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RESEARCH ARTICLE

PATHOLOGY

Lin et al.: Multiomics study of HRD in cancer

Multiomics analysis of homologous recombination deficiency across cancer types

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ABSTRACT

There remains ongoing debate regarding the association of homologous recombination deficiency (HRD) with patient survival across various malignancies, highlighting the need for a comprehensive understanding of HRD's role in different cancer types. Based on data from databases, we conducted a multivariable omics analysis on HRD in 33 cancer types, focusing mainly on 23 cancers in which HRD was significantly associated with patient overall survival (OS) rates. This analysis included the mechanisms related to patient prognosis, gene expression, gene mutation, and signaling pathways. In this study, HRD was found to be significantly associated with patient prognosis, but its impact varied among different cancers. HRD was linked to different outcomes for patients with distinct tumor subtypes and was correlated with clinical features such as clinical stage and tumor grade. Driver gene mutations, including *TP53*, *MUC4*, *KRAS*, *HRAS*, *FLG*, *ANK3*, *BRCA2*, *ATRX*, *FGFR3*, *NFE2L2*, *MAP3K1*, *PIK3CA*, *CIC*, *FUBP1*, *ALB*, *CTNNB1*, and *MED12*, were associated with HRD across specific cancer types. We also analyzed differentially expressed genes (DEGs) and differentially methylated regions (DMRs) in relation to HRD levels in these cancers. Furthermore, we explored the correlation between HRD and signaling pathways, as well as immune cell infiltration. Overall, our findings contribute to a comprehensive understanding of HRD's multifaceted role in cancer.

Keywords: Homologous recombination deficiency (HRD); prognosis; gene mutation; DNA methylation; signaling pathway; immunology.

INTRODUCTION

Despite rapid advancements in medical technology, cancer remains a leading global cause of mortality [1]. Recently, immunotherapy is developed and widely used in cancer treatment and is accepted to be one of the most promising strategies for cancer therapy [2-4]. For specific cancer subtypes, such as lung squamous cell carcinoma (LUSC) and bladder urothelial carcinoma (BLCA), immunotherapy shows well curative effect and extremely extends patient survival rate [5, 6]. Despite of these significant advances in many cancer subgroups, the clinical application of immunotherapy still faces several challenges related to efficacy and safety [7, 8], and only a small proportion of patients benefit from immunotherapy [9, 10]. For instance, studies have found that anti-PD-1 monoclonal antibodies are a promising method for treating advanced GC patients, but the response rate is still limited, and it is necessary to develop new strategies to maximize the efficacy of ICI. Therefore, further study to unveil precise biomarkers to predict the efficacy of immunotherapy and explore new effective bio-target for cancer therapy are important and urgent.

Homologous recombination is a highly conservative process, playing an important role in DNA repair, DNA replication, meiosis, chromosome separation and telomere maintenance [11].

Homologous recombination repair is one of the core methods for DNA damage repair, mainly occurs in the S phase and G2 phase of the cell cycle, and is a DNA repair mechanism to maintain genome integrity and ensures the transmission of genetic information with high fidelity [12].

When DNA damage occurs and cannot be repaired normally through homologous recombination repair process, it is considered homologous recombination deficiency (HRD) [13]. Some known

genes encoding homologous recombinant proteins include BRCA1, BRCA2, ATM, ATR, BARD1, BLM, RAD51, etc. [14, 15] As members of homologous recombinant proteins, BRCA1 and BRCA2 are widely focused on for HRD study because they are correlated with hereditary ovarian cancer and breast cancer [16, 17]. Recent studies have highlighted HRD's relevance to immunotherapy outcomes and patient prognosis across various cancers [18, 19]. BRCA1/2 mutations, for example, play a critical role in stratifying ovarian cancer subtypes based on HRD scores, influencing treatment decisions and patient outcomes [20]. While extensive research has explored HRD within specific cancers such as breast, ovarian, and prostate cancers [20-22], comprehensive pan-cancer analyses remain limited. Therefore, there is a compelling need for a detailed investigation into HRD's impact across diverse cancer types.

Drawing on public databases and bioinformatic methodologies, our study systematically examined the correlation between HRD and patient prognosis, clinical parameters, driver gene mutations, mismatch repair gene expression, DEGs, DMRs, signaling pathways, and immune cell infiltration across cancer types. As 23 in 33 cancer types showed significant association between HRD and patient OS rates, this study mainly focused on these 23 cancers (ACC, BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PCPG, READ, SARC, THYM, UCEC, OV, BLCA, GBM, LUSC, THCA). Our research aims to provide a thorough understanding of HRD's role and potential implications for patient prognostication.

MATERIALS AND METHODS

Patient cohorts

The same with our former study, we collected patient clinical parameters, HRD levels, tumor subtype, DNA methylation information, gene mutations, immune cells infiltration data etc. based on public databases including UCSC-Xena, TCGA, Firehose case datasets, TIMER2.0 and former publications in 33 cancers [23].

