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ORIGINAL RESEARCH ARTICLE

Genomic alterations of homologous recombination deficiency in Chinese NSCLC patients

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Abstract

Homologous recombination deficiency (HRD) affects genomic stability and has potential as a biomarker for the effectiveness of poly (ADP-ribose) polymerase (PARP) inhibitors and immune checkpoint inhibitors. However, the clinical and molecular profile of HRD in non-small cell lung cancer (NSCLC), particularly in the Chinese population, remains poorly characterized. Based on the next-generation sequencing data of 158 Chinese NSCLC patients, we analyzed the HRD scores of mutations in homologous recombination repair (*HRR*) genes and dissected the correlation between HRD state and programmed death-ligand 1 (PD-L1) expression. Alterations in *HRR* genes were observed in 8.9% of the patients, with *ATM* and *BRCA2* being the most commonly affected genes. HRD-high (HRD-H) status was significantly associated with advanced disease stage (≥III) and lung squamous cell carcinoma (LUSC). Transcriptomic analysis revealed distinct gene expression profiles between HRD-H and HRD-low (HRD-L) subgroups, with HRD-H tumors exhibiting predominantly downregulated genes. While *EGFR* mutations occurred at similar frequencies across HRD status, *TP53* mutations were significantly enriched in HRD-H cases. HRD-H status correlated with higher PD-L1 positivity in NSCLC overall, but not within the lung adenocarcinoma (LUAD) subgroup in our cohort. The Cancer genome atlas analysis showed higher PD-L1 protein expression in HRD-H LUAD, but not in LUSC. Kyoto Encyclopedia of Genes and Genomes analysis identified enrichment of complement and coagulation cascades, ABC transporters, and bile secretion pathways in HRD-H tumors, suggesting links to immune evasion and drug resistance. This study elucidates the genomic landscape of HRD in Chinese NSCLC patients and provides insights into its potential clinical utility for therapeutic targeting. Our findings suggest that integrated HRD scoring may guide the application of PARP inhibitors and immunotherapy in specific NSCLC patient subgroups. Further prospective clinical studies are needed to validate the predictive value of HRD scoring in NSCLC treatment and to optimize patient selection strategies.

Keywords: Homologous recombination deficiency; Immunotherapy biomarkers; Next-generation sequencing; Non-small cell lung cancer; Programmed death-ligand 1

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1. Introduction

Lung cancer is the most common type of cancer (accounting for 11.6% of all cancer cases) and the leading cause of cancer death worldwide (accounting for 18.4% of all cancer deaths). Lung cancer is one of the leading causes of cancer-related death in China, and its 5-year survival rate is only 19.8%.[1] Roughly 80 – 85% of lung cancers are categorized as non-small cell lung cancer (NSCLC), with lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) being the predominant subtypes of NSCLC.[2] Recent innovations in NSCLC management involve the use of targeted therapies, immunotherapies, and the synergistic pairing of chemotherapy and immunotherapy.[3] Nevertheless, around one-third of individuals diagnosed with LUAD and most patients with LUSC lack oncogenic driver mutations that can be targeted for treatment.[4-6] NSCLC patients without identifiable oncogenic driver alterations can undergo chemotherapy or chemo-immunotherapy, depending on the cancer subtype and the expression of the programmed death ligand-1 gene (*PD-L1*).[7] However, only a small percentage (<20%) of unselected NSCLC patients respond to immunotherapy, and some of these patients experience severe immunotoxicity.[8] Hence, there exists an unmet need to explore potential novel and effective treatment modalities to improve the therapeutic outcomes for NSCLC.

Homologous recombination is pivotal for maintaining genome stability through the repair of DNA double-strand breaks (DSBs) and stalled DNA replication forks.[9] Tumors exhibiting deficiencies in homologous recombination still require intact mechanisms for the repair of DNA lesions that are critical for cell viability, and thus shift their reliance to other functional DNA repair pathways.[10] Targeting these dependent pathways in DNA damage response (DDR)-deficient cancer cells induce a synthetic lethality effect, thereby inhibiting cancer cell proliferation.[11] Poly (ADP-ribose) polymerase (PARP) inhibitors selectively eradicate cells with homologous recombination repair (HRR) deficiencies through synthetic lethality interactions.[12] This synthetic lethality has been extensively applied in breast, ovarian, and prostate cancers with *BRCA1* and *BRCA2* mutations.[13] Therefore, *BRCA* mutations and the status of homologous recombination deficiency (HRD) serve as biomarkers for predicting the efficacy of PARP inhibitors.[14] Alterations in genes of the homologous recombination pathway have been extensively studied, leading to the development of HRD scoring algorithms utilizing various assays to quantify the extent of genomic instability. These algorithms rely on metrics associated with loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions.[15] The breast cancer susceptibility genes *BRCA1* and *BRCA2* are the most well-known genes associated with HRD.[16] Other genes, such as ataxia telangiectasia mutated (*ATM*), *BRCA1* associated RING domain 1 (*BARD1*), and *BRCA1* interacting protein C-terminal helicase 1 (*BRIP1*), have been identified as participants in homologous recombination and related pathways.[17] Numerous studies have demonstrated the presence of HRD in lung cancer.[18-20] However, the expansion of PARP inhibitors into the field of lung cancer through experimental and clinical studies is still limited, and considerable efforts are needed before their application in lung cancer.

Deficiency of homologous recombination, or HRD, arises from defects in DNA repair pathways, particularly the HRR system responsible for repairing DSBs. Abnormalities in HRR can be attributed to germline or somatic mutations of some genes including *BRCA1*, *BRCA2*, *ATM*, *RAD51*, and *BARD1*. These genetic alterations result in genomic instability, a hallmark of cancer development.[20,21] The specific HRD features include large-scale state transitions, loss of heterozygosity, and telomeric allelic imbalances. These genomic characteristics have been used to predict response to PARP inhibitors in ovarian and breast cancers, and have been associated with improved overall survival in patients with *BRCA* mutations, ovarian and breast cancers, and high HRD scores.[22] However, HRD could also be both hereditary and secondary; some tumors become HRD even without *BRCA* mutations, but this makes it useful for more cancer types.

