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Mutational landscape of DNA damage response deficiency-related genes and its association with immune biomarkers in esophageal squamous cell carcinoma

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Esophageal squamous cell carcinoma (ESCC) has limited effective treatment strategies. DNA damage response (DDR) genes are of therapeutic interest in multiple cancer types. This study aimed to depict the landscape of DDR mutations in ESCC and evaluate the association between DDR mutations and known immunotherapy biomarkers. We recruited 250 Chinese patients with ESCC and performed next-generation sequencing. A total of 107 patients underwent a PD-L1 examination. Among the 250 patients, 73 (29.2%) harbored at least one DDR gene mutation and were defined as DDR-mut. Among the six functional DDR pathways, homologous recombination (HR) accounted for 12.4% (31/250). DDR-mut patients were significantly associated with higher tumor mutational burden than those in the DDR-wt group (p=7.4e-07). Patients with PDL1-H accounted for 21.2% (36/107) of the patients. PDL1-H was more prevalent in DDR-mut than DDR-wt, although the p-value did not reach a significant level (40.5% vs. 30%, p=0.29). Further analysis revealed that BRCA1, one of the most frequently mutated genes in the HR pathway, was significantly associated with PDL1-H (p=0.01). Our data revealed a subset of patients with ESCC harbored DDR gene mutations. Patients with these DDR gene mutations are significantly associated with immune biomarkers, implying the potential feasibility of combining DDR agents with immunotherapy in patients with DDR deficiency.

Key words: esophageal squamous cell carcinoma, DNA damage, response immunotherapy, gene mutation

Esophageal cancer is one of the most prevalent cancers with a high mortality rate [1]. Esophageal squamous cell carcinoma (ESCC), which accounts for more than 90% of esophageal cancer cases, is the predominant histological subtype in China [2]. Due to its high aggressiveness, late diagnosis, and lack of effective treatment strategies, the prognosis of ESCC remains poor, with a 5-year survival rate of less than 20% [3]. Thus, there is an urgent need to develop novel treatment approaches to improve the clinical outcomes of ESCC.

Substantial evidence has proved that patients carrying DNA damage response (DDR) gene mutations are more likely to respond to platinum-based chemotherapy, and poly (adenosine diphosphate ribose) polymerase (PARP) inhibitors across multiple cancers [4-6]. DDR genes can be divided into nine pathways: base excision repair (BER), direct repair (DR), and nucleotide excision repair (NER) pathways repair DNA base damage; mismatch repair (MMR) corrects base mispairings and small loops; the Fanconi anemia (FA), homologous recombination (HR), non-homologous end joining (NHEJ), and specialized DNA polymerases in translesion synthesis (TLS) pathways repair DNA strand breaks and complex events like interstrand crosslinks. Some damage sensor genes such as ATM and ATR are also crucial to the DDR pathway, and we often define others [7]. Gene mutations in these pathways have been reported to be associated with immune biomarkers and may have a tendency to respond to immunotherapy [8, 9]. Targeting DDR pathways via DDR inhibitors can also modulate the tumor immune microenvironment [10]. The Food and Drug Administration (FDA) has approved pembrolizumab for the treatment of advanced ESCC patients whose tumors express PD-L1 (combined positive score [CPS] ≥10) when the disease has progressed based on the findings from the phase III KEYNOTE-181 and the phase II KEYNOTE-180 trials [11, 12]. Considering that few actionable molecular targets exist in ESCC, strategies to target the DDR pathways and immune checkpoints remain the most promising treatment options for ESCC. However,

the mutational pattern of DDR genes and their relevance to immune biomarkers in Chinese patients with ESCC remains to be clarified.

To depict the landscape of DDR mutations, we evaluated 25 DDR core genes covered by our next-generation sequencing (NGS) panel. We also evaluated the association between DDR mutations and immune biomarkers. Furthermore, specific DDR mutations and DDR functional pathways were found to be correlated with immune biomarkers. By identifying these DDR mutations/pathways, we hoped to identify patients who might benefit from combination therapy by targeting the DDR pathway and immune checkpoints.

Patients and methods

DDR status definition. We extracted 80 core genes from all nine DDR pathways based on a study from Cell Reports [7]. After intersecting the 80 DDR core genes with the 319 panel genes, 25 DDR core genes were used to define the DDR status. These 25 DDR core genes were enriched in six DDR pathways: BER, NER, MMR, FA, HR, and others. A detailed gene list and related DDR pathways are shown in Supplementary Table S1. Patients who were classified as having the DDR-mut phenotype were defined as having at least one nonsynonymous somatic mutation or pathogenic germline mutation in any of the 25 DDR genes. Pathogenic mutations were determined using the Clinvar database.

Patient information and sample collection. To evaluate the prevalence of DDR mutations in ESCC, we recruited 250 Chinese patients with ESCC. Additionally, we used a public dataset (TCGA, PanCancer Atlas) comprising 95 ESCC patients as a validation set. Clinical and genomic data for this cohort were obtained from cBioPortal (https://www.cbioportal.org/). The basic clinical characteristics of the two cohorts are shown in Supplementary Table S2. 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. The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of Huashan Hospital and was conducted in accordance with the ethics guidelines (protocol code KY2020-837 and date of approval 15 September 2020). All patients signed the informed consent form.

Targeted sequencing and variant calling. DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) tumor specimens. Paired whole blood was used to filter out somatic mutations. DNA library was captured with a designed 319 gene panel, which covers major tumor-associated genes. The protocol of targeted sequencing was the same as in previous studies [13]. Somatic single nucleotide variants (SNVs) and indels were detected by VarDict [14] (version 1.5.1) on both tumor samples and matched blood samples. The mutations were annotated using ANNOVAR [15]. Copy number variation (CNV) was obtained by CNVkit (v0.9.2). Genes were defined as CNV gain (copy ratio >3) or loss (copy ratio <1.5). The CNV burden was defined as the total number of genes with CNV gain/CNV loss.

Calculation of tumor mutational burden (TMB) and microsatellite instability (MSI) status. TMB value was equal to the number of somatic nonsynonymous mutations per megabase. The TMB value in the top 25th percentile in this study was defined as TMB-high (TMB-H). Seventy microsatellite loci were identified in this gene-targeted panel. After examining these loci and comparing them with healthy people in the Chinese database, MSI status was evaluated by the unstable locus/passing locus ratio. When this ratio was ≥0.2, a patient would be classified as MSI-H.

Immunohistochemical (IHC) staining of PD-L1. FFPE sections were stained with anti-PD-L1 22C3 (Dako, M3653, 1:50). PD-L1 status was determined by CPS. CPS was equal to the number of PD-L1 positive cells divided by total tumor cells and multiplied by 100. PDL1-H was defined as CPS ≥10.

Statistical analysis. Comparisons between categorical variables were using Fisher's exact test, while continuous variables were using Mann-Whitney U test. The mutational landscape was depicted using maftools [16]. All analyses were performed using R software (version 4.1.0). Statistical significance was considered at $p<0.05$.