Calculation of HRD scores

For the entire TCGA cohort, allele-specific copy numbers were estimated from SNP array data using the ASCAT algorithm [24]. The ASCAT estimates were downloaded from the GDC Data Portal (<https://gdc.cancer.gov>) [25]. The HRD scores TAI [26], LST [27], LOH [28], and HRDsum [22] (= TAI + LST + LOH) were calculated from allele-specific copy numbers using HRDscar [29]. Comparing the HRD scores of TCGA pan cancer reported in the literature, download the HRD score using the UCSCXenaTools R package (<https://xenabrowser.net/datapages/>), and finally select the data downloaded from the R package (with multiple samples).

Analysis of the relationship between HRD and patient prognosis / clinical characteristics

For correlation analysis between HRD and patient prognosis, samples of the 33 cancer types were categorized into high- and low- HRD groups, and optimal cut-point was used. Correlation between HRD and patient OS rates was analyzed by using Kaplan-Meier method and Cox regression analysis. Among these 33 cancer types, 23 cancers showed significant association between HRD and patient OS rates. These 23 cancers included ACC, BLCA, BRCA, COAD, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, READ, SARC, THCA, THYM, UCEC and they were focused on for subsequent study.

Wilcoxon rank-sum test was used for HRD level analysis in different cancer subtypes. Patient PFS rates analysis was also carried out based on the same methods. Patient clinical characteristics including sex, age, race, tumor grade, clinical stage and smoking status were involved in the correlation study with HRD. The association between HRD and patient sex was analyzed by Wilcoxon rank-sum test, and the association between HRD and the other characteristics was analyzed by a linear regression analysis. It was considered to be significant when P value < 0.05 .

Gene mutation / MMRs expression / differentially expressed gene / DNA methylation analyses

Association analyses of gene mutations, MMRs expression, differentially expressed genes and DNA methylation with HRD were carried out essentially as described in our former study [23]. For HRD grouping, top/bottom 1/3 method was used. It was considered to be significant when P value < 0.05 .

Signaling pathway and immune cell infiltration analysis

Concordant with our former study, GSEA_4.0.2 software was used for gene set enrichment analysis (GSEA); HRD was grouped according to top/bottom 1/3; “h.all.v7.2.symbols.gmt gene sets” from the MSigDB database was obtained for the reference gene set. NOM P value < 0.05 was considered to be significant for signaling pathway enrichment.

For immune cell infiltration analysis, Wilcoxon rank-sum test was used based on TCGA database and ImmuCellAI tool, and top/bottom 1/3 method was used for HRD grouping. For further study, similar analysis for immune cell infiltration was carried out based on CIBERSORT algorithm [30].

Statistical analysis

Statistical analyses in this study were performed by using R 4.0 software (<https://www.r-project.org/>). *P* value < 0.05 was considered to be statistically significant.

RESULTS

HRD levels and patient prognosis / clinical features association across 33 cancers

Using data from the UCSC-Xena database, we assessed HRD levels across 33 cancer types (ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LIHC, LUAD, LGG, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, UCEC, UCS, UVM, THCA, THYM). Ovarian cancer (OV) exhibited the highest HRD levels, followed by lung squamous cell carcinoma (LUSC) and esophageal carcinoma (ESCA); THCA showed the lowest HRD level and followed by renal tumors (including LAML, KIRP, KICH and THYM) (Figure S1).

We used Kaplan-Meier analysis and optimal cut-point method to analyze the correlation between patient OS rates and HRD in the 33 cancers. As shown in Figure S2, high HRD was associated with lower OS rates of patients with ACC (Figure 1A), BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PCPG, READ, SARC, THYM and UCEC, while it was opposite for patients with OV (Figure 1C), BLCA, GBM, LUSC and THCA. However, patients with the other 10 kinds of cancer (LAML, UVM, PRAD, DLBC, TGCT, SKCM, CESC, CHOL, STAD, UCS) showed no significant correlation between HRD and OS

rates. Based on this result, following study was conducted mainly in the 23 cancer types (HRD significantly associated with patient OS rates).

Concordantly, we performed similar analysis to investigate the association between HRD and PFS (progression free survival) in the 23 cancers. As shown in Figure S3, high HRD was associated with lower PFS rates of patients with ACC (Figure 1B), BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LGG, LIHC, MESO, PAAD, PCPG, SARC, THYM, UCEC, and was associated with higher PFS rates of patients with OV (Figure 1D), GBM. The association between HRD and patient PFS rates in the 18 cancers were all concordant with the association between HRD and patient OS rates. Patients with BLCA, LUAD, LUSC, READ or THCA showed no significant correlation between HRD and PFS rates.

For further study, we analyzed the correlation between cancer subtype and HRD. As shown in Figure S4, BRCA basal subtype, GBM IDH-wild type (IDHwt), UCEC CN-LOW subtype were associated with high HRD levels; COAD CIN and GS subtypes, ESCA ESCC subtype, HNSC HPV- and HPV+ subtypes, LGG IDHmut-non-codel subtype, READ CIN subtype, SARC DDLPS and LMS subtypes were associated with low HRD levels.

