related to immunotherapy, including tumor mutation load (TMB), PD-L1 expression, and the status of microsatellite. TMB refers to the number of somatic mutations per million bases (Mb) in the targeted sequencing coding region, including point mutations and insertion deletions. TMB was classified as high, medium, and low according to the internal database of tumor species. The high level of TMB was defined as the range of top 25%, medium level as 26%-75%, and low as 76%-100%. PD-L1 expression is detected by immunohistochemistry (IHC) of paraffin-embedded tumor tissue. Microsatellite Instability (MSI) refers to the occurrence of a new microsatellite allele due to any change in the length of a microsatellite caused by the insertion or deletion of a repeating unit in a tumor compared with normal tissue. MSI status in this study was detected by NGS or IHC staining for mismatch repair proteins.

4. Statistical Analyses

All data managements and statistical analyses were completed using GraphPad Prism software version 5.0 and R version 3.6.1. Depending on the DDR mutation status, these patients were separated into mutated and wild-type groups. Baseline characteristics were compared between the two groups using the Pearson chi-square test for categorical variables and Student t-test for continuous variables. And the respective correlation of DDR gene mutation and TMB, PD-L1 expression, or MSI status were analyzed with the t-test. The Kaplan-Meier method and log-rank test (Mantel-Cox) were used to compare the differences in OS between different groups. The R package “survival” was used to perform the Kaplan-Meier curves. The “ggplot2” and “forest plot” packages were used for graph production. Cox proportional hazard analysis was used to identify which were correlated with the prognosis of PDAC among clinical characteristics and DDR genes. For this study, $P \leq 0.05$ was considered statistically significant.

Results

1. Patient characteristics

A total of 195 PDAC patients from multiple medical centers in China were enrolled in this retrospective study. The vast majority of patients were recruited from West China Medical Center between January 2016 and December 2019, and the rest came from the Cancer Hospital of Fudan University and the People's Hospital of Sichuan Province. Demographics and clinicopathological data of the study population were listed in Table 1. The median age of all patients was approximately 60 years (range: 27–79 years), and males were moderately overrepresented compared with females (56.4% vs 43.6%). Family history of any malignancy in first-degree relatives was noted in 31 patients (15.9%). 109 resectable patients (55.9%) and 85 unresectable patients (43.6%) received curative surgery and palliative surgery or just biopsy, respectively. At any point during the disease, 60 patients (30.8%) eventually had signs of metastasis. Limited-stage patients accounted for 36.7% of overall patients and 123 patients with advanced disease (62.8%) were included in this study.

Table 1

Baseline characteristics of overall patients. Abbreviations: mut, mutant; wt, wild-type; CA, carbohydrate antigen; DDR, DNA damage repair; N, node; N, number; T, tumor. P value was calculated by χ^2 except t test for age.

Variable	Overall cohort, N = 195	DDR status		p value
		mut N = 30 (15.4%)	wt N = 165 (84.6%)	
Age at diagnosis				0.35
Median(range), years	59.3	59.6	59.2	
Sex, n(%)				0.569
Male	110(56.4%)	15(50%)	95(57.6%)	
Female	85(43.6%)	15(50%)	70(42.4%)	
Family history of cancer, n (%)	31(15.9%)	7(23.3%)	24(14.5%)	0.347
Pancreatic cancer	5(2.6%)	1(3.3%)	4(2.4%)	
Any cancer	26(13.3%)	6(20%)	20(12.1%)	
Location of primary tumor, n (%)				0.253
Head/Uncinate	123(63.1%)	15(50%)	108(65.5%)	
Body/Tail	47(24.1%)	10(33.3%)	37(22.4%)	
NA	24(12.3%)	5(16.7%)	19(11.5%)	
Surgery, n (%)				0.674
R0 (Negative margin)	109(55.9%)	16(53.3%)	93(56.4%)	
R1 (Positive margin)	83(42.6%)	13(43.3%)	70(42.4%)	
NA	3(1.5%)	1(3.3%)	2(1.2%)	
Pathological T stage, n (%)				0.596
T1 and T2	68(34.9%)	9(30.0%)	59(35.8%)	
T3 and T4	124(63.6%)	20(66.7%)	104(63.0%)	
NA	3(1.5%)	1(3.3%)	2(1.2%)	
Pathological N stage, n (%)				0.47
N0	85(43.6%)	15(50.0%)	70(42.4%)	
N1/N2	107(54.9%)	14(46.7%)	93(56.4%)	

Variable	Overall cohort, N = 195	DDR status		p value
		mut N = 30 (15.4%)	wt N = 165 (84.6%)	
NA	3(1.5%)	1(3.3%)	2(1.2%)	
Metastasis, n (%)				0.632
M0	132(67.7%)	19(63.3%)	112(67.9%)	
M1	60(30.8%)	10(33.3%)	50(30.3%)	
NA	3(1.5%)	1(3.3%)	2(1.2%)	
Stage, n (%)				0.950
Limited stage	72(36.7%)	11(36.7%)	61(37.0%)	
Advanced stage	123(62.8%)	19(63.3%)	104(63.0%)	
Perivascular invasion, n (%)				0.686
Present	40(20.5%)	6(20.0%)	34(20.6%)	
Absent	152(77.9%)	23(76.7%)	129(78.2%)	
NA	3(1.5%)	1(3.3%)	2(1.2%)	
Perineural invasion, n (%)				0.68
Present	75(38.5%)	11(36.7%)	64(38.8%)	
Absent	117(60.0%)	18(60.0%)	99(60.0%)	
NA	3(1.5%)	1(3.3%)	2(1.2%)	
CA 19–9, n (%)				0.055
Normal	44(22.6%)	7(23.3%)	37(22.4%)	
Elevated	145(74.4%)	20(66.7%)	125(75.8%)	
Unknown	6(3.1%)	3(10.0%)	3(1.8%)	
Surgery, n (%)				0.857
Curative surgery	109(55.9%)	16(53.3%)	93(56.4%)	
Unresectable cancer	85(43.6%)	14(46.7%)	71(43.0%)	
NA	1(0.5%)	0(0.0%)	1(0.6%)	
Adjuvant radiotherapy or chemotherapy, n (%)				0.094

