Identification of biomarkers complementary to homologous recombination deficiency for improving the clinical outcome of ovarian serous cystadenocarcinoma

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Research

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Abstract

Background: Ovarian cancer patients with tumors positive for homologous recombination deficiency (HRD) would benefit from PAPR inhibitor (PAPRi) therapy. However, patients with HRD-positive tumors account for less than 50% of the whole cohort. So new biomarkers still need to be developed. The aim of this study was to identify biomarkers complementary to HRD for improving the clinical outcome of ovarian serous cystadenocarcinoma (OSC).

Methods: The HRD score was established from SNP array data of OSC cohort from TCGA. The genomic landscape and transcriptome data of patients with different HRD scores were analyzed in order to identify biomarkers complementary to HRD. The candidate biomarker was validated in vivo and in vitro experiments.

Results: Based on the data from the SNP array and somatic mutation profiles in the OSC patients’ genome, we found that high frequency of actionable mutations existed in NF1 and CDK12 in patients with HRD-negative tumors. We then leveraged the gene expression profiles to screen out CXCL11 expression that was associated with the HRD score and could be used as a predictor of survival outcome. Further comprehensive analysis of the tumor immune microenvironment (TIME) revealed that CXCL11 expression was closely correlated with cytotoxic cells, neoantigen load and immune checkpoint blockade (ICB). Finally, in vivo and in vitro experiments showed that PAPRi increased the expression of CXCL11.

Conclusions: Collectively, our study not only provides biomarkers of ovarian cancer complementary to the HRD score but also introduced a potential new perspective for identifying cancer biomarkers associated with genomic instability.

Background

The incidence rate of ovarian cancer ranks third among female genital tract malignancies, but its mortality rate ranks first[1]. About 70% of ovarian cancer patients have advanced cancer at the time of initial diagnosis since there are no obvious symptoms in the initial stage of ovarian cancer[2]. Ovarian serous cystadenocarcinoma (OSC), a common type of ovarian cancer, accounts for about 90% of all ovarian cancers[3] and it is prone to peritoneal metastasis early and chemotherapy resistance. According to statistics, the five-year survival rate of ovarian cancer patients is only 30%−45%[4]. The major reason for poor prognosis of ovarian cancer is lack of effective means of early diagnosis and prognostic indicators. Discovering specific biomarkers for early screening of ovarian cancer and new therapeutic targets for ovarian cancer are the current focus of ovarian cancer research.

Emerging clinical trials have revealed the clinical value of homologous recombination deficiency (HRD) in ovarian cancer. Homologous recombination repair (HR) plays an important role in DNA repair mechanisms. BRCA (BRCA1/2), RAD (RAD50/51/51B), ATM, etc. are key components of HR mediated DNA repair[5]. Tumors with HRD-positive were recorded for the first time in patients that harbored germline mutations of BRCA gene. In the phase 3 PAOLA-1 (PAOLA-1/ENGOT-ov25) trial, the addition of
maintenance olaparib provided a significant progression-free survival benefit, which was substantial in patients with HRD-positive tumors, including those without a BRCA mutation[6]. The molecular mechanism of HRD is not fully understood. Current studies have found that mutations in genes, including BRCA gene mutations, involved in the HR signaling pathway can only explain about 14.1% of HRD ovarian cancer patients[7]. Therefore, research on transcriptome characteristics of HRD patients may fill this gap. Although several studies have investigated the relationship between the transcriptome and tumor genome instability[8, 9], HRD-associated RNAs and their clinical significance in ovarian cancer still remain largely unexplored. Moreover, HRD is present in less than 50% of serous ovarian tumors[10], so new biomarkers need to be developed for molecular typing of ovarian cancer patients with HRD-negative tumors.