In addition, we collected the patient clinical feature information of these 23 cancers and analyzed the correlation with HRD. As shown in Figure 1E, HRD was positively correlated with clinical stages in patients with ACC, BLCA, BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LUSC, PAAD, READ, THCA and UCEC; HRD was positively correlated with tumor grades in patients with BLCA, HNSC, KIRC, LGG, LIHC, OV, PAAD, UCEC; while HRD was negatively correlated with human races in patients with BRCA, ESCA, GBM, LIHC, PAAD and UCEC.

Moreover, in several cancer types, HRD levels were associated with patient gender: male patients with LIHC, COAD, HNSC, and ESCA tended to have high levels of HRD, while female patients with KIRP tended to have high HRD levels (Figure 1F).

Therefore, we demonstrated the heterogeneous status of the correlation between patient prognosis / clinical features and HRD in different cancer types.

Driver gene mutations and mismatch repair gene expression associated with HRD

Next, we analyzed the correlation between driver gene mutations / mismatch repair gene expression and HRD in the 23 cancers.

As shown in Figure 2A-D, many driver gene mutations were positively correlated with HRD, including TP53 in LUAD (Difference=14), HNSC (Difference=13), COAD (Difference=9), BLCA (Difference=13), LGG (Difference=4), LIHC (Difference=7), LUSC (Difference=5), PAAD (Difference=10), GBM (Difference=1) and READ (Difference=7.5); MUC4 in BRCA (Difference=8); MUC17 in LUAD (Difference=9) and HNSC (Difference=8); KRAS in PAAD (Difference=14); HRAS in PCPG (Difference=5); FLG in LUAD (Difference=6); ANK3 in LUAD (Difference=8.5); BRCA2 in BLCA (Difference=12.5); ATRX in LGG (Difference=4) and SARC (Difference=10) (all P value < 0.01). However, several driver gene mutations were negatively correlated with HRD, including CTNNB1 (Difference=-18) and CTCF (Difference=-12) in UCEC (Figure 2A, E); CDH1 (Difference=-13), GATA3 (Difference=-6), MAP3K1 (Difference=-14) and PIK3CA (Difference=-12) in BRCA (Figure 2A, F); FGFR3 (Difference=-15) in BLCA; CIC (Difference=-3) in LGG (Figure 2A); ALB (Difference=-6.5) and CTNNB1 (Difference=-6) in LIHC (Figure 2A) (all P value < 0.01). In addition, samples of KICH, ACC,

KIRC, KIRP, MESO, OV, THCA and THYM exhibited low HRD level, and there were no significant association between HRD and driver gene mutation (Figure 2A).

Loss of function caused by MMR has been proved to induce irreparable DNA replication errors.

We used Spearman method to calculate the correlation between MMR genes expression and HRD. As shown in Figure 2G, in most of these 23 cancers, MMR genes expression was negatively correlated with HRD. Especially, the MMR genes negatively correlated with HRD included POLD4 in BLCA ($\rho = -0.11$, P value = 0.04) and in GBM ($\rho = -0.19$, P value = 0.04); MLH3 in BRCA ($\rho = -0.23$, P value = $1.22E-12$), in KIRC ($\rho = -0.14$, P value = 0.01) and in MESO ($\rho = -0.23$, P value = 0.05); MLH1 in BRCA ($\rho = -0.18$, P value = $6.39E-08$), in HNSC ($\rho = -0.12$, P value = 0.01) and in LUAD ($\rho = -0.12$, P value = 0.01). On the other hand, we found MMR genes expression was positively correlated with HRD including RFC5 in ACC ($\rho = 0.35$, P value = 0.01), in KICH ($\rho = 0.56$, P value = 0.02) and in MESO ($\rho = 0.31$, P value = 0.01); POLD3 in ACC ($\rho = 0.56$, P value = $2.10E-05$), in KICH ($\rho = 0.50$, P value = 0.05) and in GBM ($\rho = 0.25$, P value = 0.01); EXO1 in KICH ($\rho = 0.56$, P value = 0.03), in KIRP ($\rho = 0.56$, P value = $1.71E-07$) and in MESO ($\rho = 0.32$, P value = 0.01) (all $P < 0.05$).

In addition, we found that MLH3 is positively correlated with HRD of THCA and UCEC, but negatively correlated with HRD of BRCA, KIRC, and MESO; POLD4 is positively correlated with HRD of ACC, PCPG, and UCEC, but negatively correlated with HRD of BLCA, BRCA, and HNSC (Figure 2G). However, only 21 out of 23 cancers were found to be associated with

HRD in terms of MMR gene expression, with no significant correlation observed between MMR gene expression and HRD in READ and THYM cancers.

Therefore, these results indicated that the correlation between driving gene mutations / MMR genes expression and HRD varied among different types of cancer.