Beyond conferring sensitivity to PARP inhibitors, HRD is also linked to increased tumor immunogenicity. Several investigations have shown that the mutation rate in HRD-positive tumors is higher and that these mutations may give rise to neoantigens and increased infiltration of tumor tissue by immune cells.[23] HRD is associated with increased expression of immune checkpoint proteins such as PD-L1 and activation of interferon signaling pathways, suggesting a potential interaction between HRD and immunotherapy.[24] For example, in microsatellite stable cancers, it has been demonstrated that the utilization of HRD increases the effectiveness of immune checkpoint inhibitors (ICI), including anti-PD-1 and anti-PD-L1 agents.[25] The combination of PARP inhibitors and ICIs is based on pre-clinical evidence that PARP inhibition releases intracellular DNA to activate the cGAS-STING pathway, enhancing immunity against the tumor. That is why today there are many clinical trials of such combinations for various malignancies. For example, the MEDIOLA trial exposed a relatively favorable outcome of combining olaparib and durvalumab in *BRCA*-mutant metastatic breast cancer with a high response rate and disease control.[26]

Despite these advances, studies specifically addressing the clinical and molecular landscape of HRD in NSCLC, particularly in East Asian populations, remain scarce. Most large-scale HRD studies have focused on breast, ovarian, and prostate cancers, where HRD is more prevalent and its prognostic and predictive value is well established.[27] In NSCLC, the prevalence, clinical significance, and biomarker potential of HRD, especially the commonly mutated genes such as *EGFR*, *TP53*, and ALK are not well characterized, and evidence from Chinese populations is particularly limited. At present, the mainstream HRD score is based on the Food and Drug Administration-approved Myriad HRD assay, which uses a threshold of 42.[28] In this study, the HRD threshold was set at 43, determined according to the genomic database of the Chinese population. This threshold better reflects the practical value of our research in Asian NSCLC patients.

In addition, evidence has shown that cancers with HRD exhibit enhanced immunogenicity, and checkpoint inhibitors demonstrate potential efficacy.[29] Numerous oncological studies have illustrated the potential of HRD as a biomarker for immunotherapy.[30,31] One previous study observed the disparities in PD-L1 expression status, genetic backgrounds, and exposure to the environment between Asia and the United States.[32] Thus, there is a need for region-specific research on the applicability and predictive/prognostic values of HRD. Further investigation into HRD could be crucial for identifying new therapeutic targets in NSCLC.

In this study, we aim to describe the correlation between HRD scores and clinical characteristics in Chinese patients with NSCLC, as well as the mutation status in HRD-related genes. Furthermore, we attempted to analyze the relationship between HRD scores and PD-L1 expression, providing foundational data for selecting biomarkers in future clinical targeted therapies for NSCLC.

2. Materials and methods

2.1. Patient cohort and sample collection

This study is a retrospective study conducted on 158 pathologically proven NSCLC patients treated at the Chongqing University Three Gorges Hospital. The cohort included 148 patients with LUAD and 10 patients with LUSC, which are the two most common subtypes of NSCLC.

Patients were enrolled consecutively according to the order of their thoracic surgery admissions over a 6-month period, from August 2023 to January 2024. The inclusion criteria are as follows: (i) age between 18 and 75 years; (ii) histologically confirmed diagnosis of NSCLC (LUAD or LUSC); (iii) availability of sufficient formalin-fixed paraffin-embedded (FFPE) tumor tissue for DNA extraction, with tumor cellularity >30%; (iv) availability of matched germline peripheral blood samples; (v) ability to provide written informed consent; and (vi) complete clinicopathological and demographic data.

The exclusion criteria are as follows: (i) prior neoadjuvant chemotherapy, radiotherapy, or targeted therapy before tissue sampling, to avoid treatment-induced genomic alterations; (ii) insufficient tumor cellularity (<30%) in FFPE samples; (iii) poor DNA quality or quantity after extraction; (iv) diagnosis of small cell lung cancer or other rare NSCLC subtypes; and (v) presence of medical or psychiatric conditions that precluded the possibility of obtaining informed consent.

These criteria were established to ensure high-quality genomic data and accurate HRD assessment and to reduce confounding factors that could artificially alter the observed HRD-related mutation frequency. By excluding patients with prior systemic therapy, inadequate tissue or DNA, or inability to provide informed consent, we aimed to capture the true prevalence of HRD-related mutations in treatment-naïve, representative Chinese NSCLC patients. Informed consent was obtained from all patients, and the study protocol was approved by the institutional ethical review committee.

2.2. DNA extraction and quality control

Total genomic DNA from FFPE tumor tissues was isolated using the Paraffin-Embedded Tissue DNA Extraction Kit (centrifugal column method) from Novogene Biotechnology, Tianjin, China; it is a modified protocol of a standard DNA extraction kit that provides high yield and quality of purified DNA from the fragmented genomic DNA from FFPE samples. For germline DNA, leukocyte from the peripheral blood sample was used, and the DNA was extracted using the Tienken Blood DNA kit of Tiangen Biotech, Beijing, China. DNA yield and quality were determined by using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, USA) and Agilent 2,100 Bioanalyzer (Agilent Technologies, Santa Clara, USA).

2.3. Gene panel design and sequencing

Targeted next-generation sequencing (NGS) was done on a DNAseq custom hybrid capture panel consisting of 188 cancer genes and 37,000 genome-wide single nucleotide polymorphism (SNP) markers. This panel was selectively used to detect somatic and germline mutations in the genes related to HRR and to assess the HRD score based on genomic instability. The panel includes comprehensive coverage of *EGFR* mutation hotspots and other driver

alterations that occur at significantly higher frequencies in East Asian NSCLC patients compared to Western populations. The selected genes include some known oncogenes and DDR-associated genes that are involved in tumor development and therapy response. Sequencing libraries were prepared with 0.5 μg of good-quality DNA per sample. DNA was sheared to 180 – 280 bp using a Covaris M220 ultrasonicator (Massachusetts, USA). End-repair was then performed, followed by A-tailing and adapter ligation, and the samples were finally subjected to polymerase chain reaction enrichment using index primes. DNA samples were purified using AMPure XP beads (Beckman Coulter, USA) and all the libraries were then quantified using a high-sensitivity DNA assay kit from Agilent. DNA sequencing was done in the Illumina NovaSeq 6,000 where the tool used was 2 × 150 bp paired-end. With a significance level at $p<0.05$ and power of 0.90, a minimum average coverage depth was set for targeted genes and SNPs loci at ×1,000 and ×200, respectively, to ensure an adequate HRD scoring and mutation detection.