For the first time in the present study, by taking advantage of both the Cancer Genome Atlas (TCGA)/Gene Expression Omnibus (GEO) database and the algorithm for quantifying HRD score, we found that high frequency of actionable mutations existed in patients with HRD-negative tumors. Through transcriptome analysis, we identified and validated C-X-C motif chemokine 11 (CXCL11) that predicted the survival and prognosis of OSC patients. Furthermore, we discovered a relationship between CXCL11 expression and tumor immune microenvironment (TIME), including cytotoxic cells, neoantigen load and immune checkpoint blockade (ICB). Finally, in vivo and in vitro experiments confirmed that olaparib could upregulate the expression of CXCL11 in ovarian cancer cell lines and induce changes in the TIME. Our research perspectives and methods provide a possible direction for molecular typing of ovarian cancer. The results of this study may be valuable for understanding the relationship between the HRD and TIME and predicting the prognosis of OSC patients.

Materials And Methods

Data collection and processing

OSC patients’ RNA sequencing data, somatic mutation data, SNP array data and corresponding clinical follow-up information were downloaded from the publicly available the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov) and the NCBI GEO database[11]. RNA sequencing data were normalized as transcripts per million (TPM) by using the R. SNP array data were processed using Affymetrix Power Tools and PennCNV. The somatic mutation counts, copy number variation (CNV), fraction genome altered scores and MSIsensor score were obtained from the cBioPortal database(https://www.cbioportal.org/). In total, 348 TCGA samples data were extracted; 296 GEO samples data were extracted (GSE140082 and GSE30161).

HRD score analysis

Loss of heterozygosity (LOH) was defined as the number of counts of chromosomal LOH regions shorter than whole chromosome and longer than 15 Mb[12]. Large-scale State Transitions (LST) were defined as chromosome breakpoint (change in copy number or allelic content ) between adjacent regions each of at least 10 megabases obtained after smoothing and filtering shorter than 3 Mb small-scale copy number
Telomeric Allelic Imbalance (TAI) was defined as the number of regions with allelic imbalance which extend to the sub-telomere but do not cross the centromere[14]. The HRD score was defined as the sum of TAI, LST, and LOH scores[15]. The HRD score of each patient was shown in Supplementary Table 1.

**Genomic landscape and neoantigen load**

The datasets of the somatic mutations for OSC in TCGA were obtained from the MC3 TCGA dataset and analyzed using the TCGA mutations package of R[16]. Somatic mutation alterations were analyzed by using the maftools package of R[17]. The antigen peptides resulting from non-synonymous mutated HLA sequences with predicted binding affinities below 500 nM are defined as neoantigens[18].

**Gene set enrichment analysis (GSEA)**

RNA-seq data (raw counts) analysis was conducted using the “edgeR” package of R[19]. Fold change > 1.5, adj. p < 0.05, TPM > 1 and genes with the first 75% of median absolute deviation were set as the cutoffs to screen for differentially expressed genes (DEGs). Heatmaps and clustering were generated using an open source web tool ClustVis[20]. GSEA was performed using GSEA software from the Broad Institute (MIT, Cambridge, MA) to identify differential signaling pathways in different groups[21]. The normalized enrichment score was calculated for each gene set. GSEA results with a nominal P < 0.05 were considered significant.

**Identification of prognostic related genes associated with the HRD score**

Univariable Cox regression analysis was performed to select the prognostic related genes using the computing environment R with the survival package[22]. The prognosis-related genes with a p value < 0.05 in the univariate Cox regression analysis were considered as candidate variables. The results were further analyzed through the LASSO regression approach to seek a balance between the maximization of prediction accuracy and the minimization of interpretation[23]. The screening process was shown in Fig. 1.

**Immune cells infiltration in bulk tumor gene expression data**

In order to study the enrichment of immune cells in CXCL11-negative and CXCL11-positive groups, we used TIMER[24], an efficient algorithm for predicting immune cell infiltration of bulk tumor gene expression data (https://cistrome.shinyapps.io/timer/ ). For each sample, TIMER quantified the relative abundance of six types of infiltrating immune cells, including T cells, B cells, macrophages, neutrophiles and dendritic cells.