Results

Mutational landscape of DDR genes in Chinese patients with ESCC. Among 250 patients with ESCC, 248 had at least one mutation. The top three mutated genes were *TP53* (90%), *NOTCH1* (32%), and *FAT1* (21%) (Supplementary Figure S1A). Compared with the Chinese cohort, the frequencies of *NOTCH1* (14%) and *FAT* (4%) were much lower in TCGA cohort (Supplementary Figure S1B). Of the 250 patients, 73 (29.2%) had DDR-related mutated genes (Figure 1A), while 23 (9.2%) patients harbored two or more DDR mutations. *ATM* (4.8%, 12/250), *ATR* (3.6%, 9/250), and *BRCA1* (3.6%, 9/250) were the most frequently mutated DDR genes. Of the 73 DDR mutated patients, 68 patients only had somatic DDR gene mutations (13 patients had pathogenic somatic DDR gene mutations), while 5 patients only had pathogenic germline DDR gene mutations. One patient had both somatic and pathogenic germline DDR mutations (Figure 1B). Six pathogenic germline mutations occurred in five DDR genes: *ATM* (2/6), *BRIP1* (1/6), *MRE11A* (1/6), *MSH3* (1/6), and *RAD50* (1/6). Pathogenic germline mutations in *BRIP1* and *MRE11A* were found at the splicing sites. Other pathogenic germline mutations were in the exon region and belonged to nonsense mutations or frameshift deletion/insertion (Figure 1C). The mutational frequencies of DDR genes in TCGA cohort (27.37%) were very similar to our Chinese cohort (Supplementary Figure S2A). We also counted mutations in the corresponding DDR pathway (Figures 1A, 1D). Among the six functional DDR pathways, HR (12.4%, 31/250) and others (11.6%, 29/250) were the most common

mutated pathways. In the HR pathway, *BRCA1/2* showed 16 mutations in 14 patients. No pathogenic germline mutations were detected in *BRCA1/2* cells. Nine patients harbored *BRIP1* mutations, and six patients had *RAD50* mutations. The remaining HR genes such as *BRRD1*, *BLM*, *MRE11A*, *NEB*, and *PALB2*, were mutated in patients with no more than two mutations. For the other pathways, *ATM* was the most prevalent mutated DDR gene (4.8%, 12/250), followed by *ATR* (3.6%, 9/250) and *CHECK2* (3.2%, 8/250). Interestingly, we observed that these other pathway gene mutations were mutually exclusive.

Differential mutation/pathway distributions between DDR-mut and DDR-wt groups. According to our DDR status definition, we classified each patient as DDR-mut or DDR-wt. No significant differences were shown in gender, age, or stage between the two groups (Supplementary Figure S3). The landscape of the top 20 mutated genes in DDR-mut and DDR-wt are shown in Figure 2A. By comparing the mutation frequencies between the DDR-mut and DDR-wt groups, significant differences were found in 13 genes (*CIC*, *ERBB2*, *POLD1*, *BAP1*, *CARD11*, *ERG*, *FLT1*, *MPL*, *PDGFRB*, *SF3B1*, *TP53*, *ARID1A*, and *GRIN2A*). All these genes were more likely to mutate in the DDR-mut group (Figure 2B). Despite the high mutational frequency of *TP53* in the ESCC cohort (90%), mutations in *TP53* still showed significant distributional differences between the DDR-mut and DDR-wt groups. The lollipop charts of *TP53* in the DDR-mut or DDR-wt cohorts are shown in Figure 2C. p.R136H (in 6 patients) and p.R243W (in 10 patients) were the most prevalent mutation sites in the DDR-mut and DDR-wt cohorts, respectively. In both DDR-mut and DDR-wt groups, TP53 and NOTCH pathways were the most frequently mutated pathways. Comparing the pathway mutation frequencies between the two groups, we found Wnt (p=0.0081) and p53 (p=0.0441) pathway mutations were significantly prevalent in DDR-mut patients (Figure 2D).

Association of immune biomarkers with DDR-mut group. We next examined the potential relationship between DDR deficiency and immunotherapy using conventional immune biomarkers. Only one patient was identified as having MSI-H, and this patient also had the DDR-mut phenotype. More than 99% of patients with ESCC were diagnosed with MSS. We found that TMB values in the DDR-mut group were significantly higher than those in the DDR-wt group (p=7.4e-07, Figure 3A). A significant association was also found in TCGA cohort (Supplementary Figure 2B). When