2.4. Bioinformatics pipeline and variant calling

The raw sequencing data were then filtered using FASTP version 0 (HaploX Biotechnology, China) with adapters trimming and removing low-quality reads. The clean reads were then mapped to the target human genomic reference sequence, specifically the hg19 or the NCBI Build 37. Sambamba was used in the process of sorting the BAM files and Samblaster in identifying the duplicate reads. Somatic and germline single nucleotide variants, as well as small insertion-deletions were called from the tumor and normal samples using VarScan2 (Washington University, USA). All variants were filtered with an in-house pipeline and were then validated using Integrative Genomics Viewer (Broad Institute, USA) to ensure that these were accurate.

2.5. Calculation of HRD score and classification

For the evaluation of HRD, we utilized the scarHRD R package (https://github.com/sztup/scarHRD) to calculate HRD scores based on three genomic instability metrics: loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions. The use of 37,000 genome-wide SNP markers provided high-resolution detection of these events, tailored to the genetic background of Chinese patients. The patients were further divided into two subgroups according to their scores of the HRD as follows:

- HRD-High (HRD-H): Score ≥43
- HRD-Low (HRD-L): Score <43.

This threshold has been taken from other previously validated works[24] and is a biologically significant cut-off to signify patients with high genomic instability and therefore possible responders to therapeutics.

2.6. Biomarker and clinical feature integration

Expression of PD-L1 was assessed using immunohistochemistry, and mutation status for key oncogenes was determined from sequencing data. This comprehensive biomarker assessment allows for an analysis of HRD's clinical significance in the Chinese context.

2.7. Statistical analysis

In this study, we performed the statistical analysis and visualization using the R package map tools. Landscape analyses, statistical tests, and other pertinent studies were made easier by this package.[1] For comparisons between two categorical and continuous variables, Fisher's exact test and the Mann–Whitney *U* tests were used. In addition, the software used for differential analysis of the cancer genome atlas (TCGA) transcriptomic data was limma, and the software used for differential analysis of HRD-RNAseq data was DESeq2. The criteria for selecting differentially expressed genes (DEGs) were $|\log2FC| > 1$ and adjusted $p<0.05$, and all other tests were also performed at the statistically significance level <0.05.

3. Results

3.1. Mutational landscape of *HRR* genes in Chinese NSCLC patients

To study the association between HRD score and NSCLC clinicopathological and genetic features, we employed an NGS test covering 188 cancer-related genes and more than 37,000 SNPs distributed across the human genome. Among 158 NSCLC patients, 8.9% (14/158) harbored somatic genomic alterations in *HRR* genes. Within these 14 patients, we identified 17 mutations in *HRR* genes, with the majority (88.2%) being missense mutations. In addition, 24.7% (39/158) of patients exhibited germline genomic alterations in the *HRR* genes. Among these 39 patients, we identified 49 mutations in the *HRR* genes, with 18.4% being classified as pathogenic mutations (Figure 1A). In our cohort of Chinese NSCLC patients, *ATM* emerged as the most frequently somatically mutated *HRR* gene, occurring in 4.4% of cases. The most common germline mutated gene among the *HRR* genes was *BRCA2,* present in 7.0% of cases, followed by *ATM* at 3.2%, *BRIP1* at 3.2%, and *BARD1* at 2.5% (Figure 1A). Our analysis revealed that *EGFR* alterations were mutually exclusive with many other genetic alterations, suggesting that *EGFR* mutations represent the most potent driver events in NSCLC. Conversely, we observed that changes in multiple

[1] Accessible at http://bioconductor.org/packages/release/bioc/vignettes/maftools/inst/doc/maftools.html