Variable	Overall cohort, N = 195	DDR status		p value
		mut	wt	
		N = 30 (15.4%)	N = 165 (84.6%)	
Yes	84(43.1%)	15(50%)	69(41.8%)	
No	44(22.5%)	7(23.3%)	37(22.4%)	
Unknown	67(34.4%)	8(26.7%)	59(35.8%)	

In total, thirty patients (15.4%) were identified as mutant germline or somatic DDR gene in our study by NGS. The remaining 165 patients (84.6%) were therefore confirmed as DDR wild-type genotype and were matched by several clinical characteristics to the DDR mutated patients (Table 1). Generally, no significant difference in baseline characteristics of patients between the two groups. For example, there were equal numbers of male and female patients in DDR mutated groups, and male patients were slightly overrepresented in the wild-type group (50% DDR mut vs 57.6% DDR wt, $p = 0.569$). The percentage of family cancer history was similar in each group, also for the presence of pancreatic cancer (3.3% DDR mut vs 2.4% DDR wt, $p = 0.347$). Regarding the TNM staging, the composition of each stage of patients was similar between the two groups (T1-T2: 30.0% DDR mut vs 35.8% DDR wt; T3-T4 66.7% DDR mut vs 63.0% DDR wt; $p = 0.596$). More importantly, there was no difference between patients in limited stage or advanced stage ($p = 0.950$). In conclusion, these results showed that no significant difference in other variables between the two groups, except for the DDR gene mutation status.

2. Mutation profiles of main driver genes

We performed NGS for 195 Chinese PDAC patients enrolled in our study, which has revealed a complex mutational landscape about genes known to be important in pancreatic cancer. 565 deleterious mutations were detected in all patients. The average mutations per cancer sample were 2.9 and 13 patients (6.6%) did not have any alterations in the genes of our panel. The whole mutation landscape of our cohorts is illustrated in **Figure 1**. Kirsten-ras protein (KRAS) was the most prevalent mutating gene, which occurred in 83.6% patients of our cohorts. Other frequent genomic alterations were listed as follows: tumor protein p53 (TP53) (62.05% in our cohorts vs 51% in TCGA), cyclin-dependent kinase inhibitor 2A (CDKN2A) (27.18% vs 11%), drosophila mothers against decapentaplegic homolog 4 (SMAD4) (17.44% vs 15%). Next, we investigated the influence of mutations in driver genes to the clinical outcomes of advanced patients. KRAS mutated patients with significantly lower overall survival (OS) than wild-type patients (**Figure 2A**). Interestingly, in this study, no significant correlation was found between the number of drive gene mutations and OS (**Figure 2B**), which differed from the report of other studies that more drive gene mutations may lead to shorter survival in PDAC patients [14-15]. As the fifth most common genetic alteration, a handful of genes related to DNA damage repair were identified in 15.38% of patients in our study. Among the genetic alterations, BRCA2 germline mutation was the most prevalent mutation of DDR deficiency. BRCA2, ATM, RAD50 and MLH1 genes were mutated in 9 (4.62%),

8 (4.10%), 3 (1.54%) and 2 (1.03%) of all patients, respectively. Other mutant DDR genes, such as BRCA1, MSH, RAD51, PMS2, PALB2, FANCA, FANCE, BLM, CHEK2, and FANCD2, were found in one patient (0.51%), respectively (**Figure 2C**).

3. Survival Analyses Based On Ddr Mutation Status

The mutation profiles of all DDR gene mutations detected in our study has shown in Fig. 3A. There were several different alteration types among these mutations, including missense, nonsense, frameshift, intron mutation, and copy number variation (CNV)-loss. The detailed mutational information (mutation level, amino acid change, and corresponding functions) was listed in Table 2. A total of 36 mutations of DDR genes were identified in 30 patients, including 19 somatic mutations and 17 germline mutations (Fig. 3B). Six (3.07%) patients had more than one mutation. We recorded 12 germline and somatic deleterious BRCA1/2 mutations in 9 patients, 1 of which (0.5%) occurred in BRCA1, and 11 (4.7%) occurred in BRCA2. Among them, 2 patients had 2 sites of BRCA2 mutation simultaneously.

Table 2
Mutation details of DNA damage repair gene in 30 patients of our cohort.

Patient ID	Age	Sex	Mut Level	Amino acid change	Function
Patient 167	M	55	ATM germline	p.R1882*	Nonsense
Patient 185	M	61	ATM germline	p.C1899*	Nonsense
Patient 186	F	71	ATM germline	p.K468Efs*18	Frameshift
Patient 187	F	71	ATM germline	p.Q441Afs*45	Frameshift
Patient 195	M	55	ATM somatic	p.K2811Sfs*46	Frameshift
Patient 189	F	63	ATM somatic	R1898*	Nonsense
Patient 190	F	69	ATM somatic	p.G509*, p.L1651*	Nonsense
Patient 188	M	63	ATM somatic	c.2921 + 1G > A	Intron mutation
Patient 170	F	54	BLM germline	p.L258Efs*7	Frameshift
Patient 183	F	45	BRCA1 somatic	p.R1443*	Nonsense
Patient 173	F	67	BRCA2 somatic	p.T3033Nfs*11, p.K437Ifs*22	Frameshift
Patient 182	F	34	BRCA2 somatic	p.R3128*	Nonsense
Patient 180	M	63	BRCA2 somatic	D2723N	Missense
Patient 181	M	55	BRCA2 somatic	p.R2494*	Nonsense
Patient 182	F	34	BRCA2 germline	p.N2137Kfs*29	Frameshift
Patient 177	M	55	BRCA2 germline	c.3847_3848del	Indel
Patient 184	F	49	BRCA2 germline	Y1894*	Nonsense
Patient 192	F	73	BRCA2 germline	p.Q1073Rfs*4	Frameshift
Patient 113	M	34	BRCA2 germline	p.V1283Kfs*2	Frameshift
Patient 172	F	68	FANCA somatic	-	CNV-amplification
Patient 166	F	79	FANCE somatic	-	CNV-loss
Patient 191	M	60	FANCD2 germline	p.Q718*	Nonsense
Patient 174	M	47	MLH1 somatic	p.N287Kfs*10	Frameshift
Patient 175	M	57	MLH1 somatic	-	CNV-loss
Patient 194	M	62	MSH2 somatic	p.N566Ifs*24	Frameshift
Patient 194	M	62	MSH6 somatic	p.F1088Sfs*2	Frameshift