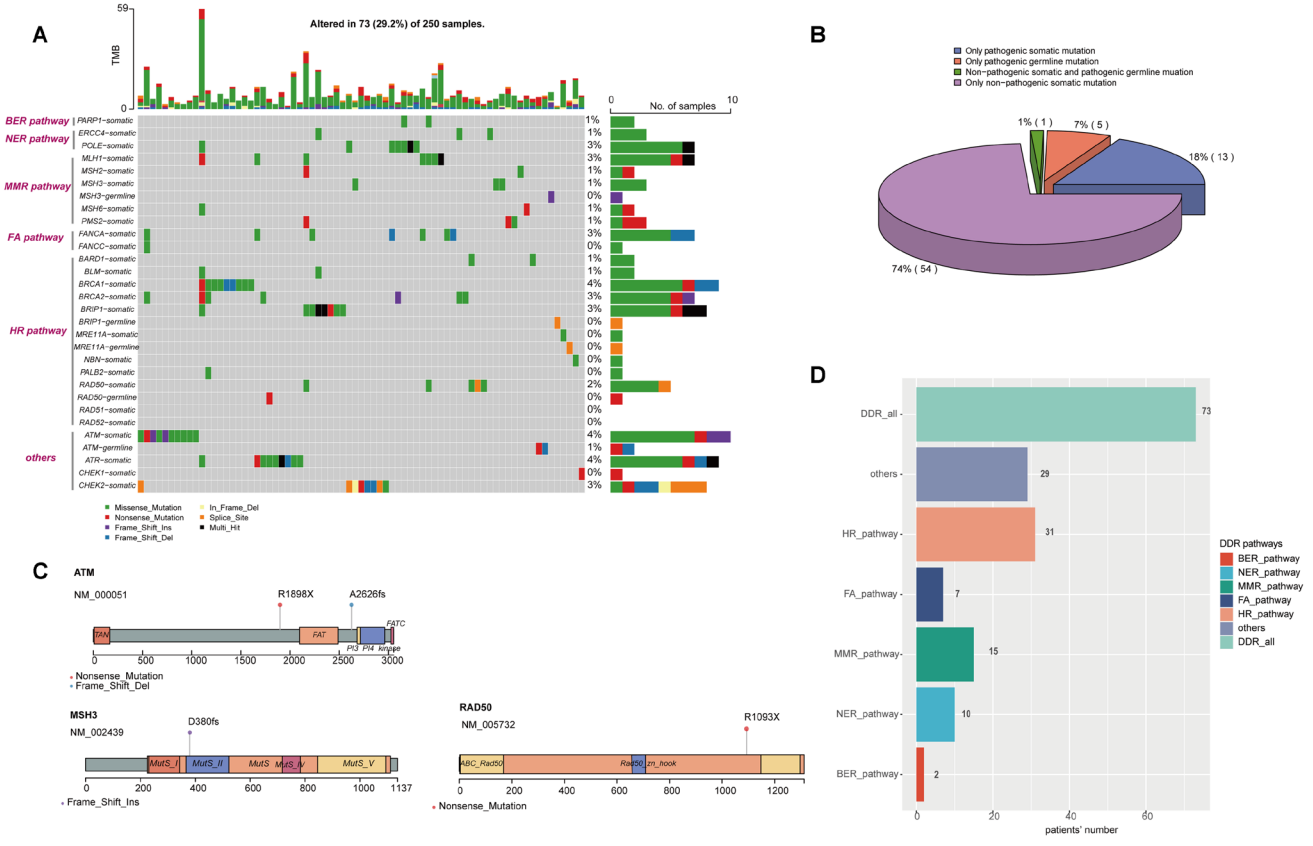

Figure 1. 25 DDR core genes and related DDR functional pathways in 250 Chinese ESCC patients. A) Mutational landscape of 25 DDR core genes and corresponding DDR functional pathways. B) Mutation types of DDR mutations. C) Lollipop charts of pathogenic germline mutations. D) Number of patients with DDR mutations in DDR functional pathways.

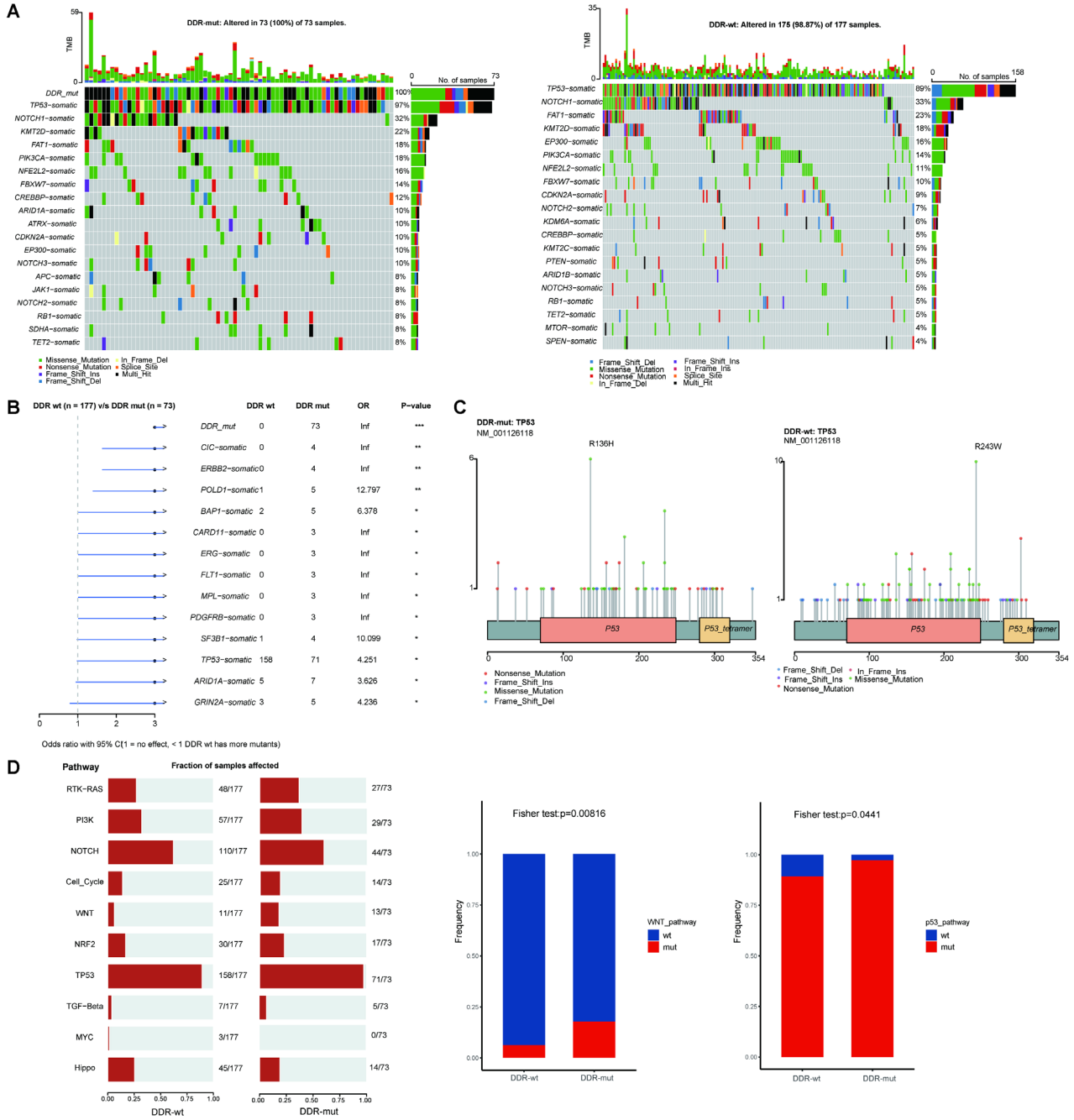

Figure 2. Comparisons between DDR-mut and DDR-wt groups. A) Top 20 mutated genes in DDR-mut (left) and DDR-wt (right) groups. B) Forest plot of differential mutated genes between two groups. C) Lollipop charts of TP53 mutations in DDR-mut (left) and DDR-wt (right) groups. D) Different distributions of tumor pathway mutations in the DDR-wt and DDR-mut groups.

using the binary variable, TMB-H was more common in the DDR-mut group than in the DDR-wt group (49.3% vs. 22.6%, p=6.7e-05, Figure 3B). The BER pathway was excluded from further analysis due to its small sample size. All the mutated DDR pathways (NER, MMR, FA, and HR) were significantly related to higher TMB (p<0.01), except for the other pathways (Supplementary Figure S4A). Additionally, we compared the CNV burden between DDR-mut and DDR-wt groups and found that patients with DDR-mut tended to have a higher CNV burden than DDR-wt patients (p=0.069, Figure 3C). This difference was significant in the FA (p=0.05) and MMR (p=0.017) pathways but disappeared in the HR pathway (Supplementary Figure S4B). Of the 250 patients with ESCC, 107 patients were examined for PD-L1 expres-

sion. Patients with PDL1-H (CPS ≥ 10) accounted for 21.2% (36/107) of the patients. The DDR-mut group tended to have more PDL1-H patients than the DDR-wt group, although the p-value did not reach a significant level (40.5% vs. 30%, p=0.29) (Figure 3E). When further focusing on the five DDR pathways (excluding BER), NER, MMR, and other pathways showed similar tendencies (Supplementary Figure S4C), especially the HR pathway (52.9% vs. 30%, p=0.09, Figure 3F). We then examined the correlation between specific DDR-mutated genes and PD-L1 expression. BRCA1, one of the most prevalent HR genes, was the only DDR gene that was significantly associated with PD-L1 expression status (p=0.01, Figure 3G).