Differentially expressed genes (DEGs) and differentially methylated regions (DMRs) related to HRD

We identified DEGs and DMRs in high- and low-HRD groups (top and bottom 1/3 by HRD) of 23 cancers. Among the 23 cancers (Figure 3A, Figure S5), BRCA showed the most DEGs and DMRs (Figure 3A and 3B), and the proportion of DEGs related to abnormal methylation was also the highest in BRCA (Table S1). The clustering profile software package of R was used to conduct KEGG analysis of genes related to DNA methylation in BRCA, and the P value < 0.05 was considered to be significant. As shown in Figure 3C and Table S2, there were 10 of 45 genes enriched in BRCA including Cytokine-cytokine receptor interaction, Fluid shear stress and atherosclerosis, Alcoholic liver disease pathways etc. For DMRs analysis, most DMRs were located on chromosome 1, 6 and X sex chromosome in LUAD and UCEC (Figure 3D, Table S3). However, most DMRs in other cancers were mainly located on chromosomes 1, 2, 6, 8 and 19 (Figure 3D, Table S3). Especially, in MESO, approximately 15.4% of DMRs were located on chromosome 1; in KIRC, approximately 14.7% of DMRs were located on chromosome 6; in PAAD, approximately 10.6% of DMRs were located on chromosome 19 (Figure 3D, Table S3). In several cancers including GBM, KICH, OV, READ and THCA, no DMRs were found. Overall, gene expression differences were widely observed between high HRD and low HRD

groups in different cancers, but the frequency of differential methylation in the promoter regions was lower.

Signaling pathway and immune cell infiltration associated with HRD

To better understand the impact of HRD on cell signaling pathways and tumor microenvironment, we used the top/bottom 1/3 method for analyses.

In signaling pathway analysis, as shown in Figure 4A, we found G2M checkpoint, E2F target, and DNA repair pathways were enriched in high HRD groups of ACC, BLCA, BRCA, GBM, LGG, LIHC, LUAD, LUSC, MESO and SARC. On the other hand, Coagulation and P53 pathways were enriched in low HRD groups of BRCA, LUAD and LUSC. However, in many cancer types, signaling pathway enrichments in samples with different HRD levels were more complex: Glycolysis pathway was enriched in high HRD groups of BRCA, HNSC, KIRC, LIHC, LUAD, MESO, PAAD, SARC and THYM, but was enriched in low HRD group of COAD; Estrogen response late pathway was enriched in high HRD groups of THYM, PAAD, OV, MESO and LIHC, but was enriched in low HRD groups of BRCA, COAD, LUSC and UCEC; Allograft rejection pathway was enriched in high HRD group of KIRC, but was enriched in low HRD groups of GBM, HNSC and LUSC. Therefore, HRD associated cell signaling pathways varied in different cancers, and G2M checkpoint, E2F target, and DNA repair pathways might be important in many cancers associated with HRD.

Based on ImmuCellAI tool and CIBERSORT algorithm, we investigated the relationship between immune cell infiltration and HRD. The heat maps showed immune cells infiltration including macrophages, T cell follicular helper, T cell CD4 + memory activated, T helper cell 1

(Th1), regulatory T cells (nTreg, iTreg) and monocytes were positively associated with HRD; immune cells infiltration including CD4 + T cells, CD4 + naive T cells, naive B cells, and mast cell activated were negatively associated with HRD. In addition, we found macrophage M0 infiltration was positively associated with HRD in BLCA, BRCA, HNSC, KIRC, LIHC, LUAD, PAAD and THYM, but negatively associated with HRD in KIRP; macrophage M1 infiltration was positively associated with HRD in BLCA, BRCA, KIRP, LUAD, LUSC, OV and THYM, but negatively associated with HRD in HNSC (Figure 4B and 4C, Table S4). Therefore, the correlation between HRD and immune cells infiltration varied in different cancer types.

DISCUSSION

Historically, studies on HRD have predominantly focused on specific cancer types such as breast, ovarian, and prostate cancers [20-22, 31]. Comprehensive pan-cancer analyses of HRD across diverse malignancies remain limited. Based on data obtained from database, we conducted a multivariable omics analysis encompassing 33 cancer types, with a particular focus on 23 cancers where HRD significantly correlated with overall survival (OS) rates, including its mechanisms related to patient prognosis, gene expression, gene mutation, gene methylation and signaling pathway. We uncovered the roles of HRD in different cancer types and shed light on the possible reasons for its correlation with patient prognosis.

Through Kaplan-Meier analysis, we found in most cancers (including ACC, BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LGG, LIHC, MESO, PAAD, PCPG, SARC, THYM and UCEC), high levels of HRD were correlated with both lower OS and PSF rates. As reported previously, high-HRD patients with low grade glioma (LGG) had significantly worse overall

survival compared with low-HRD patients [32]; for breast cancer (BRCA), HRD high tumors were more clinically aggressive and associated with higher hazards for recurrence especially in ER+ tumors [33]. Moreover, in ACC, STAD, UCEC, KIRC, SARC, PRAD, PAAD and BRCA, it was reported that patients with high HRD scores exhibited worse prognosis compared with those with low HRD scores [34-38]. These results were concordant with our present data. On the other hand, we found in OV and GBM patients, high levels of HRD were correlated with both higher OS and PSF rates, which was concordant with Knijnenburg TA et al's and Shi Z et al's studies [39, 40]. It was reported that HRD high tumors were more responsive to platinum-based chemotherapies, especially in high-grade serous ovarian cancer [41-43]. Furthermore, we revealed that in BLCA, HNSC, KIRC, PAAD and UCEC, high levels of HRD were associated with both higher clinical stages and higher tumor grades. As reported in former studies, in HNSC patients, clinical stage, clinical T stage, pathological T stage and lymphovascular invasion were associated with HRD high score (HRD-H), and HRD-H was associated with poor outcomes [44]; in kidney renal clear cell carcinoma and endometrial cancer, patients with high HRD exhibited worse prognosis [45, 46]. Therefore, HRD was an important factor for patient prognosis prediction, and their correlations varied among cancer types.