Patient ID	Age	Sex	Mut Level	Amino acid change	Function
Patient 176	F	50	PALB2 somatic	p.F440Lfs*12	Frameshift
Patient 176	F	50	PALB2 germline	p.R753*	Nonsense
Patient 171	F	56	PMS2 somatic	-	CNV-loss
Patient 168	M	51	RAD50 germline	p.Q826*	Nonsense
Patient 169	M	67	RAD50 germline	c.3618 + 1G > A	Intron mutation
Patient 179	M	76	RAD50 germline	p.R1077*	Nonsense
Patient 171	F	56	RAD51 somatic	-	CNV-loss

Survival analyses were conducted to confirm the predictive and prognostic value of mutations in DDR-related genes. In our cohort, there were 123 patients (63.1%) in the advanced cohorts and 102 of advanced patients had survival data. Among them, 104 patients were DDR wildtype, while 19 patients were identified as DDR deficiency. The median OS of advanced patients was 11.69 months. The patients with DDR deficiency showed no benefit in OS compared to wild-type patients ($p = 0.71$) (Fig. 3C).

4. The effects of olaparib, platinum-based chemotherapy and PD-1/PD-L1 blockade on overall survival to patients with the DDR deficiency.

Of all the 195 patients, 22 have ever received any one of these DDR targeting drugs (olaparib, platinum-based chemotherapy and PD-1 blockades) and ten of them harbored DDR deficiency. Most patients who received these drugs harbored BRCA1/2 or ATM mutations (Fig. 4A). In the 18 advanced DDR-mutated patients, 4 patients received the second-line olaparib treatment after the failure of chemotherapy with gemcitabine and nab-paclitaxel or platinum. An improvement of OS was observed in the group with olaparib treatment compared to those without ($p = 0.034$; Fig. 4B).

There were overall 15 patients treated with platinum-based chemotherapy in our study, 9 of them were DDR wild-type while 6 were in the DDR mutated group. In advanced patients with DDR mutations, a total of 5 patients have received the platinum chemotherapy during the whole therapeutic course. 3 of them received second-line platinum-based chemotherapy, including 1 patient with mFOLFIRINOX (modified 5-Fluorouracil, leucovorin, irinotecan and oxaliplatin) regimen, after the tumor progression of gemcitabine plus nab-paclitaxel. The other two patients both received the gemcitabine and platinum chemotherapy as the first-line treatment. However, one patient had progression disease after 3 cycles of platinum chemotherapy and another patient had the disease recurrence in two years. In the advanced patients, platinum-based chemotherapy was also found to result in favorable OS ($p = 0.0096$, Fig. 4C). Next, we investigated the correlation between DDR deficiency and response to PD-1 inhibitors. Although PD-L1 overexpressed in tumors of 6 advanced patients, the efficacy of PD-1 blockades was a little disappointing: 1 patients with intact DDR genes had stable disease (SD), meanwhile, of the remaining 5 patients with DDR deficiency, 1 was evaluated as partial response (PR), 3 as SD, and 1 as progression

disease (PD) (based on RECIST 1.1). However, in the advanced patients with DDR deficiency, the OS was not significantly prolonged after treatment of PD-1 blockades ($p = 0.14$; Fig. 4D).

13 advanced patients with DDR deficiency had the treatment and survival records. Detailed data of these individual patients were summarized in Fig. 5A. Matched therapy was defined as precise treatment according to the molecular profiling of the individual patient. For example, the matched therapy of DDR mutations included olaparib and platinum-based chemotherapy, and PD-1 blockade was matched therapy for positive PD-L1 expression. As shown in Fig. 5B, the participation of molecularly matched therapy in the treatment course significantly improved the overall survival of patients compared to those treated with unmatched therapy.

5. Correlations Between Hypermutation Phenotype And Ddr Mutation

In our study, TMB could be evaluated in 87 patients who profiled by the “OK partner” panel. The median level of TMB was 4.9 mutations/Mb (range, 0.81–15.32 mutations/Mb). By analyzing the sequencing data of enrolled patients, we identified no significant difference of TMB between patients with DDR mutations and those in wild-type status ($P = 0.384$; Fig. 6A). However, in the DDR mutated group, a higher proportion of patients had medium or high level of TMB (56.25% DDR mut vs 38.23% DDR wt), and fewer patients were located at the low level of TMB (31.25% DDR mut vs 47.06% DDR wt).

In order to meet the need to respond appropriately to different kinds of DNA damage, mammalian cells have evolved intricate DNA repair pathways to repair a large variety of structurally genotoxic damages: mismatch repair (MMR), base-excision repair (BER), nucleotide excision repair (NER), homologous recombination (HR), non-homologous DNA end joining (NHEJ) pathway, translesion synthesis (TLS), Fanconi anemia (FA) and checkpoint factors (CPF). In this study, the mutational genes were associated with five pathways (Fig. 3B). To further disclose the main contributing components affecting the connection between DDR mutations and TMB, we investigated whether the mutations among these pathways of DDR system may affect the TMB levels. As shown in Fig. 6B-E, patients with genetic alterations in CPF ($p = 0.424$), HRR ($p = 0.590$), and FA pathways ($p = 0.099$) failed to show significant differences with corresponding wide-type patients. However, NHEJ pathway alterations demonstrated a comparably higher level of TMB than the NHEJ wild-type groups ($p < 0.001$).

In our study, 89 of 195 patients had the available information of microsatellite status. One patient was evaluated as MSI-high by known NGS sequencing sites and another MSI-low was confirmed by immunohistochemistry (IHC) detection. And the remaining patients were all microsatellite stable (MSS). In contrast to our hypothesis, the two MSI patients were both DDR wild-type. IHC information of PD-L1 protein was available in 102 patients, and 23 of them (22.5%) were positive. In the DDR mutated group, the proportion of patients with PD-L1 overexpression was a little higher than that in the wild-type group (29.17% DDR mut vs 20.51% DDR wt) (Fig. 6F).