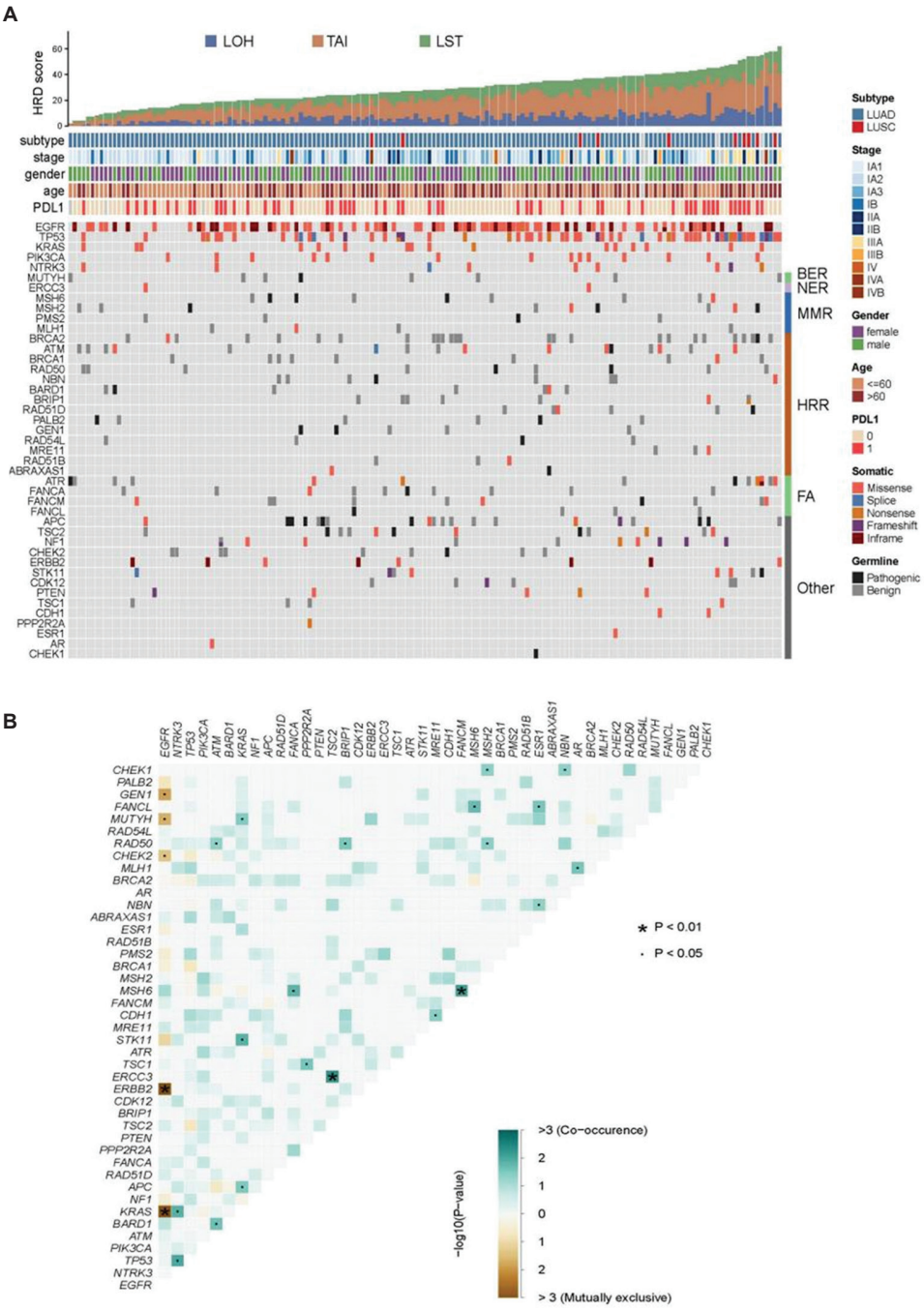

Figure 1. Genomic HRD score and mutation landscape in Chinese non-small cell lung cancer (NSCLC) patients. (A) Landscape of *HRR* genes and clinical characteristics in 158 Chinese NSCLC patients sorted by HRD score. In this cohort, 8.9% (14/158) exhibited somatic genomic alterations in *HRR* genes, identifying 17 mutations predominantly of the missense type (88.2%). Germline genomic alterations were observed in 24.7% (39/158) of the cohort with 49 mutations identified, and 18.4% were categorized as pathogenic. *ATM* was the most frequently mutated somatic *HRR* gene (4.4%), while *BRCA2* was the most commonly mutated germline *HRR* gene (7.0%), followed by *ATM* (3.2%), *BRIP1* (3.2%), and *BARD1* (2.5%). (B) Co-occurring and mutually exclusive somatic mutations in the cohort are illustrated. *EGFR* alterations showed mutual exclusivity with many other genetic alterations, underscoring its potential as a major driver event in NSCLC. Significant co-occurrences of mutations were found particularly between *ERCC3* and *TSC2*, and between *MSH6* and *FANCM* (p<0.01).

Note: p-values were calculated using Fisher's exact test: *p<0.01, p<0.05.

Abbreviations: BER: Base-Excision Repair; FA: Fanconi Anemia; HRD: Homologous recombination deficiency; HRR: Homologous recombination repair; LOH: Loss of heterozygosity; LST: Large-scale state transition; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MMR: Mismatch Repair; NER: Nucleotide Excision Repair; NSCLC: Non-small cell lung cancer; TAI: Telomeric allelic imbalance.

genes often occur simultaneously, suggesting potential synergistic effects among these genes. Particularly, the concurrent mutations in the *ERCC3* and *TSC2* genes, as well as the co-mutations in the *MSH6* and *FANCM* genes, demonstrated significant statistical importance ($p<0.01$) (Figure 1B).

3.2. The relationship between clinicopathological characters and the HRD score in NSCLC

To further illustrate the clinical value of HRD score in NSCLC, we analyzed the relationship between clinicopathological characteristics and HRD score (Figure 1A). Patients with advanced-stage (stage III and above) NSCLC are more likely to have high HRD scores compared to those with early-stage (stage I and II) disease (Table 1), but not in the TCGA cohort. Furthermore, individuals with LUSC have a higher incidence of HRD than patients with LUAD, which was consistent with the TCGA cohort. No significant differences in clinical characteristics, including age, smoking history, and gender, were observed between HRD-H and HRD-L patients (Table 1).

To further analyze the transcriptomic differences between HRD-H and HRD-L subgroups, RNA sequencing (RNA-seq) data from both the TCGA dataset and our NSCLC cohort were analyzed. Volcano plots (Figure 2A and C) revealed a higher number of DEGs in HRD-H compared to HRD-L, with a predominance of downregulated genes. This suggests that HRD-H may suppress certain transcriptional programs, potentially linked to genomic instability caused by homologous recombination defects. Heatmaps (Figure 2B and D) validated these findings, demonstrating consistent expression patterns of DEGs across distinct samples. These results highlight systematic transcriptomic disparities between HRD-H and HRD-L subgroups, underscoring their potential as biomarkers for NSCLC stratification.

Table 1. Clinical characteristics of HRD-H and HRD-L patients

Characteristics	HRD-H (n=19)	HRD-L (n=139)	p-value
Age (%)			0.3354
Young (≤60 years)	8 (42.11)	76 (54.68)	
Old (>60 years)	11 (57.89)	63 (45.32)	
Gender (%)			0.9999
Female	9 (47.37)	70 (50.36)	
Male	10 (52.63)	69 (49.64)	
Stage (%)			***<0.0001
Early (<III)	10 (55.56)	122 (93.13)	
Late (≥III)	8 (44.44)	9 (6.87)	
Histology (%)			**0.0032
LUAD	14 (73.68)	126 (96.18)	
LUSC	5 (26.32)	5 (3.82)	
Smoking (%)			0.3075
Yes	9 (47.37)	47 (33.81)	
No	10 (52.63)	92 (66.19)	

Notes: **$p<0.01$, ***$p<0.001$.
Abbreviations: HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma.

3.3. Genetic alterations between HRD-H and HRD-L patients in LUAD

To gain deeper insights into the genomic alteration spectrum associated with the HRD phenotype in LUAD patients, we assessed and contrasted the frequencies of gene mutations between individuals classified within HRD-H and HRD-L groups. *EGFR* and *TP53* were the most commonly mutated genes in both the HRD-H and HRD-L groups (Figure 3A). In the HRD-H group, 10 out of 14 cases exhibited mutations in the *EGFR* gene. *TP53* gene was more frequently mutated in HRD-H than in HRD-L patients (Figure 3B and Figure A1 in the Appendix).

3.4. Association between HRD status and PD-L1 expression

In our study, we assessed the PD-L1 expression status in 154 patients. Out of these patients, 36.4% (56/154) tested positive for PD-L1 expression. We found that the positive rate of PD-L1 expression was significantly higher in the HRD-H group compared to the HRD-L group in NSCLC (Figure 4A and Figure A2 in the Appendix). Nevertheless, there was no correlation of HRD phenotype with PD-L1 expression in either the HRD-H or the HRD-L group in LUAD (Figure 4B). In addition, we analyzed the PD-L1 expression in the TCGA database. The TCGA PD-L1 expression data of LUSC and LUAD datasets were directly pulled down from the UCSC Xena RPPA TCGA hub.[2] These data include 352 LUAD patients and 321 LUSC patients. Since the PD-L1 status in the TCGA database cannot be classified as positive or negative, we examined its relationship with HRD status by comparing numerical values. Our analysis revealed that in TCGA-LUAD, the mean protein expression of PD-L1 was significantly higher in the HRD-H group compared to the HRD-L group (Figure 4C). However, in TCGA-LUSC, there was no significant difference observed (Figure 4D).