Discussion

In this study, we examined the mutational landscape of 25 core DDR genes in Chinese patients with ESCC. Of the 250 patients, 29.2% presented with at least one DDR mutation and might benefit from multiple treatments.

Platinum-based chemotherapy has been accepted as the first-line treatment for ESCC. Patients with DDR deficiency are considered platinum-sensitive in breast and pancreatic cancer [17, 18]. The clinical significance of DDR deficiency in ESCC is still under investigation. Both cell lines and clinical studies of ESCC have reported that *BRCA1/2* knockdown dramatically increased the sensitivity to cisplatin and could be a predictive marker in ESCC patients who underwent cisplatin treatments [19, 20]. The platinum-based treatment induces DNA damage, and DDR-targeted agents play an important role in maintenance treatment. DDR-targeted agents can maximize DNA damage by affecting DNA repair. Previous research has confirmed that PARP inhibitors such as olaparib showed great efficacy against ovarian and breast tumors harboring *BRCA1/2* mutations [21]. Considering the high frequency of DDR mutations in ESCC across all cancer types [22], patients with DDR mutations have great prospects in clinical applications.

By comparing the mutation frequencies between the DDR-mut and DDR-wt groups, 13 genes were significantly mutated in the DDR-mut group. Five (*POLD1*, *BAP1*, *SF3B1*, *TP53,* and *ARID1A*) of them play critical roles in DNA replication and repair. *SF3B1* mutations have been proven to affect HR and could be targeted with PARP inhibitors. In a

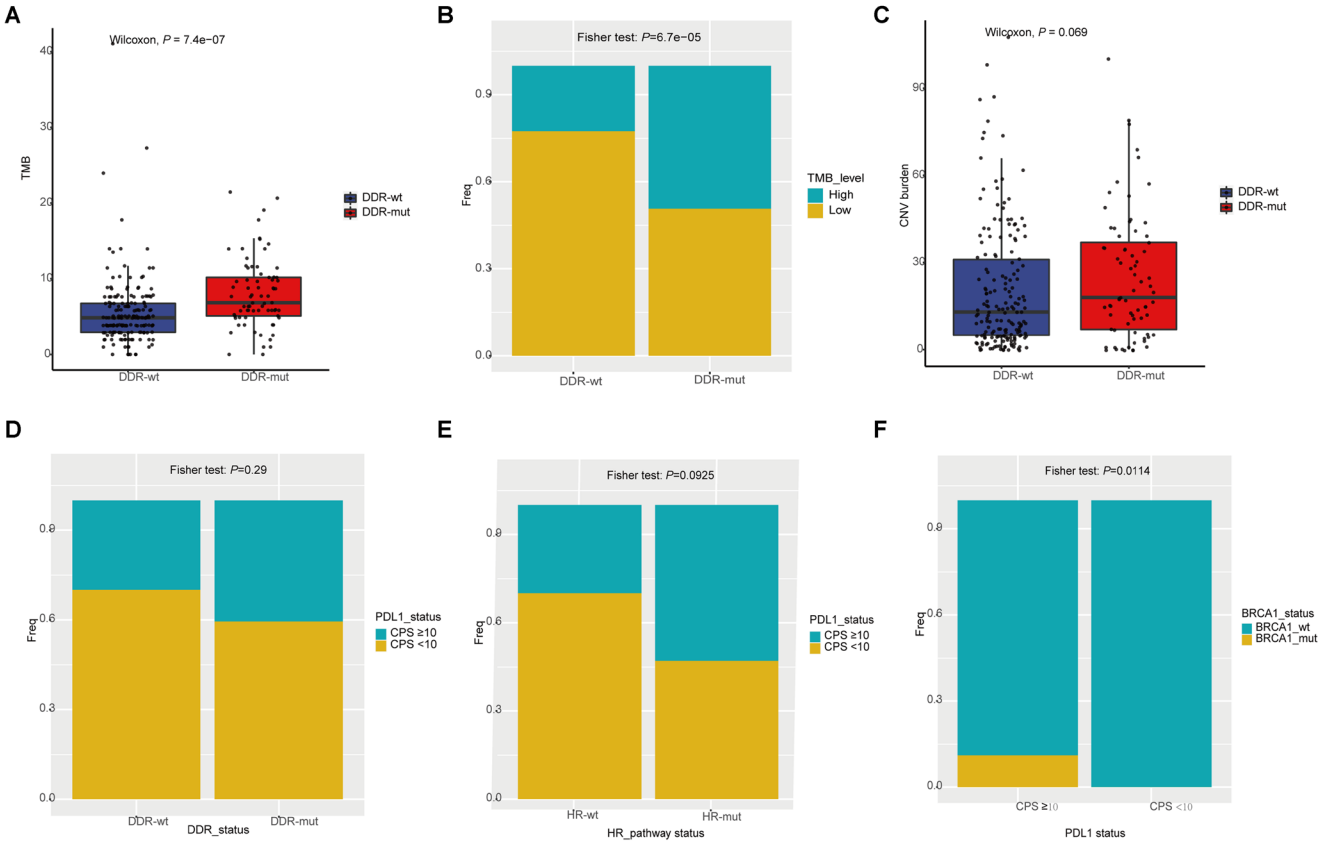

Figure 3. Association of immune biomarkers with DDR-mut group. A, B) TMB value (A)/TMB status (B) was significantly associated with DDR status. C) DDR-mut patients had a higher CNV burden than DDR-wt patients. D, E) DDR-mut (D)/HR-mut (E) groups had more PDL1-H patients than DDR-wt /HR-wt patients. F) *BRCA1* was significantly associated with PDL1-H. Significant level was p<0.05.

large pan-cancer study, *POLD1* mutations were promising potential predictive biomarkers for positive ICI outcomes [23]. Meanwhile, *POLD1*-relevant clinical trials are ongoing (NCT05103969, NCT03810339). These studies suggested that DDR-related gene *SF3B1* and *POLD1* had a good prospect in clinical applications. For patients who received platinum and PARP inhibitors, many would eventually develop the required resistance [24]. However, they also exhibit increased immunogenicity in tumor cells. In gastrointestinal cancer, patients with HR gene mutations show enhanced immune activity and might be an immunotherapy response predictor [25]. Theoretically, DDR deficiency can lead to enhanced genomic instability and tumor immunogenicity in all cancer types. In our Chinese ESCC cohort, DDR deficiency was significantly associated with high TMB. Even divided into five DDR functional pathways, patients with pathway-mut have higher TMB than pathway-wt. Only the other pathways did not reach a significant level (p=0.13). Moreover, the DDR-mut group had a higher CNV burden than the DDR-wt patients, especially in the FA and MMR pathways. MSI-H was a rare event in our Chinese patients with ESCC (0.4%), much lower than that in patients with esophageal cancer from TCGA (1.63%) [26]. PD-L1 expression has been reported as a prognostic biomarker associated with unfavorable clinical outcomes in esophageal cancer [27]. It has also served as a predictive biomarker for immunotherapy [11, 12]. According to the FDA approval, PD-L1 positivity was defined as CPS ≥ 10. There were 21.2% PD-L1 positive patients in our Chinese ESCC cohort. Patients with *BRCA1* mutations were significantly associated with PD-L1 positivity (p=0.01). Although a limited number of *BRCA1* mutated patients were detected in our study, numerous studies have reported an association between BRCA1 and PD-L1, including ovarian cancer [28] and pancreatic cancer [29]. In cell lines and mouse models, DDR inhibitors could induce PD-L1 upregulation [30]. Therefore, DDR deficiency could play an important role in making tumors more receptive to immunotherapy.