Discussion

To our knowledge, this multicenter retrospective study is the first study focusing on the germline or somatic DDR mutations of PDAC patients in the Chinese population.

First of all, the top 4 commonly mutated genes between different racial population were almost the same: KRAS, TP53, CDKN2A, and SMAD4, which was in accord with previous studies about western PDAC patients [16–17]. However, different racial cohorts may have different tendencies of the fifth most frequent mutation. ARID1A was supported by some research to be the fifth most altered gene with more than 10% incidence rate [18]. A whole-genome sequencing conducted in 100 Australian PDAC patients showed that ARID1A mutation was prevalent, which was in consistent with our cohorts [4]. And other candidates including FLG [19] and myeloid/lymphoid or mixed-lineage leukemia protein 3 (MLL3) [20]. ARID1A, however, was not listed as the top 5 mutated genes in the current understanding of PDAC molecular type [21]. Despite the molecular status of main driver genes being considered to potentially influence OS in some studies [22–23], in our research, there was no survival difference between patients with or without any mutations of the top 4 mutated genes except KRAS. KRAS mutation also were confirmed to shorten survival term of PDAC patients by other studies [14]. And the phenomenon that patients with altered driven genes may had worse OS, which was reported in other studies [15, 24], was not observed in our research.

The overall incidence of DDR mutation in our cohort was 15.38%, which is a little higher than what has been reported in other NGS studies: Matthew B et al enrolled 289 resected PDAC patients of the USA and found that 7.3% carried the germline variants in 24 detected DDR genes [25]. A large-scale study of targeted genomic profile analyses showed that BRCA and FANC mutations were detected in 14% of 3594 international PDAC patients [26]. This figure was similar to the 17% prevalence of the DDR mutations in the high-risk population (Ashkenazi Jews) [27]. Although further validations in a larger-scale population were required, it is hypothetical that the Chinese population was also at high risk for overall DDR mutations. The results emphasized that the DDR gene mutations were relatively common in Chinese PDACs, which required us to pay more attention.

Secondly, the clinical outcomes of DDR mutations was controversial. In the western cohorts, the OS of patients carried with BRCA1/2 or PALB2 mutation was prolonged compared to that of non-carriers (21.8 months DDR mut vs 8.1 months DDR wt) [28]. However, some studies showed that there was no prognostic difference between the two groups, and others even suggested that germline BRCA mutation may induce worse prognosis [29]. No significant difference in OS was observed between the patients with or without DDR mutations in our study. The inconsistency of results may ascribe to the different characteristics of the enrolled population and the different therapeutic regimens they received.

Next, to further investigate the impact of DDR mutations as actionable genes to guide precision medicine. In the advanced patients with DDR deficiency, we found that molecularly matched therapy significantly improved the OS of patients than those with unmatched therapy. The Know Your Tumor (KYT) trial also

reported that the median PFS of patients with the matched therapy was significantly longer than the patients in the unmatched therapy group [30].

Additionally, no significant difference of TMB between patients with DDR mutations and those in wild-type status in our study. Of the four specific sub-pathways, mutation of the NHEJ pathway was the only one to induce higher TMB. In the DDR mutated group, a higher proportion of patients had medium or high level of TMB and fewer patients were located at the low level of TMB. However, some studies found the positive correlation of TMB and DDR mutations. In Korean SCLC patients, higher TMB level was identified in the DDR mutated group than DDR wild-type group [31]. Another study revealed that deleterious alterations in 34 DDR related genes may exhibit high TMB levels and be independently related to better response to ICIs in metastatic urothelial cancer [32]. Furthermore, only 2 patients in our cohorts were identified with MSI-high but with the intact DDR genes. Contrary to other studies, DDR mutations were observed to positively correlate to MSI in a study [33]. These differences mainly derived from the a lot absence of information on MSI status in our study. According to the latest ASCO clinical practice guideline, pembrolizumab is suggested for patients with MMR deficiency or MSI-high metastatic pancreatic cancer [34]. A study reported that PDAC patients with DDR mutations had a higher percentage of positive PD-L1 expression than wild-type [35]. In conclusion, the correlation between DDR mutations, MSI status, and PD-L1 expression needs to be further verified by large-scale research.

There were some limitations in this study. The first issue included controversial definitions of DDR genes and the relationship between these genes and their corresponding pathways. Due to insufficient knowledge about the intricate regulation mechanism of the DDR pathways, current research concerning PDAC used unrecognized standards to classify the DDR genes. For example, some studies defined 14 or 16 genes as the members of the DDR system [29, 36]. Patients in this study profiled using three different gene panels, which had specific probes covering a different range of gene sets. Obviously, the different spectrum of detected genes may lead to diverse classification of DDR mutations and affect the results of studies. Additionally, a low number of patients who received the DDR related treatment limited the development of statistical analyses. Although there were difference in median OS between two groups of small-sample comparison, it was still difficult to reach significant P value according to statistic analyses. As a result, the lack of information and small sample size may lead to some deviations in conclusions. Another limitation is the retrospective nature of our study. The impact of other treatment regimens the patients received, such as radioactive particle implantation, arterial infusion chemotherapy or traditional Chinese medicine were beyond the scope of this study, which may lead to a large degree of therapeutic heterogeneity within the total cohorts. Even with these above-mentioned limitations, genomic data generated separately from some platforms validated our findings [25, 37–39]. Large-scale randomized controlled trials are expected to prospectively verify the predictive role of DDR mutations.

Conclusions

In conclusion, our study described mutation profiling of the currently largest Chinese PDAC population. The main driver genes of Chinese PDAC patients were KRAS, TP53, CDKN2A, and SMAD4. Patients with

KRAS mutation showed worse OS than those without. DDR deficiency was identified in 15.38% of overall patients, mainly involving BRCA2, ATM, and RAD50 genes. Furthermore, our results portrayed a probably positive association between DDR mutations and the better therapeutic efficacy of olaparib and platinum-based chemotherapy in advanced PDAC patients. DDR mutations were limited in inducing highmutation status of patients and higher sensitivity to PD-1 blockades. Our study provided a relatively comprehensive profile of DDR mutations in Chinese PDAC patients and suggested the potential connection between DDR mutation and therapeutic effects, which may catalyze further biomarker studies targeting impaired DNA pathways or immunotherapies.