In driver gene mutations / mismatch repair gene expression analysis, we found many driver gene mutations were positively correlated with HRD, including TP53 in LUAD, HNSC, COAD, BLCA, LGG, LIHC, LUSC, PAAD, GBM, READ and SARC. As reported, mutations in the tumor suppressor TP53 gene are one of the most common genetic alterations present at high frequency in human cancers [47, 48], and TP53 alterations were associated with higher HRD

scores [39, 49]. In hepatocellular carcinoma (LIHC), up to 30% patients carried TP53 mutation, and it acts as a key significant risk factor in LIHC patients [50, 51]. In non-small cell lung cancer (NSCLC) patients, TP53 alterations were correlated with poor overall survival rates and were relatively more resistant to chemotherapy and radiation [47, 52]. Moreover, TP53 mutations were related with poor prognosis in patients with HNSC, COAD, BLCA, LGG, GBM and SARC [53-57]. Based on these results, the poor prognosis of these cancer patients with high HRD might be caused by the high frequency of TP53 mutations. We demonstrated that KRAS mutation was positively correlated with HRD in PAAD. As reported, KRAS has been proved to be mutated in approximately 75% of pancreatic ductal adenocarcinoma, and genomic alterations in KRAS were associated with worse outcomes in pancreatic ductal adenocarcinoma patients [58, 59]. Our data showed BRCA2 mutation was positively correlated with HRD in BLCA. As known, BRCA2 mutations deteriorate the genomic stability and predispose to malignant transformation [60]. However, previous studies found BRCA2 gene could inhibit the occurrence and development of cancer, but patients with BRCA2 gene mutation were more sensitive to chemotherapy and radiotherapy [60-62]. Therefore, BRCA2 mutation was associated with better prognosis in breast cancer [63], ovarian cancer [64] and bladder cancer patients [60]. In our herein OS rate analysis, we found high HRD was associated with higher OS rates of OV and BLCA patients. These results were concordant.

In the present study, we found immune cells infiltration including macrophages, T cell follicular helper, T cell CD4 + memory activated, T helper cell 1 (Th1), regulatory T cells (nTreg, iTreg) and monocytes were positively associated with HRD; whereas CD4 + T cells, CD4 + naive T

cells, naive B cells, and mast cell activated were negatively associated with HRD. As reported previously, tumors with high HRD scores bore increased leukocyte infiltration and lymphocyte fraction with immune-sensitive microenvironment [65]. In breast cancer patients, high infiltration of immune cells including TAMs (tumor-associated macrophages), neutrophils, Tregs, myeloid derived stem cells (MDSCs) correlated with a worse cumulative survival rate. Macrophages M2, neutrophils, and Tregs infiltration was negatively correlated with prognosis in colorectal cancer patients [66]. Researches have shown that Tregs contributed to antitumor immunity suppressing and deterring immune surveillance, and thereby associated with poor prognosis in cancer (including breast cancer, colorectal cancer) patients [67-69]. Moreover, increased numbers of Tregs and TAMs infiltration were also associated with poor prognosis in patients with NSCLC, hepatocellular carcinoma, clear cell renal cell carcinoma, etc [70-72]. Therefore, the TAMs and Tregs infiltration might mediate the association between HRD and poor prognosis in patients with BRCA, COAD, KIRC, LUAD, LIHC, etc. However, as demonstrated in former studies, T follicular helper (Tfh) cells infiltration was associated with favorable outcomes in patients with lung adenocarcinoma, breast cancer and colorectal cancer [73-75], which was not concordant with our HRD related data, thereby Tfh cells might play minor role compared with other immune cells infiltration related with HRD.

CONCLUSIONS

In conclusion, our pan-cancer analysis highlights the heterogeneous impact of HRD on patient prognosis, genetic alterations, and immune microenvironment across 33 cancer types. These findings underscore HRD as a critical biomarker for predicting clinical outcomes and guiding

personalized treatment strategies in oncology. Future studies integrating multi-omics approaches and prospective clinical trials are warranted to validate our observations and translate them into improved patient care.

Data availability

All relevant data are available within the article and its supplementary information.