3.5. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis in TCGA and study cohorts

KEGG pathway enrichment analysis of HRD-H and HRD-L

[2] https://xenabrowser##net/datapages/

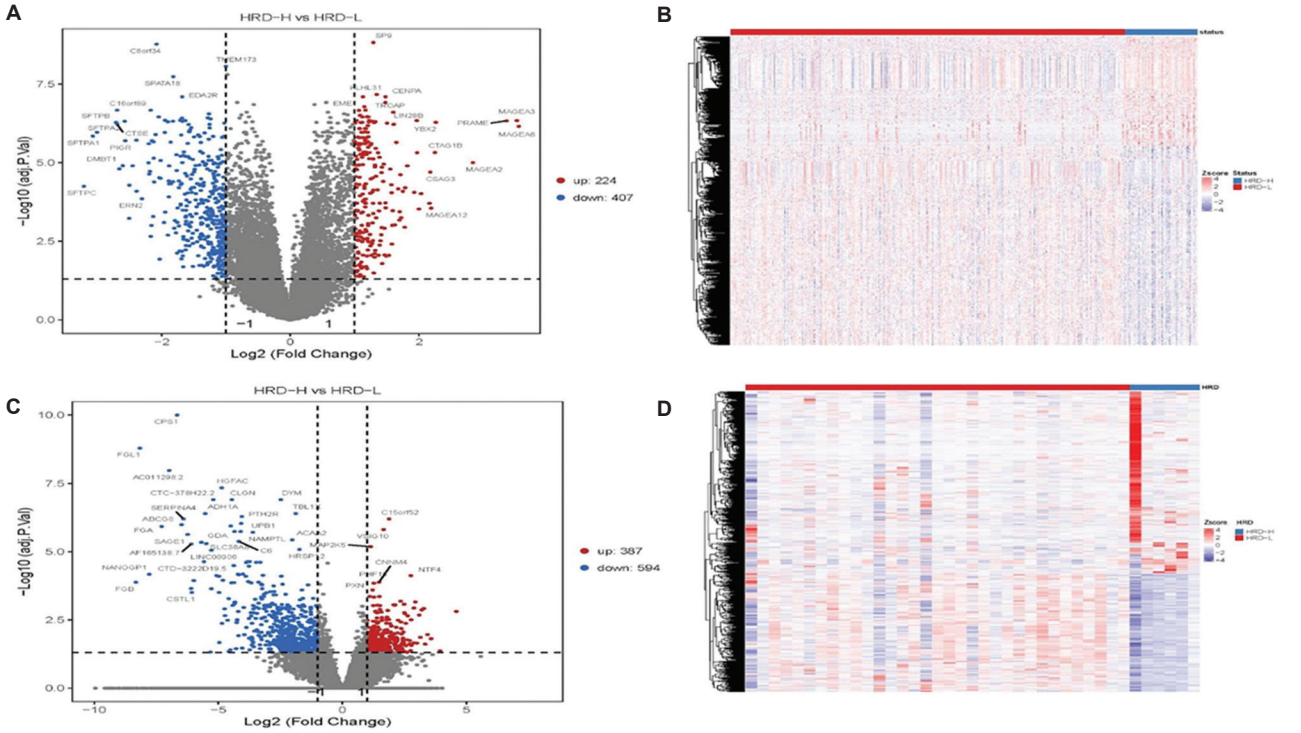

Figure 2. Differentially expressed genes between HRD-H and HRD-L RNA-seq. (A) Volcano plot depicting DEGs between HRD-H and HRD-L subgroups in the TCGA database. A greater number of DEGs are observed in HRD-H compared to HRD-L, with predominance of downregulated genes, indicative of potentially suppressed transcriptional programs linked to homologous recombination defects. Genes with significant differential expression (upregulated, red; downregulated, blue) are highlighted. (B) Heat map illustrating the expression patterns of DEGs between HRD-H and HRD-L groups in the TCGA dataset. Consistent expression patterns are noted across samples, validating the differential expression observed in the volcano plot. (C) Volcano plot of DEGs between HRD-H and HRD-L in RNA-seq data from 40 NSCLC cases in China. Similar to the TCGA analysis, HRD-H samples exhibit more DEGs, with a significant number of downregulated genes highlighted (blue) compared to upregulated genes (red). (D) Heat map showing consistent expression patterns of DEGs in HRD-H and HRD-L groups in the cohort of 40 Chinese NSCLC patients, reinforcing the observed transcriptomic disparities between the HRD subgroups.

Abbreviations: DEGs: Differentially expressed genes; HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low; NSCLC: Non-small cell lung cancer; RNA-seq: RNA sequencing; TCGA: The Cancer Genome Atlas.

subgroups in both the TCGA cohort and our study cohort (Figure 5A-D) revealed distinct metabolic, immune, and drug-response signatures in HRD-H tumors. In the TCGA cohort (Figure 5A and B), HRD-H tumors exhibited significant downregulation of the protein digestion and absorption pathway ($P_{adjust} \approx 0.10$) and pancreatic secretion pathway, suggesting impaired nutrient metabolism that may exacerbate genomic instability. Concurrently, the complement and coagulation cascades pathway (Rich factor >10) was markedly enriched in HRD-H tumors (Figure 5B), indicating complement-dependent immune evasion. In our cohort (Figure 5C and D), recurrent enrichment of the complement and coagulation cascades pathway ($P_{adjust} < 0.05$) further validated the immunosuppressive phenotype of HRD-H. Strikingly, upregulation of the ABC transporters pathway (Rich factor >15) and bile secretion pathway (Figure 5D) highlighted enhanced drug efflux as a potential resistance mechanism in HRD-H tumors. These pathway-level insights underscore the potential value of HRD as a biomarker for guiding precision therapy in NSCLC.

4. Discussion

In this study, we investigated the epidemiologic characteristics of HRD in Chinese NSCLC patients and discussed the rationale for using HRD as a biomarker in treatment strategies. The results showed that 8.9% of NSCLC patients had somatic variants in *HRR* genes, of which *ATM* and *BRCA2* were most affected. This observation is clinically significant, as patients with HRD, particularly those harboring *BRCA1/2* or *ATM* mutations may be candidates for PARP inhibitor therapy. PARP inhibitors have been known to be effective in patients with HR-positive breast and ovarian cancers and its applicability to NSCLC patient has been explored and found effective in patients who meet genomic instability criteria.