Declarations

Ethics approval and consent to participate

Written consent and institutional approval were obtained.

Consent for publication

All authors read and approved the manuscript as submitted.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Dan Cao, Lin Shui and Xiaofen Li designed the investigation and contributed to writing the paper. Lin Shui, Jiangfang Tian and Shuangshuang Li collected the detailed information and performed the investigation. Du He, Ang Li, Bole Tian and Mao Li provided essential assistance to collect clinical information of patients. Dao Cao and Cheng Yi participated in correction of the final manuscript.

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Figures

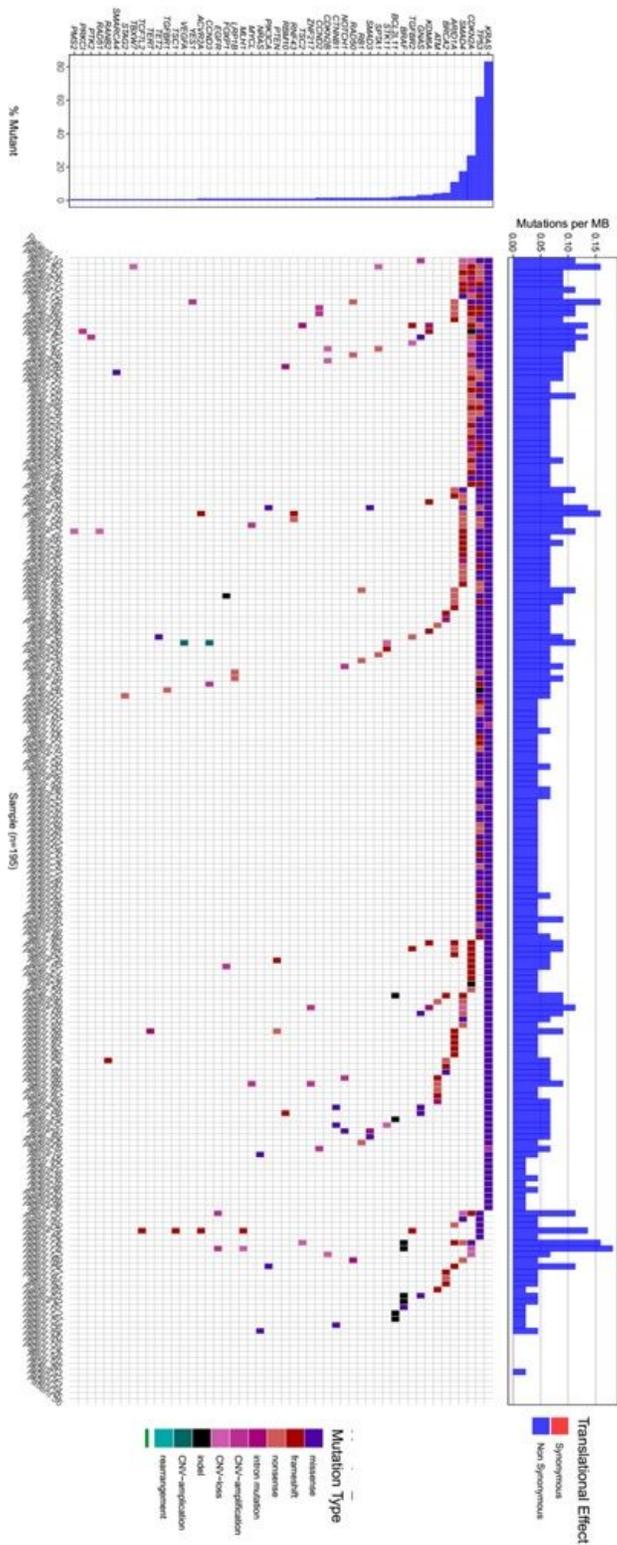


Figure 1

Mutation landscape of the 195 Chinese PDAC patients in our study.