Inspired by the synergistic effect of DDR agents and immune checkpoint inhibitors, combined DDR agents with immunotherapy drugs may be a good choice for patients with ESCC who have DDR deficiency or experience required resistance. In fact, a series of clinical trials are ongoing to examine the efficacy of this combination therapy. Early data released from these clinical trials supported the feasibility of the combination strategy [31-34]. Overall, with the development of new DDR inhibitors and immunotherapy drugs, we believe that more patients will benefit from clinical treatment in the future.

This study has several limitations. Firstly, restricted by our panel data, DR, NHEJ, and TLS pathways were not included in our study. As NHEJ is one of the major DDR pathways in ESCC, patients with DDR core gene mutations should be more than 29.2%. Secondly, a larger Chinese cohort is needed to verify the association between PD-L1 positive and *BRCA1* mutations in ESCC. Finally, this is a retrospective study, and we did not obtain prognostic data. The prognostic-related analysis could not be performed.

This study revealed the mutational landscape of 25 DDR core genes and relevant 6 functional pathways in 250 ESCC patients from China. DDR deficiency is significantly associated with immune biomarkers, implying the potential feasibility of combining DDR agents with immunotherapy in patients with DDR deficiency.

Supplementary information is available in the online version of the paper.

References

[1] BRAY F, FERLAY J, SOERJOMATARAM I, SIEGEL RL, TORRE LA et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424. https://doi.org/10.3322/caac.21492

[2] ZHAO J, HE YT, ZHENG RS, ZHANG SW, CHEN WQ. Analysis of esophageal cancer time trends in China, 1989-2008. Asian Pac J Cancer Prev 2012; 13: 4613-4617. https://doi.org/10.7314/apjcp.2012.13.9.4613

[3] THEN EO, LOPEZ M, SALEEM S, GAYAM V, SUNKARA T et al. Esophageal Cancer: An Updated Surveillance Epidemiology and End Results Database Analysis. World J Oncol 2020; 11: 55-64. https://doi.org/10.14740/wjon1254

[4] LORD CJ, ASHWORTH A PARP inhibitors: Synthetic lethality in the clinic. Science 2017; 355: 1152-1158. https://doi.org/10.1126/science.aam7344

[5] WATTENBERG MM, ASCH D, YU S, O'DWYER PJ, DOMCHEK SM et al. Platinum response characteristics of patients with pancreatic ductal adenocarcinoma and a germline BRCA1, BRCA2 or PALB2 mutation. Br J Cancer 2020; 122: 333-339. https://doi.org/10.1038/s41416-019-0582-7

[6] BASOURAKOS SP, LI L, APARICIO AM, CORN PG, KIM J et al. Combination Platinum-based and DNA Damage Response-targeting Cancer Therapy: Evolution and Future Directions. Curr Med Chem 2017; 24: 1586-1606. https://doi.org/10.2174/0929867323666161214114948

[7] KNIJNENBURG TA, WANG L, ZIMMERMANN MT, CHAMBWE N, GAO GF et al. Genomic and Molecular Landscape of DNA Damage Repair Deficiency across The Cancer Genome Atlas. Cell Rep 2018; 23: 239-254e236. https://doi.org/10.1016/j.celrep.2018.03.076

[8] MORSE CB, TOUKATLY MN, KILGORE MR, AGNEW KJ, BERNARDS SS et al. Tumor infiltrating lymphocytes and homologous recombination deficiency are independently associated with improved survival in ovarian carcinoma. Gynecol Oncol 2019; 153: 217-222. https://doi.org/10.1016/j.ygyno.2019.02.011

[9] MOUW KW, GOLDBERG MS, KONSTANTINOPOULOS PA, D'ANDREA AD. DNA Damage and Repair Biomarkers of Immunotherapy Response. Cancer Discov 2017; 7: 675-693. https://doi.org/10.1158/2159-8290.CD-17-0226

[10] SEN T, RODRIGUEZ BL, CHEN L, CORTE CMD, MORIKAWA N et al. Targeting DNA Damage Response Promotes Antitumor Immunity through STING-Mediated T-cell Activation in Small Cell Lung Cancer. Cancer Discov 2019; 9: 646-661. https://doi.org/10.1158/2159-8290.CD-18-1020

[11] KOJIMA T, SHAH MA, MURO K, FRANCOIS E, ADENIS A et al. Randomized Phase III KEYNOTE-181 Study of Pembrolizumab Versus Chemotherapy in Advanced Esophageal Cancer. J Clin Oncol 2020; 38: 4138-4148. https://doi.org/10.1200/JCO.20.01888

[12] SHAH MA, KOJIMA T, HOCHHAUSER D, ENZINGER P, RAIMBOURG J et al. Efficacy and Safety of Pembrolizumab for Heavily Pretreated Patients With Advanced, Metastatic Adenocarcinoma or Squamous Cell Carcinoma of the Esophagus: The Phase 2 KEYNOTE-180 Study. JAMA Oncol 2019; 5: 546-550. https://doi.org/10.1001/jamaoncol.2018.5441

[13] WANG Z, ZHENG X, WANG X, CHEN Y, LI Z et al. Genetic differences between lung metastases and liver metastases from left-sided microsatellite stable colorectal cancer: next generation sequencing and clinical implications. Ann Transl Med 2021; 9: 967. https://doi.org/10.21037/atm-21-2221

[14] LAI Z, MARKOVETS A, AHDESMAKI M, CHAPMAN B, HOFMANN O et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. Nucleic Acids Res 2016; 44: e108. https://doi.org/10.1093/nar/gkw227

[15] WANG K, LI M, HAKONARSON H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic acids research 2010; 38: e164. https://doi.org/10.1093/nar/gkq603

[16] MAYAKONDA A, LIN DC, ASSENOV Y, PLASS C, KOEFFLER HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res 2018; 28: 1747-1756. https://doi.org/10.1101/gr.239244.118