EARLY ACCESS

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FIGURES WITH LEGENDS

Figure 1

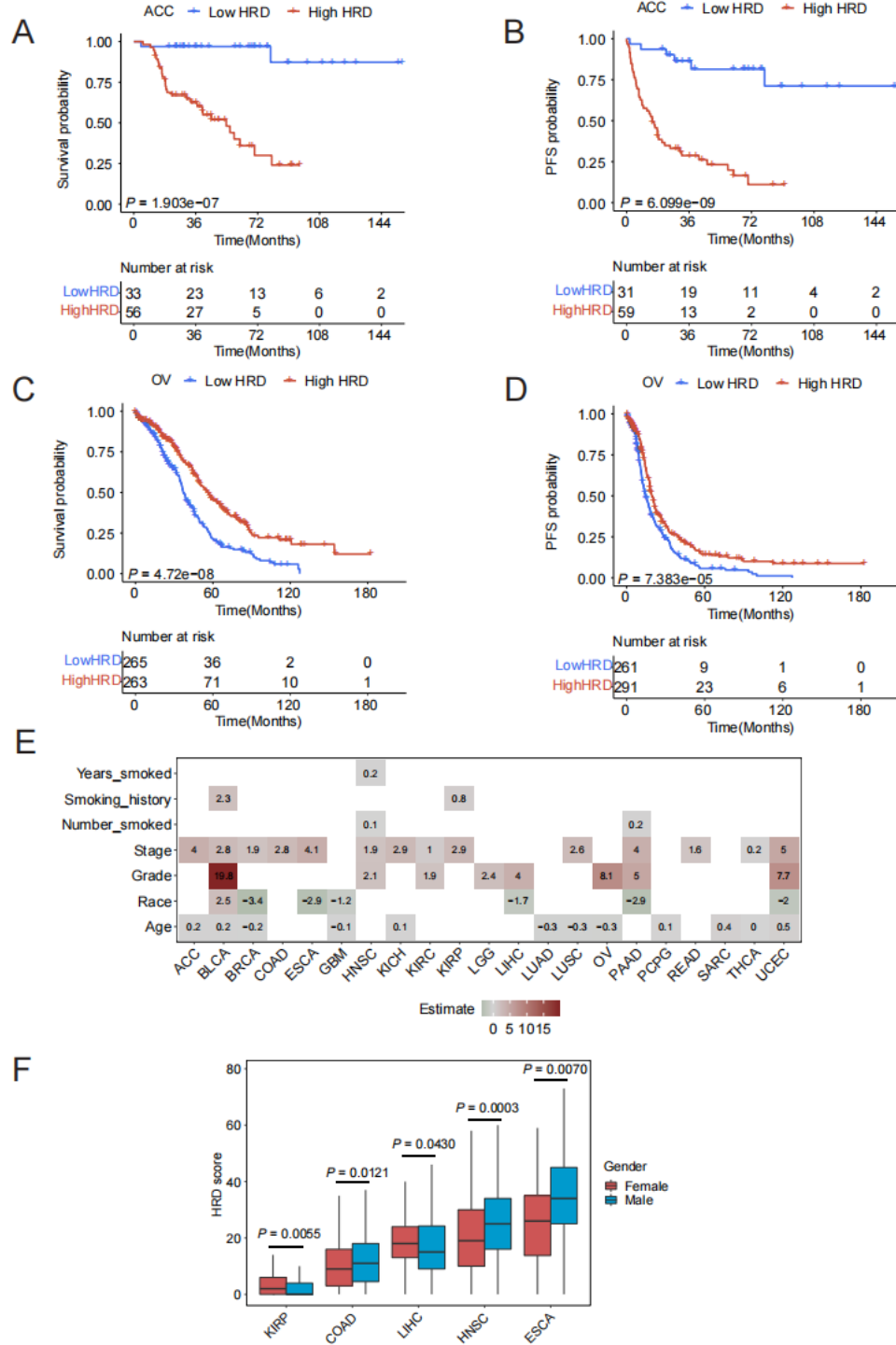


FIGURE 1. Association of survival rates and clinical features with HRD in respective cancer types.

(A) Association of OS rate with HRD in patients with ACC; (B) Association of PFS rate with HRD in patients with ACC; (C) Association of OS rate with HRD in patients with OV; (D) Association of PFS rate with HRD in patients with OV; (E) Correlation of patient clinical features including age, race,

tumor grade, clinical stage and smoking status with HRD; (F) HRD levels in male and female patients with different cancers.