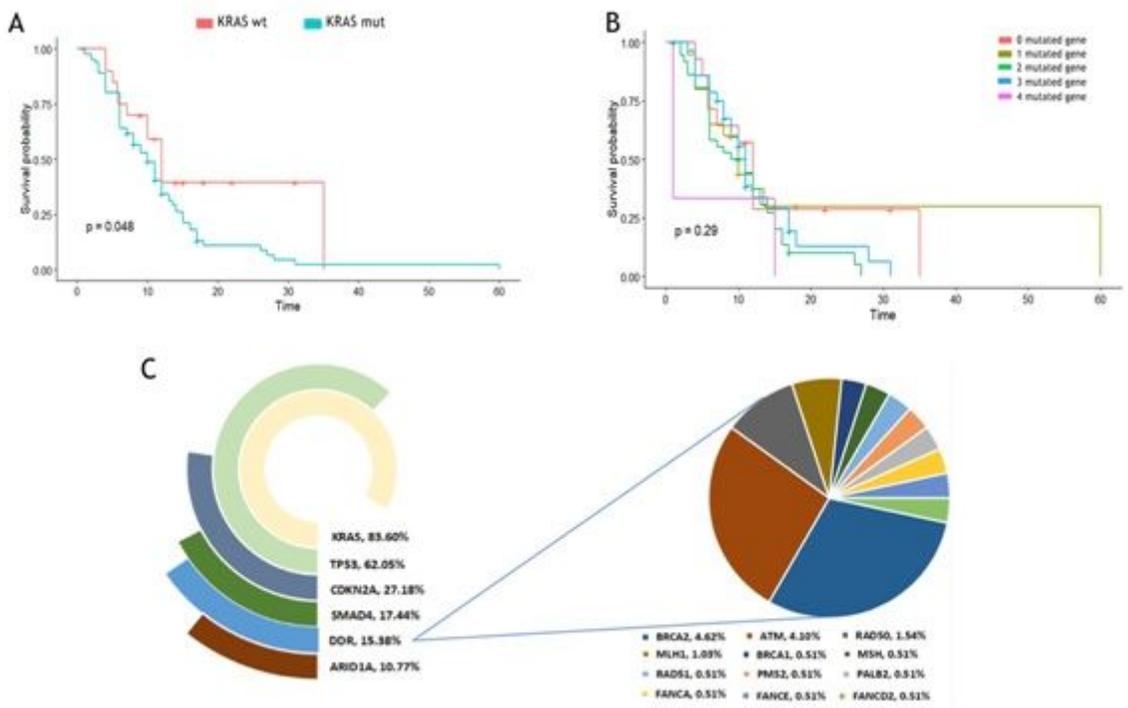


Figure 2

The association between driver genes and prognosis and the mutational landscape of DDR gene in our study. 2A. The overall survival (OS) was shorter in advanced patients with KRAS mutation compared to those without. 2B. The K-M survival analysis of advanced patients with 0 to 4 mutated driven gene. 2C. The most frequently altered mutation genes and the percentage of mutations in DNA damage repair (DDR) gene systems.

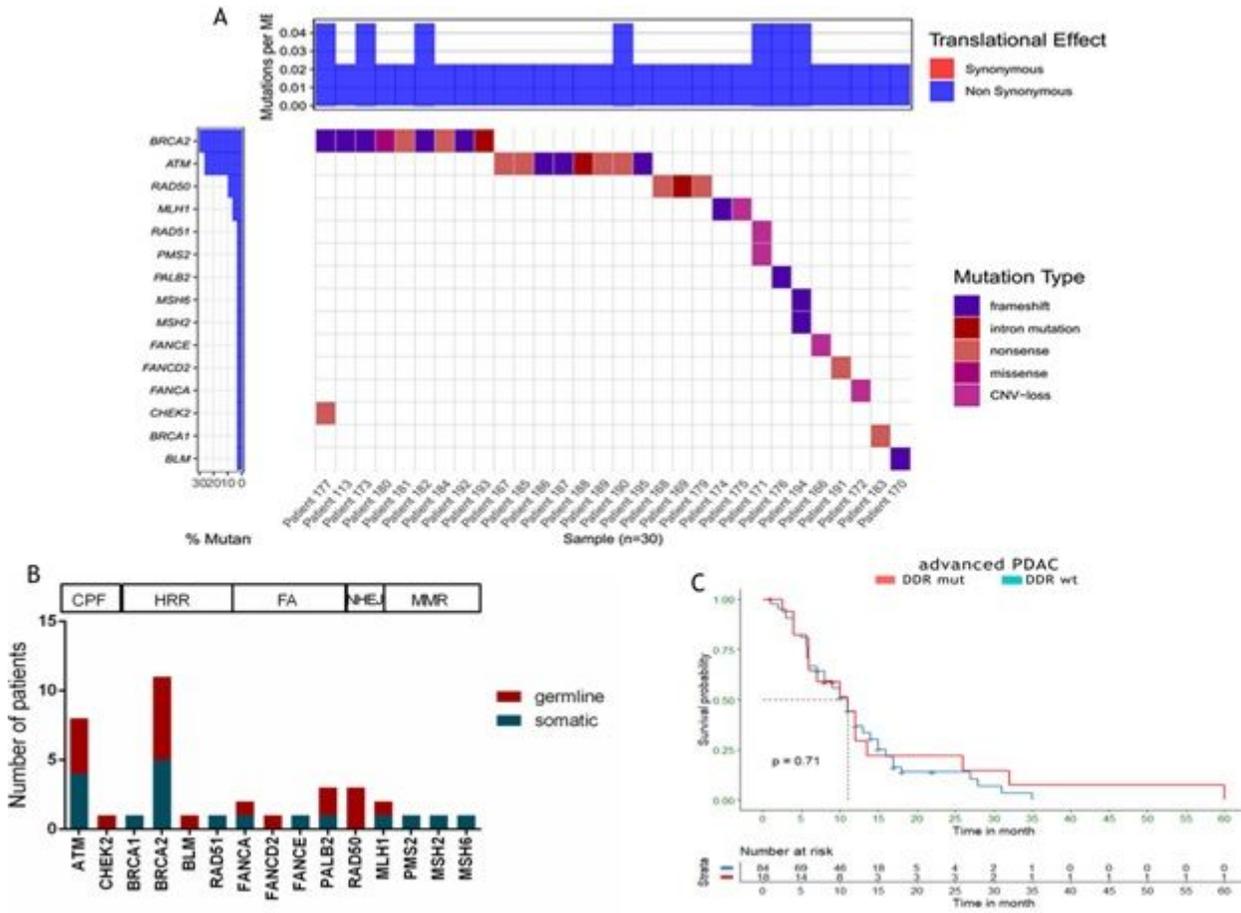


Figure 3

The mutation profile of DDR mutation in our cohorts and the correlation of DDR mutation status and overall survival of patients. 3A. Mutation profile of DDR gene mutations in 30 patients. 3B. The distribution and numbers of germline and somatic gene mutations in each individual sub-pathways of DDR systems. 3C. The overall survival of advanced patients with DDR deficiency and those with intact DDR genes.