[17] ZHAO EY, SHEN Y, PLEASANCE E, KASAIAN K, LEELAKUMARI S et al. Homologous Recombination Deficiency and Platinum-Based Therapy Outcomes in Advanced Breast Cancer. Clin Cancer Res 2017; 23: 7521-7530. https://doi.org/10.1158/1078-0432.CCR-17-1941

[18] POKATAEV I, FEDYANIN M, POLYANSKAYA E, POPOVA A, AGAFONOVA J et al. Efficacy of platinum-based chemotherapy and prognosis of patients with pancreatic cancer with homologous recombination deficiency: comparative analysis of published clinical studies. ESMO Open 2020; 5: e000578. https://doi.org/10.1136/esmoopen-2019-000578

[19] GAO Y, ZHU J, ZHANG X, WU Q, JIANG S et al. BRCA1 mRNA expression as a predictive and prognostic marker in advanced esophageal squamous cell carcinoma treated with cisplatin- or docetaxel-based chemotherapy/chemoradiotherapy. PLoS One 2013; 8: e52589. https://doi.org/10.1371/journal.pone.0052589

[20] YAN T, CUI H, ZHOU Y, YANG B, KONG P et al. Multi-region sequencing unveils novel actionable targets and spatial heterogeneity in esophageal squamous cell carcinoma. Nat Commun 2019; 10: 1670. https://doi.org/10.1038/s41467-019-09255-1

[21] ROSE M, BURGESS JT, O'BYRNE K, RICHARD DJ, BOLDERSON E. PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. Front Cell Dev Biol 2020; 8: 564601. https://doi.org/10.3389/fcell.2020.564601

[22] HEEKE AL, PISHVAIAN MJ, LYNCE F, XIU J, BRODY JR et al. Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. JCO Precis Oncol 2018; 2018. https://doi.org/10.1200/PO.17.00286

[23] LAPPIN KM, BARROS EM, JHUJH SS, IRWIN GW, MCMILLAN H et al. Cancer-Associated SF3B1 Mutations Confer a BRCA-Like Cellular Phenotype and Synthetic Lethality to PARP Inhibitors. Cancer Res 2022; 82: 819-830. https://doi.org/10.1158/0008-5472.CAN-21-1843

[24] LHEUREUX S, GOURLEY C, VERGOTE I, OZA AM. Epithelial ovarian cancer. Lancet 2019; 393: 1240-1253. https://doi.org/10.1016/S0140-6736(18)32552-2

[25] WANG Y, JIAO X, LI S, CHEN H, WEI X et al. Alterations in DNA damage response and repair genes as potential biomarkers for immune checkpoint blockade in gastrointestinal cancer. Cancer Biol Med 2021; 19: 1139-1149. https://doi.org/10.20892/j.issn.2095-3941.2020.0708

[26] BONNEVILLE R, KROOK MA, KAUTTO EA, MIYA J, WING MR et al. Landscape of Microsatellite Instability Across 39 Cancer Types. JCO Precis Oncol 2017; 2017: PO.17.00073. https://doi.org/10.1200/PO.17.00073

[27] YAGI T, BABA Y, ISHIMOTO T, IWATSUKI M, MIYAMOTO Y et al. PD-L1 Expression, Tumor-infiltrating Lymphocytes, and Clinical Outcome in Patients With Surgically Resected Esophageal Cancer. Ann Surg 2019; 269: 471-478. https://doi.org/10.1097/SLA.0000000000002616

[28] WIESER V, GAUGG I, FLEISCHER M, SHIVALINGAIAH G, WENZEL S et al. BRCA1/2 and TP53 mutation status associates with PD-1 and PD-L1 expression in ovarian cancer. Oncotarget 2018; 9: 17501-17511. https://doi.org/10.18632/oncotarget.24770

[29] SEEBER A, ZIMMER K, KOCHER F, PUCCINI A, XIU J et al. Molecular characteristics of BRCA1/2 and PALB2 mutations in pancreatic ductal adenocarcinoma. ESMO Open 2020; 5: e000942. https://doi.org/10.1136/esmoopen-2020-000942

[30] JIAO S, XIA W, YAMAGUCHI H, WEI Y, CHEN MK et al. PARP Inhibitor Upregulates PD-L1 Expression and Enhances Cancer-Associated Immunosuppression. Clin Cancer Res 2017; 23: 3711-3720. https://doi.org/10.1158/1078-0432.CCR-16-3215

[31] KARZAI F, VANDERWEELE D, MADAN RA, OWENS H, CORDES LM et al. Activity of durvalumab plus olaparib in metastatic castration-resistant prostate cancer in men with and without DNA damage repair mutations. J Immunother Cancer 2018; 6: 141. https://doi.org/10.1186/s40425-018-0463-2

[32] THOMAS A, VILIMAS R, TRINDADE C, ERWIN-COHEN R, ROPER N, et al. Durvalumab in Combination with Olaparib in Patients with Relapsed SCLC: Results from a Phase II Study. J Thorac Oncol 2019; 14: 1447-1457. https://doi.org/10.1016/j.jtho.2019.04.026

[33] LEE JM, ANNUNZIATA CM, HOUSTON N, KOHN EC, LIPKOWITZ S et al. A phase II study of durvalumab, a PD-L1 inhibitor and olaparib in recurrent ovarian cancer (OvCa). Ann Oncol 2018; 29: viii334. https://doi.org/10.1093/annonc/mdy285.145

[34] BANG YJ, KAUFMAN B, GEVA R, STEMMER SM, HONG SH et al. An open-label, phase II basket study of olaparib and durvalumab (MEDIOLA): Results in patients with relapsed gastric cancer. J Clin Oncol 2019; 37: 140. https://doi.org/10.1200/JCO.2019.37.4_suppl.140

https://doi.org/10.4149/neo_2022_220721N738

Mutational landscape of DNA damage response deficiency-related genes and its association with immune biomarkers in esophageal squamous cell carcinoma