**Cells and culture**

A2780 and A2780cisR (cisplatin resistant) human ovarian cell lines were gifts from Fudan University Shanghai Cancer Center. IOSE-80 and HEY-T30 ovarian cell lines originated from a gift from Dr Luopei Guo (Obstetrics and Gynecology Hospital of Fudan University). ES-2 and SKOV3 ovarian cell lines were purchased from GeneChem (Shanghai, China). All cell lines were cultured according to ATCC guidelines at 37 °C in a 5% CO2 incubator. The Olaparib (Selleck, catalog number S1060) was dissolved in DMSO, and
the final concentration of DMSO in the medium was 0.1%. After the cells were plated for 24 hours, cells were overlaid with Olaparib (0uM, 2uM, 10uM, 25uM, 50uM) conditioned medium and harvested for 24 h.

**Real time-quantitative PCR (RT-qPCR) analysis**

For cDNA synthesis, 1ug total RNA was processed using the HiScript RT SuperMix for qPCR (+ gDNA wiper) kit (Vazyme). The ChamQ Universal SYBR qPCR Master Mix (Vazyme) was used for the thermocycling reaction. The RT-qPCR analysis was carried out in triplicate times. Primer sequences were as follows:

*Beta-ACTIN*: Forward: 5’-GTGGCCGAGGACTTTGATTG-3’
Reverse: 5’-CCTGTAACAACGCATCTCATATT-3’

*CXCL11*: Forward: 5’-GACGCTGTCTTTGCATAGGC-3’
Reverse: 5’-GGATTTAGGCATCGTCTTTT-3’

**Results**

The HRD Score was significantly correlated with the prognosis and molecular characteristics of TCGA-OSC cohort.

According to the HRD-algorithm, LOH, TAI and LST were used as the basis for calculating the HRD score. Optimal cutoff scores were determined by assessing the score which had the minimum p value of the log-rank test (Fig. 2A). HRD status was defined as HRD-positive if HRD score was ≥57; HRD status was defined as HRD-negative if HRD score was ≤57. The Kaplan-Meier survival curve (Fig. 2A) showed that overall survival of patients in the HRD-positive group is much longer than the cases in the HRD-negative group (hazard ratio (HR) = 0.49 (0.37,0.65), log-rank test, \( P < 0.00001 \)). Subsequently, we investigated the correlation between the HRD score and other hallmarks of genomic instability, including somatic mutation counts, fraction genome altered and MSI. The somatic mutation pattern was significantly different between the HRD-positive group and the HRD-negative group. The median value of somatic cumulative mutations in the HRD-positive group was significantly higher than that in the HRD-negative group (Wilcoxon signed-rank test, \( P < 0.0001 \); Fig. 2B). We next compared the fraction genome altered scores between the HRD-positive group and the HRD-negative group. As shown in Fig. 2C, the fraction genome altered scores in the HRD-positive group were higher than those in the HRD-negative group (Wilcoxon signed-rank test, \( P < 0.05 \); Fig. 2C). The plot of “somatic mutation counts vs fraction genome altered” clearly showed that the distribution of points in the HRD-positive group was concentrated in the upper right of the coordinate system, while that of the HRD-negative group was scattered (Fig. 2D). The results of transcriptome level analysis were consistent with those at the genome level: through GSEA analysis, it was found that the three signal pathways with the most significant differences between the two groups were DNA replication, homologous recombination and mismatch repair (Supplementary
Fig. 1A). There was no difference in the microsatellite instability (MSI) status of the two groups (Supplementary Fig. 1B).

**The genomic landscape of the HRD-negative group showed a high proportion of actionable mutations in NF1 and CDK12**

Genomic characteristics, such as the oncogene activation (e.g., ERBB2 amplification, EGFR tyrosine kinase mutation) and inactivation of tumor suppressor genes (e.g., MMR, BRCA1/2) have shown a strong correlation with clinical response to target therapy. Therefore, we compared the genomic mutational landscape between the HRD-positive group and the HRD-negative group. The results showed that the genomic landscape of the HRD-negative group was significantly different from that of the HRD-positive group. Only nine of the top twenty genes with the highest mutation rate in the two groups overlapped (Supplementary Fig. 1C). The mutational landscapes of these two subgroups displayed a distinct mutation ratio in TP53 [94.0% (HRD-negative) vs 62% (HRD-positive)], and the mutation classification of the HRD-negative group was more abundant, including a higher proportion of frame shift del, nonsense mutation and so on (Fig. 3A, B). Through the screening of actionable genes in the OncoKB database (https://www.oncokb.org/actionableGenes), among the 20 genes with the highest mutation frequency in the HRD-negative group, two genes were biomarkers for targeted drugs (NF1 and CDK12). Moreover, most of the variant classifications of these two genes were those affecting gene structure. Patients with mutations in these two genes accounted for 13% of the HRD-negative group. The mutational landscapes of HR genes in the two subgroups also exhibited a distinct difference. HR gene mutations in the HRD-positive group were mainly concentrated in BRCA1/2 (7%), while the HRD-negative group was scattered (Supplementary Fig. 2A, B). Besides, we also compared the copy number variation of HR genes in the two subgroups, and observed BRCA2 homozygous deletion was only present in the HRD-positive group (Supplementary Fig. 2C).