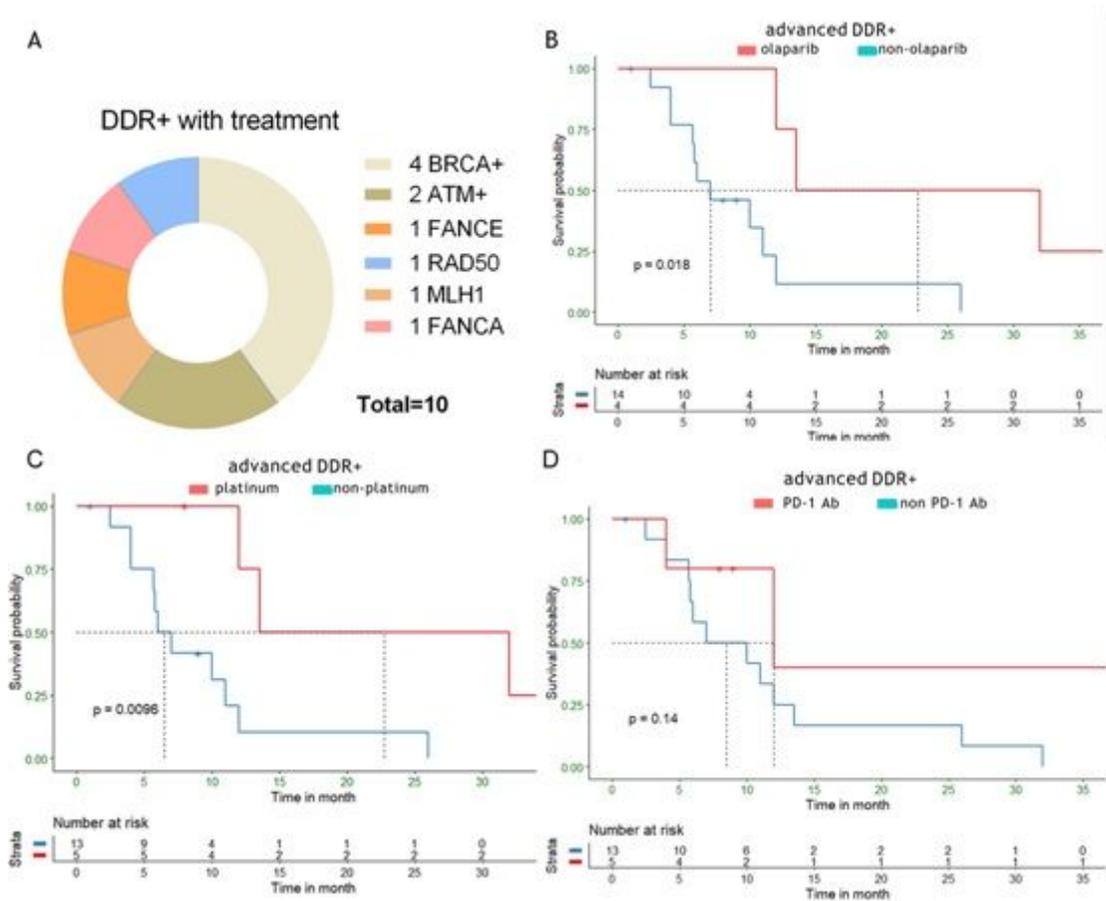


Figure 4

The respective relationship between OS and olaparib, platinum-based chemotherapy or PD-L1 blocking therapy in defective DDR patients. 4A. The constitution of mutant DDR genes in patients with any one treatment of olaparib, platinum-based chemotherapy and PD-1/PD-L1 blockade. 4B. In the subgroup of locally advanced or metastatic PDACs, there were significant difference between defective DDR patients with or without olaparib treatment. 4C. Application of platinum-based chemotherapy positively correlated with the prolonged OS in locally advanced or metastatic PDAC patients. 4D. The difference of OS between locally advanced or metastatic patients who treated with PD-L1 blockade and those without.