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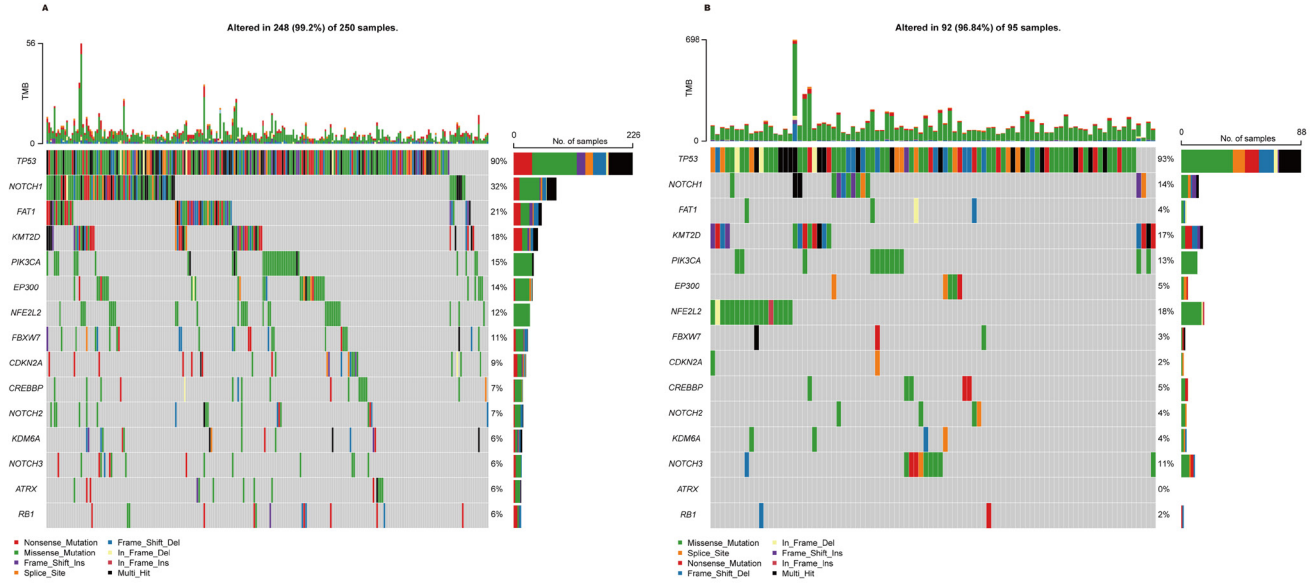
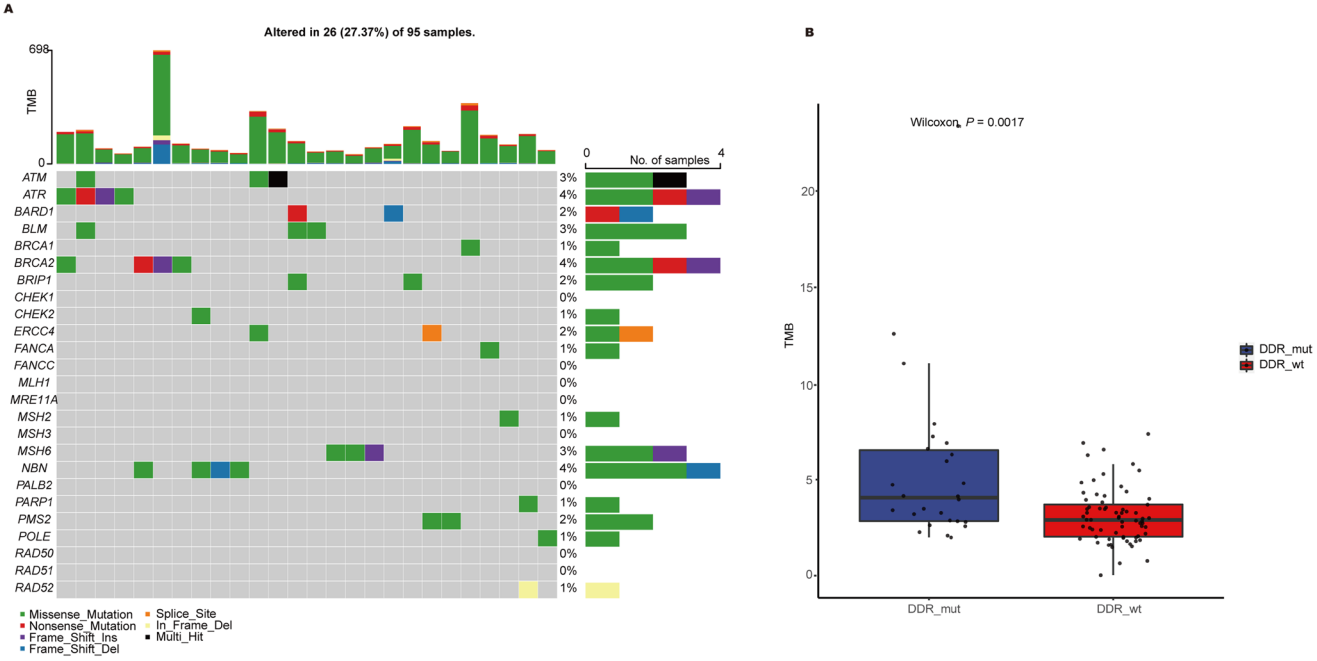
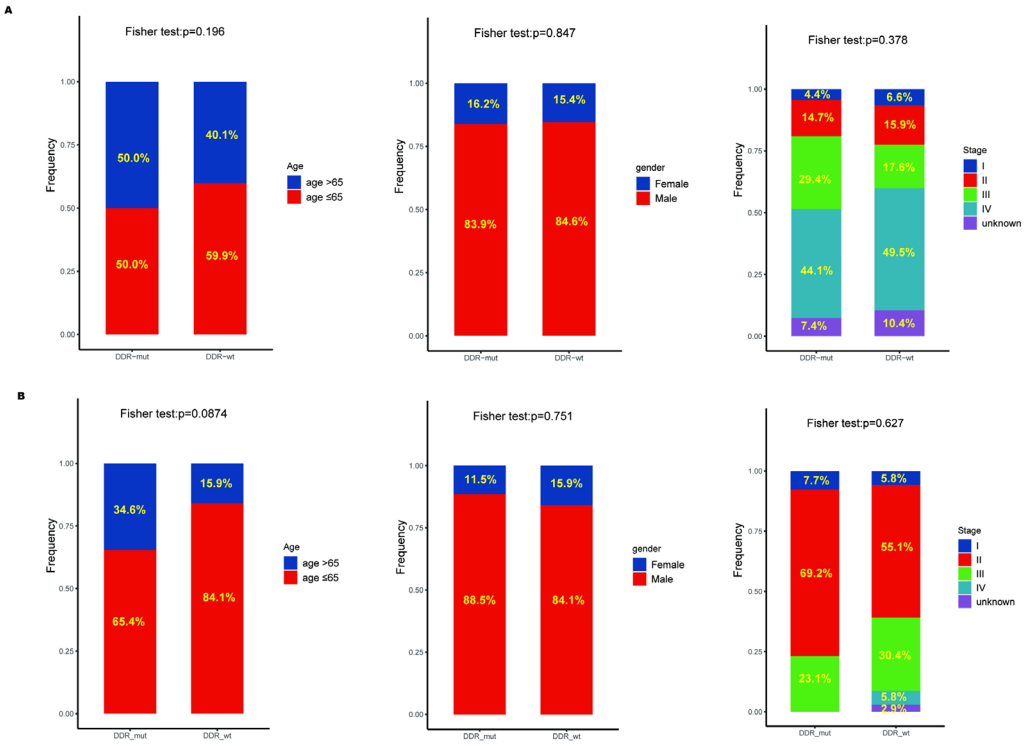
Supplementary Information



Supplementary Figure S1. Mutational landscape in ESCC. A) Top 15 mutated genes in 250 Chinese ESCC patients. B) Mutational landscape of the 15 mutated genes (Chinese cohort) in TCGA cohort.