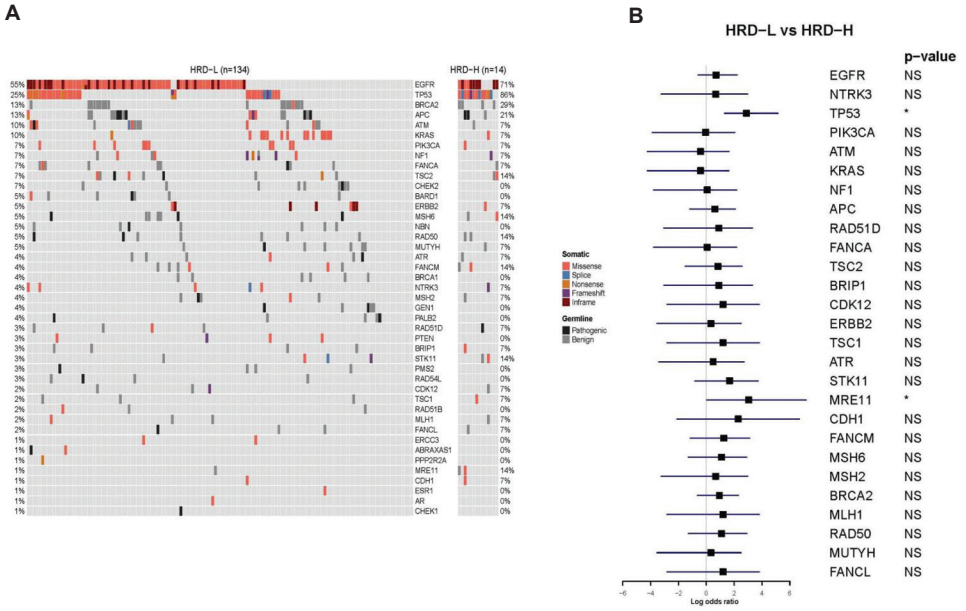

Figure 3. Comparison of genomic alterations between the HRD-L and HRD-H groups of Chinese NSCLC patients. (A) Mutational landscapes of HRD-L (n = 134) and HRD-H (n = 14) groups highlighting the frequencies of gene mutations. *EGFR* and *TP53* genes were the most frequently mutated in both HRD-H and HRD-L groups. Notably, 71% of HRD-H cases harbored mutations in the *EGFR* gene, and *TP53* was more frequently mutated in HRD-H (86%) than in HRD-L patients (26%). (B) Forest plot depicting the enrichment of gene mutations in HRD-L and HRD-H groups, measured by logarithmic odds ratio (*p<0.05). The x-axis shows the log odds ratio. Despite the higher mutation rates of *EGFR* and *TP53* in the HRD-H group, these did not reach statistical significance in the comparative analysis. Other genes, including *PIK3CA*, *ATM*, *KRAS*, and *MSH6*, also showed variations in mutation frequency between the two groups, without statistically significant differences.
Note: NS denotes not significant.
Abbreviations: HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low; NSCLC: Non-small cell lung cancer.

Furthermore, this study provides valuable insights into the genetic and clinical differences between HRD-H and HRD-L patients. Notably, HRD-H patients were more frequently diagnosed at advanced stages and exhibited a higher prevalence of *TP53* mutations, further supporting the association between HRD and genomic instability.[33] The co-occurrence of HRD-H status and *TP53* mutations is particularly noteworthy, as both features are individually associated with aggressive tumor biology and poor prognosis in NSCLC.[32] *TP53* mutations can compromise cell cycle control and apoptosis,[34] while HRD leads to defective DNA repair and increased genomic instability.[23] The combination of these alterations may synergistically promote tumor progression, resulting in more advanced disease at diagnosis and potentially poorer clinical outcomes. This synergistic effect is evident in our cohort, where patients with HRD-H status exhibited significantly higher rates of advanced disease (stage IIIB-IV) compared to HRD-L patients (approximately 60% vs. 40%). This association likely reflects the aggressive phenotype driven by extreme genomic instability when both alterations are present.

From a therapeutic perspective, this co-occurrence may have complex implications. On the one hand, tumors harboring both HRD-H and *TP53* mutations might be more sensitive to DNA-damaging agents, such as platinum-based chemotherapy and PARP inhibitors,[35] due to their impaired ability to repair DNA damage. On the other hand, the presence of *TP53* mutations has been associated in some studies with resistance to certain therapies and with a more immunosuppressive tumor microenvironment, which could influence response to ICIs.[36] Therefore, the dual presence of HRD-H and *TP53* mutations may define a subset of patients with both high therapeutic vulnerability and high risk, showing the need for tailored treatment strategies and close clinical monitoring. Further research is warranted to clarify the prognostic and predictive value of this co-occurrence and to optimize therapeutic approaches for these patients.

Apart from the management of PARP inhibitors, HRD may have applicability in identifying the patients who would benefit from the use of ICIs. In contrast to the TCGA-LUAD set, *a priori*-defined HRD was not associated with PD-L1 expression in our LUAD cohort, although previous reports also indicated that the latter had higher immunogenicity in cancers with HRD.[37] Molecularly, HRD results in DNA damage that can activate the cGAS-STING