EARLY ACCESS

Figure 2

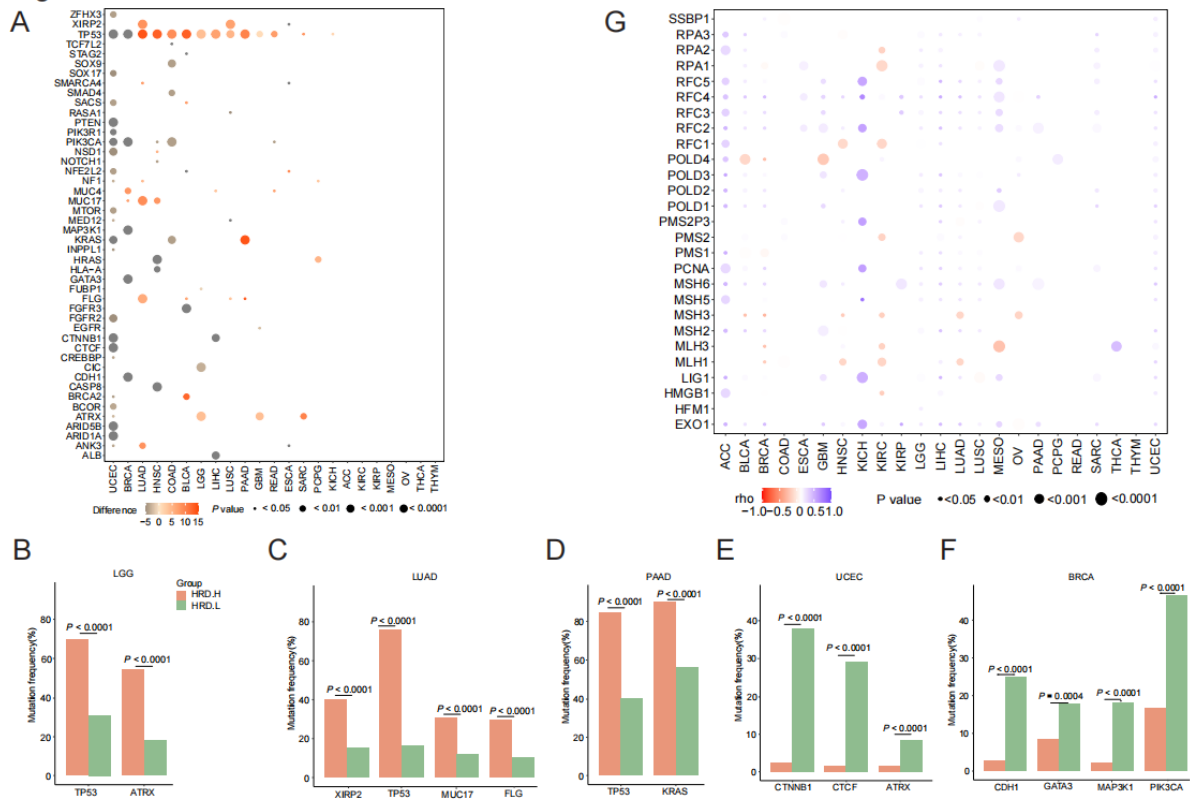


FIGURE 2. Association of driver gene mutations and mismatch repair gene expression with HRD.

(A) Association of driver gene mutations with HRD in 23 cancer types analyzed by Wilcoxon rank-sum test; (B-F) Driver gene mutations in high/low HRD groups of LGG (B), LUAD (C), PAAD (D), UCEC (E), and BRCA (F); (G) Correlation between the expression of mismatch repair genes and HRD by Spearman analysis.

Figure 3

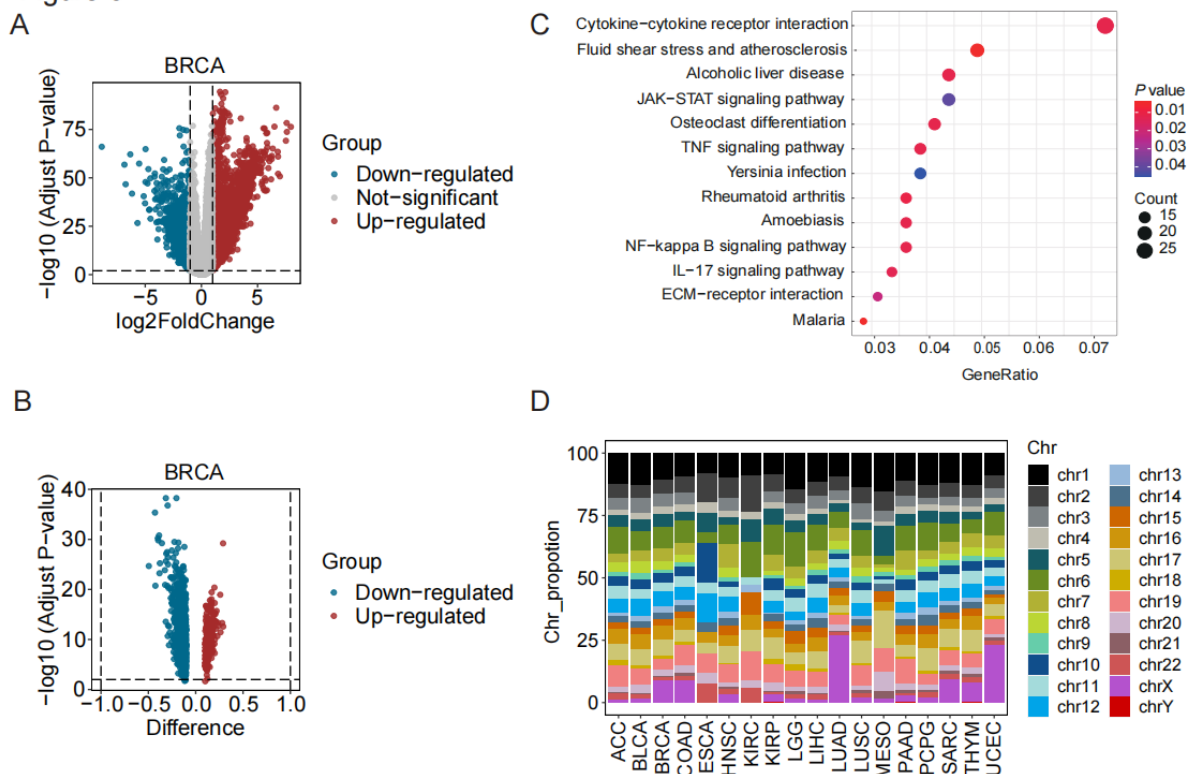
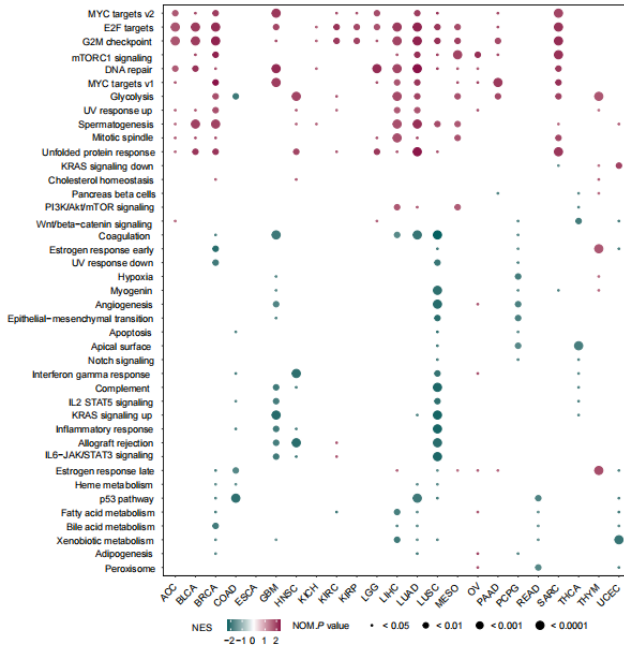