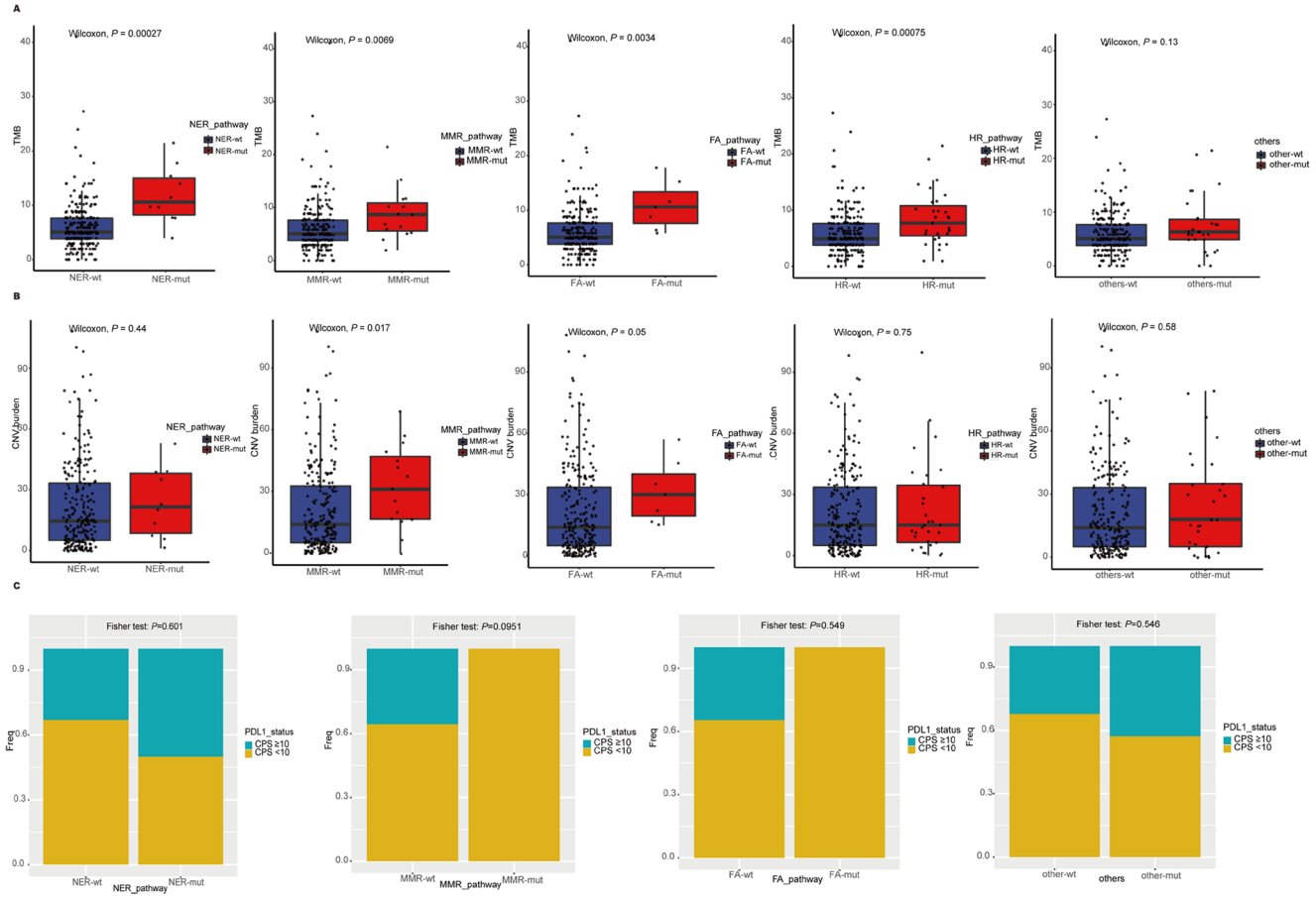
Supplementary Figure S2. DDR mutations in ESCC of TCGA cohort. A) Mutational landscape of 25 DDR core genes in TCGA. B) TMB value was significantly associated with DDR status.



Supplementary Figure S3. Distribution of clinical information (Age, gender and stage) in DDR-wt and DDR-mut groups. A) Chinese ESCC patients. B) TCGA patients.

Supplementary Table S1. 25 DDR core genes and corresponding functional DDR pathways.

Gene Symbol	BER pathway	NER pathway	MMR pathway	FA pathway	HR pathway	NHEJ pathway	DR pathway	TLS pathway	others	DDR all
PARP1	1	0	0	0	0	0	0	0	0	1
ERCC4	0	1	0	0	0	0	0	0	0	1
POLE	0	1	0	0	0	0	0	0	0	1
MLH1	0	0	1	0	0	0	0	0	0	1
MSH2	0	0	1	0	0	0	0	0	0	1
MSH3	0	0	1	0	0	0	0	0	0	1
MSH6	0	0	1	0	0	0	0	0	0	1
PMS2	0	0	1	0	0	0	0	0	0	1
FANCA	0	0	0	1	0	0	0	0	0	1
FANCC	0	0	0	1	0	0	0	0	0	1
BARD1	0	0	0	0	1	0	0	0	0	1
BLM	0	0	0	0	1	0	0	0	0	1
BRCA1	0	0	0	0	1	0	0	0	0	1
BRCA2	0	0	0	0	1	0	0	0	0	1
BRIP1	0	0	0	0	1	0	0	0	0	1
MRE11A	0	0	0	0	1	0	0	0	0	1
NBN	0	0	0	0	1	0	0	0	0	1
PALB2	0	0	0	0	1	0	0	0	0	1
RAD50	0	0	0	0	1	0	0	0	0	1
RAD51	0	0	0	0	1	0	0	0	0	1
RAD52	0	0	0	0	1	0	0	0	0	1
ATM	0	0	0	0	0	0	0	0	1	1
ATR	0	0	0	0	0	0	0	0	1	1
CHEK1	0	0	0	0	0	0	0	0	1	1
CHEK2	0	0	0	0	0	0	0	0	1	1



Supplementary Figure S4. Association of immune biomarkers with DDR functional pathways. A) Association with TMB. B) Association with CNV burden (C) Association with PD-L1.

Supplementary Table S2. Clinical characteristics of Chinese cohort and TCGA cohort.

Characteristics	Chinese cohort (N=250)		TCGA cohort (N=95)	
	n (%)	Mean (SD)	n (%)	Mean (SD)
Age		63.6 (8.33)		58.3 (10.2)
Gender				
Female	39 (15.6)		14 (14.7)	
Male	211 (84.4)		81 (85.3)	
Stage				
I	15 (6.0%)		6 (6.32%)	
II	39 (15.6%)		56 (58.9%)	
III	52 (20.8%)		27 (28.4%)	
IV	120 (48.0%)		4 (4.21%)	
unknown	24 (9.6%)		2 (2.11%)	