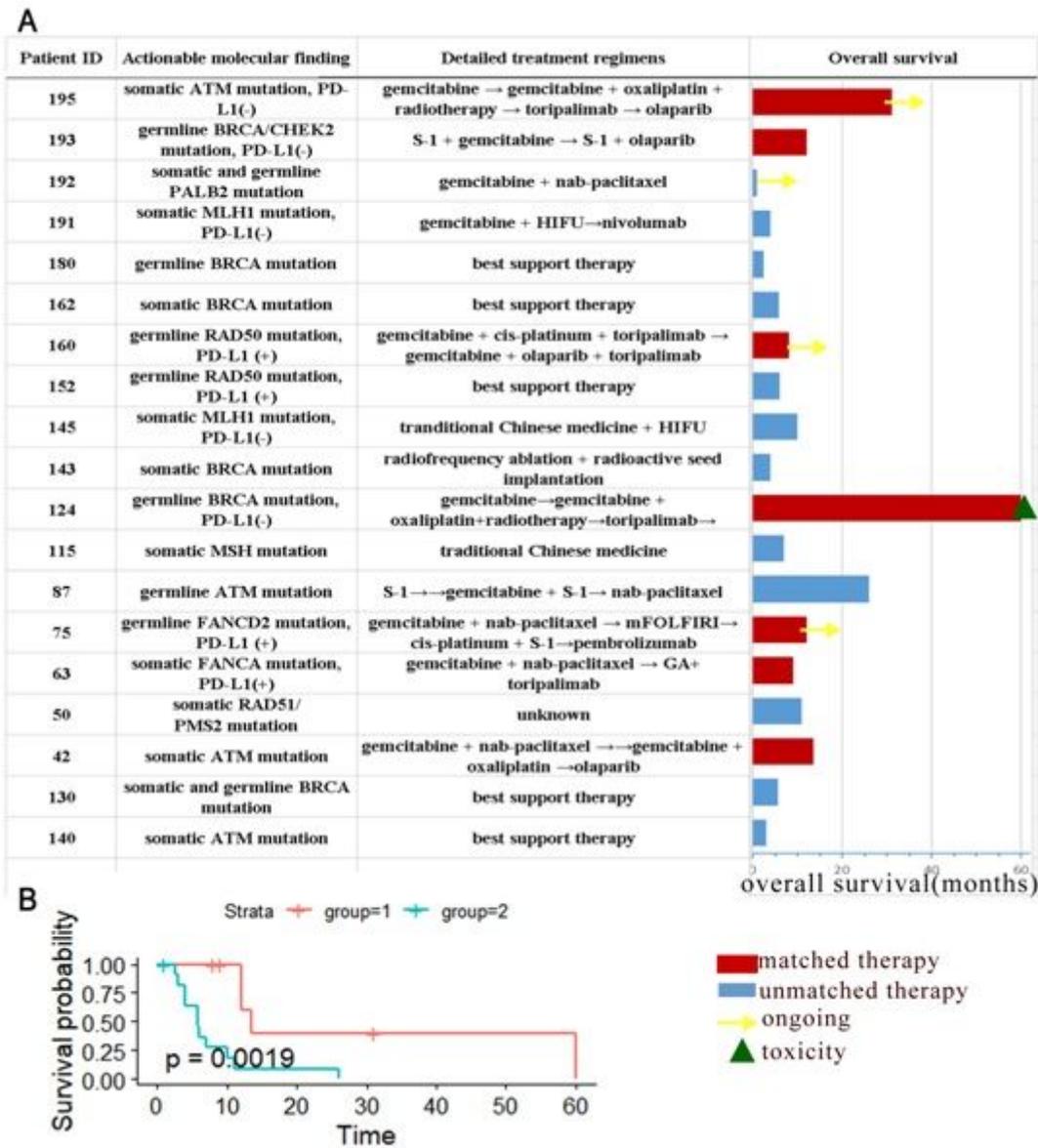


Figure 5

Actionable alterations combined with overall survival for molecularly matched and unmatched therapies. 5A. The molecular mutations and therapeutic regimens in advanced patients with DDR deficiency and detailed clinical and survival data. 5B. Matched therapy significantly improved the OS of advanced patients with DDR mutations than those with unmatched therapy. Matched therapy was defined as precise treatment according to the molecular profiling of the individual patient (olaparib and platinum-based chemotherapy for DDR mutations, PD-1 blockade for positive PD-L1 expression). FOLFIRI: fluorouracil and irinotecan. FOLFIRINOX: fluorouracil, irinotecan, and oxaliplatin. HIFU: high intensity focused ultrasound.

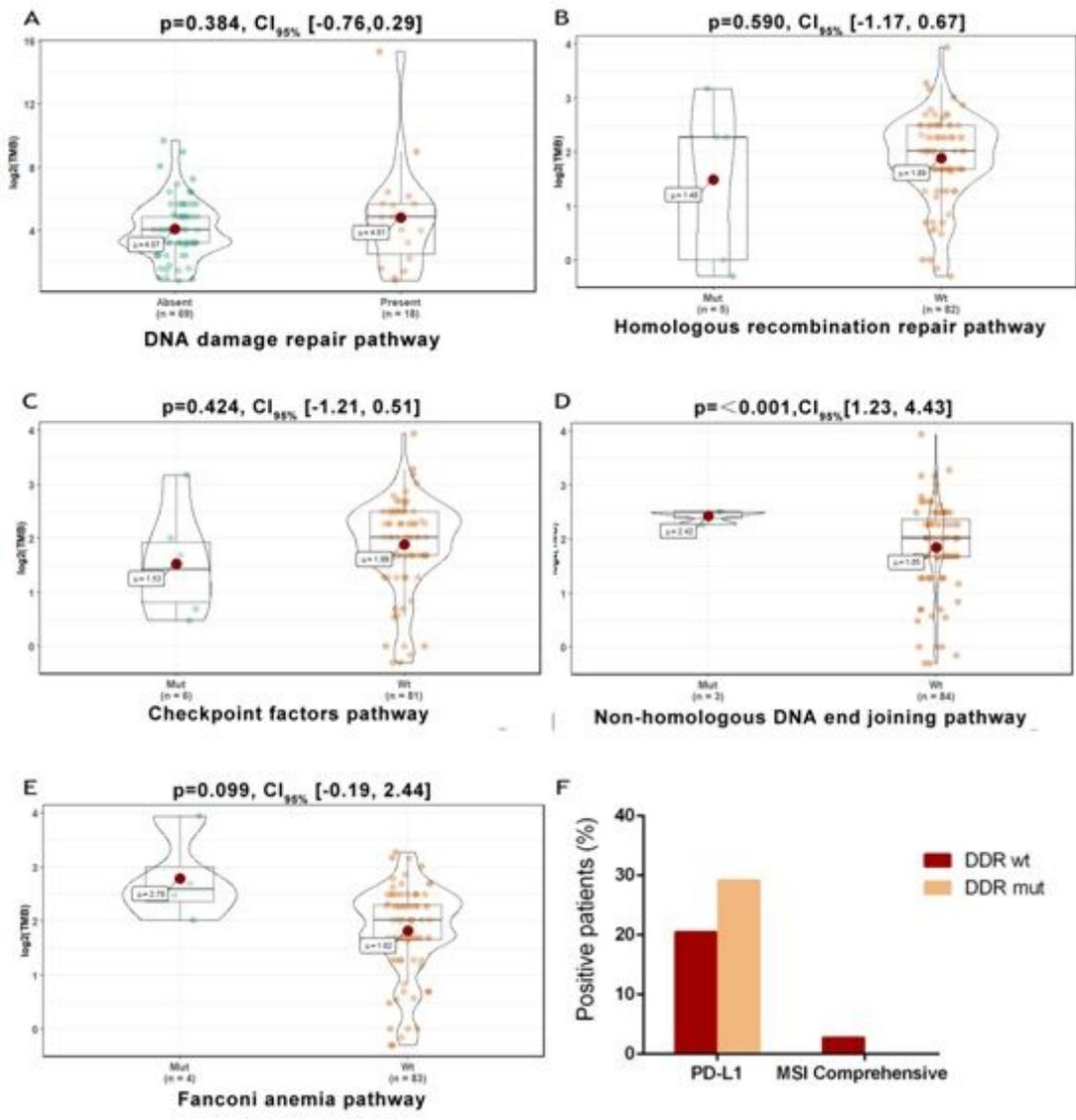


Figure 6

Distribution of TMB based on DNA damage-related gene-set alteration. 6A. The relationship between tumor mutation burden (TMB) and the DDR gene status. 6B-6E. Comparison of the TMB levels between patients with the mutation of respective subways of DDR systems, HRR (6B), CPF (6C), NHEJ (6D), and FA (6E), and those without corresponding mutations. The y axis is indicating TMB per megabase in log2 scale. 6F. Patients with DDR deficiency harbored higher percentage of PD-L1 overexpression compared to those with DDR wild-type. Abbreviations: DDR, DNA damage repair; FA, Fanconi anemia; HR, homologous recombination; NHEJ, non-homologous end joining; CPF, checkpoint factors; TMB, tumor mutation burden.

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