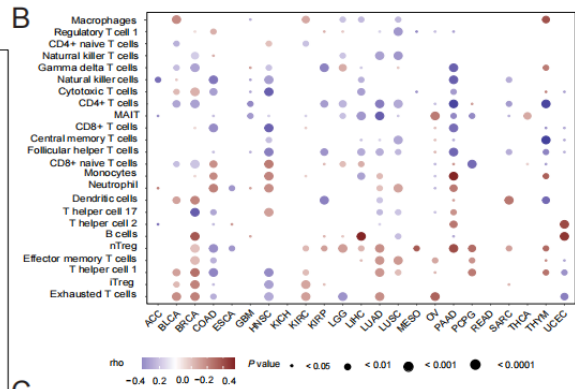
FIGURE 3. Association of DEGs and DMRs with HRD. (A) The DEGs in high-HRD group compared with low-HRD group in BRCA ($FDR < 0.01$ and $|\log_2(FC)| > 1$; up-regulated, red; not-significant, gray; down-regulated, blue); (B) The DMRs in the promoter regions in high-HRD group compared with low-HRD group in BRCA ($FDR < 0.05$ and $|\text{difference}| > 0.1$; up-regulated, red; down-regulated, blue); (C) KEGG pathway enrichment associated with DNA methylation in BRCA was showed by bubble plot (all $P < 0.05$); (D) Distribution map of DMRs in respective chromosomes.

Figure 4

A



B



C

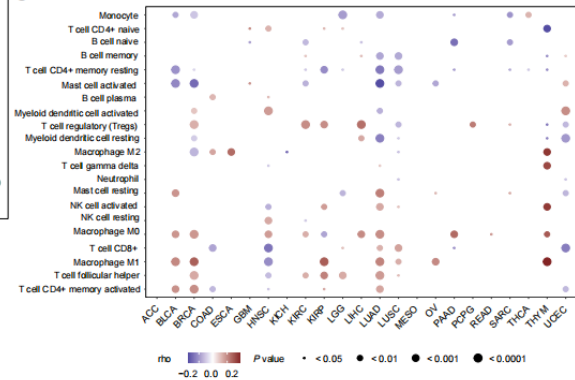


FIGURE 4. Association of signaling pathway and immune cell infiltration with HRD. (A) Signaling pathways related to HRD in 23 cancer types were analyzed by Wilcoxon rank-sum test; (B and C) Immune cells infiltration related to HRD in 23 cancer types were analyzed by Wilcoxon rank-sum test based on the ImmuCellAI (B) and CIBERSORT (C) algorithm respectively.

SUPPLEMENTAL DATA

Supplemental tables are available at the following link:

<https://www.bjbms.org/ojs/index.php/bjbms/article/view/10448/3393>

Supplemental figures are available at the following link:

<https://www.bjbms.org/ojs/index.php/bjbms/article/view/10448/3391>

The legends for the supplemental figures are provided in the following text:

FIGURE S1. HRD levels calculated among 33 cancers.

FIGURE S2. Association of OS rates with HRD among cancers.

FIGURE S3. Association of PFS rates with HRD among cancers.

FIGURE S4. Association of OS rates with HRD in 13 molecular subtypes of 9 cancers.

FIGURE S5. Volcano map showing the DEGs in high-HRD group compared with low-HRD group among the 22 cancers (FDR < 0.01 and $|\log_2(\text{FC})| > 1$; up-regulated, red; not-significant, gray; down-regulated, blue).