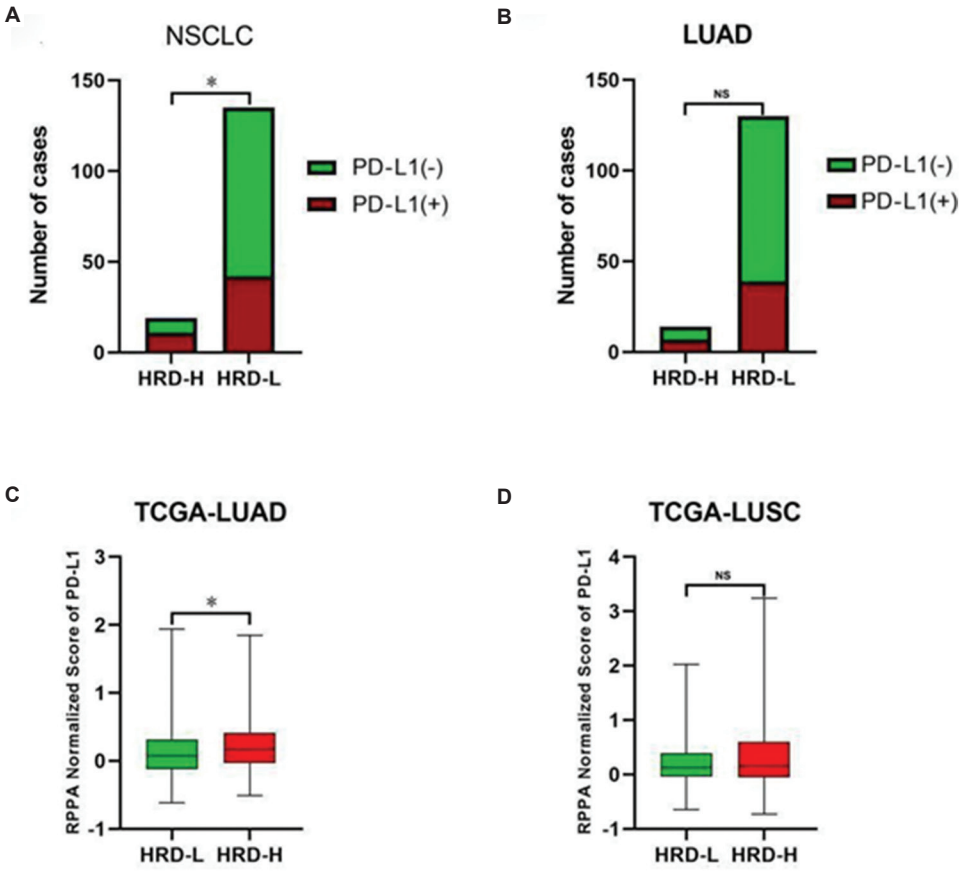

Figure 4. The association of HRD status with PD-L1 expression in this cohort and TCGA cohort. (A) Comparison of the number of PD-L1 positive and negative cases between the HRD-H (n = 14) and HRD-L (n = 140) groups in NSCLC. The HRD-H group exhibited a significantly higher rate of PD-L1 positive expression compared to the HRD-L group. (B) Comparison of the number of PD-L1 positive and negative cases between the HRD-H and HRD-L groups in LUAD. No significant correlation was observed between HRD phenotype and PD-L1 expression in LUAD. (C) Comparison of normalized scores of PD-L1 expression between the HRD-L (n=321) and HRD-H (n = 31) groups in the TCGA-LUAD dataset. The mean PD-L1 protein expression was significantly higher in the HRD-H group compared to the HRD-L group. (D) Comparison of normalized scores of PD-L1 expression between the HRD-L (n = 317) and HRD-H (n = 31) groups in the TCGA-LUSC dataset. No significant difference in PD-L1 expression was observed between the HRD-L and HRD-H groups in the TCGA-LUSC dataset.
Note: *p<0.05, NS denotes not significant.
Abbreviations: HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; NSCLC: Non-small cell lung cancer; PD-L1: Programmed death-ligand 1; RPPA: Reverse phase protein array; TCGA: The Cancer Genome Atlas.

pathway and release type I interferons, thus increasing immune cell infiltration.[38] This process may upregulate immune checkpoint molecules, including PD-L1, thereby increasing tumor immunogenicity. Clinically, this suggests that HRD-positive tumors could be more responsive to ICIs. Recent clinical evidence strongly supports this dual biomarker approach. In the CheckMate-9LA study, patients with both HRD positivity and PD-L1 ≥1% who received nivolumab plus ipilimumab combined with chemotherapy achieved a remarkable 3-year overall survival rate of 38%, significantly outperforming patients positive for only one biomarker.[39] Therefore, HRD status, in combination with PD-L1 expression, could serve as a composite biomarker to identify patients most likely to benefit from immunotherapy or combination regimens.

Although in our LUAD cohort, pre-defined HRD was not significantly associated with PD-L1 expression, this does not rule out the potential for synergy between PARP inhibitors and ICIs in NSCLC. Previous studies in other solid tumors have demonstrated that combining PARP inhibitors with ICIs can enhance anti-tumor immune responses, as seen in the MEDIOLA and TOPACIO trials.[40] Therefore, integrating HRD status and PD-L1 expression into clinical decision-making could help stratify patients: Those with both high HRD scores and elevated PD-L1 expression may be prioritized for combination therapies, while those with only one or neither biomarker may be directed toward alternative strategies. This approach could optimize therapeutic outcomes and minimize unnecessary toxicity.

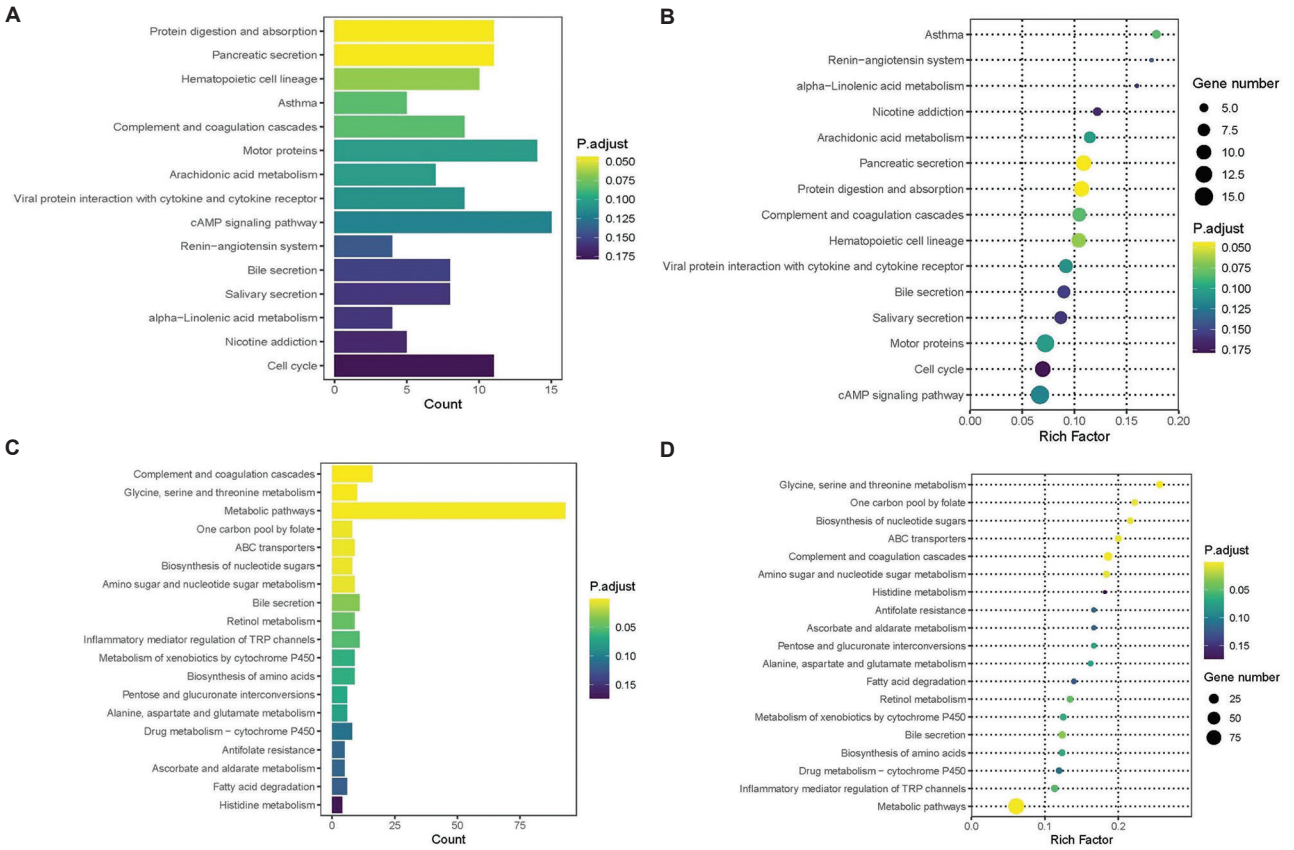

Figure 5. KEGG pathway analysis. (A and B) KEGG pathway analysis for TCGA databases. (C and D) KEGG pathway analysis for our study participant's RNA-seq data. (A and C) Bar plots display the number of genes involved in significantly enriched KEGG pathways for HRD-H tumors compared to HRD-L tumors. The bar color gradient indicates the adjusted *p*-value significance, from yellow (less significant) to purple (more significant). (B and D) Bubble plots summarizing KEGG pathway enrichment, with the y-axis listing pathways and the x-axis showing the Rich factor, a measure of pathway enrichment. Abbreviations: HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low; KEGG: Kyoto Encyclopedia of Genes and Genomes; RNA-seq: RNA sequencing; TCGA: The Cancer Genome Atlas.

However, there are several limitations in this study. First, the sample size was relatively small for certain subgroups, such as HRD-H and LUSC, limiting the statistical power to detect additional associations. Second, this is a retrospective study. Without prospective data collection, we were unable to systematically capture important clinical outcomes such as survival data and treatment response rates. The retrospective design also precluded the assessment of the HRD score as a direct predictor of treatment efficacy or as a prognostic factor under specific therapeutic regimens. Moreover, the discrepancy between our cohort's data and the TCGA-LUAD data on PD-L1 mainly implies that genetic factors such as ancestry, tumor microenvironment, and immune system might affect the association between HRD and immunogenicity on a population level. These limitations underscore the necessity for larger, prospective, population-specific studies to fully elucidate the clinical utility of HRD as a biomarker in NSCLC and to validate our preliminary findings. Despite these constraints, our study provides valuable insights into the genomic landscape of HRD in Chinese NSCLC patients and lays important groundwork for future investigations into targeted therapeutic approaches.

5. Conclusion

The present study provides preliminary evidence supporting the implementation of HRD status to subclassify NSCLC and to guide precision therapy strategies. HRD may serve not only as a biomarker for selecting patients likely to benefit from PARP inhibitors but also as a potential predictor of immunotherapy efficacy. Future large-scale, prospective studies that integrate comprehensive clinical data and evaluate combination treatment regimens are warranted to further validate the prognostic and therapeutic value of HRD in NSCLC.

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Conflict of interest

Mingzhu Yin is an Editor-in-Chief of this journal but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

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Writing – review & editing: Song Duan, Mingzhu Yin

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of the Chongqing University Three Gorges Hospital (Institutional Review Board approval number: 2022-Scientific Research No. 145). Written informed consent was obtained from the patients before their participation.

Consent for publication

Patients consented on the publication of their data.

Availability of data

The sequencing data were obtained from the public datasets of TCGA and affiliated research centers.

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Appendices

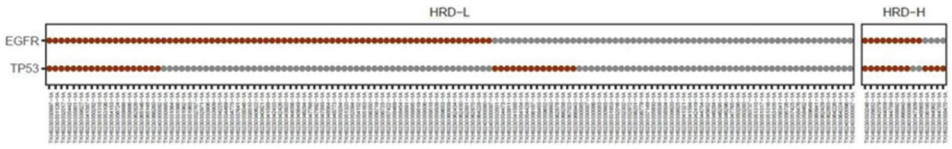

Figure A1. Distribution of gene mutations in the HRD-H and HRD-L groups, showing the distribution of mutation status (mutated/unmutated) of different genes in patients. X-axis: Group (HRD-H, HRD-L). Y-axis: Gene name (*EGFR*, *TP53*). Each point represents a patient, with mutation/unmutated marked by color.
Abbreviations: HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low.

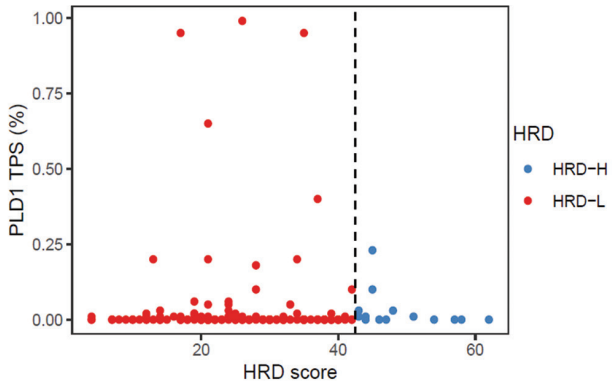

Figure A2. The difference in PD-L1 expression distribution between the HRD-H and HRD-L groups, visually presenting the PD-L1 expression status of each data point (patient). X-axis: Group (HRD-H, HRD-L). Y-axis: PD-L1 expression level (percentage). Each point represents a patient.
Abbreviations: HRD-H: Homologous recombination deficiency-high; HRD-L: Homologous recombination deficiency-low; PD-L1: Programmed death-ligand 1; TPS: Tumor proportion score.