**CXCL11 expression associated with the HRD score and its prognostic value in OSC**

To identify RNAs associated with the HRD score, the TCGA-OSC cohort was sorted in ascending order of HRD scores, and the last 20% (n = 70) and the top 20%(n = 70) of the patients were selected to identify DEGs. Utilizing the edgeR method, a total of 124 DEGs were screened out. Among them, 38 RNAs were found to be upregulated and 86 to be downregulated in the HRD-positive group (Supplementary Table 2). Then, 124 differentially expressed RNAs were used to perform unsupervised cluster analysis on 348 TCGA-OSC samples. As shown in Fig. 4A, we found that not all DEGs clustered the HRD-positive and the HRD-negative groups well in the entire TCGA-OSC cohort. Only the DEGs in the red block region were able to cluster the HRD-positive group and the HRD-negative group well. To further screen out DEGs related to the HRD score and prognosis of the patients, the univariate analysis was conducted in the 124 DEGs for the whole TCGA-OSC cohort. A total of 17 genes with prognostic potentiality were identified by the univariate analysis and log-rank test ($P < 0.05$) (Supplementary Table 3). The 17 HRD-related genes were then subjected to Lasso-Cox proportional hazards regression and tenfold cross-validation to identify the best gene model. The Lasso coefficient profile plot was produced against the log(lambda) sequence, and
the optimal lambda method resulted in only one optimal coefficient (Fig. 4B, C). The only one HRD-related gene was \textit{CXCL11}. A heatmap of \textit{CXCL11} expression and the HRD score and the scatterplot of overall survival (OS) with corresponding risk scores were illustrated in Fig. 4D. Kaplan–Meier analysis displayed that the survival outcomes of TCGA-OSC patients with high \textit{CXCL11} expression (\textit{CXCL11}-positive) were significantly better than patients with low \textit{CXCL11} expression (\textit{CXCL11}-negative) (hazard ratio (HR) = 0.39 (0.29,0.51), log-rank test $P<0.00001$) (Fig. 4E). To verify whether the \textit{CXCL11} expression signature has similar prognostic value in different OSC cohorts, we further confirmed this phenomenon in two independent OSC cohorts in the GEO database (including GSE140082 and GSE30161): results from Kaplan–Meier analysis also showed that patients in the \textit{CXCL11}-positive group demonstrated a better prognosis than those in the \textit{CXCL11}-negative group (Supplementary Fig. 3A, B).

\textbf{Comparison of immune cells infiltration within the \textit{CXCL11}-positive group and the \textit{CXCL11}-negative group}

Gene expression of \textit{CXCL11} is essential for activating immune cells\cite{25}, suggesting that tumor infiltrating immune cells might be different in the \textit{CXCL11}-positive group and the \textit{CXCL11}-negative group. To validate this assumption, the TIMER algorithm\cite{26} was applied to estimate enrichment of various immune cell types within different subgroups. We developed a heatmap with TIMER results to visualize the relative abundance of 6 immune infiltrating cell subpopulations from the TCGA-OSC cohort (Fig. 5A). As depicted in the heatmap, there were significant differences in immune cell infiltration between the two subgroups. Anti-tumor lymphocyte cell subpopulations, such as CD4$^+$/CD8$^+$ T cells and dendritic cells were enriched in the \textit{CXCL11}-positive group (Wilcoxon signed-rank test, $P<0.01$). The neutrophils were also enriched in the \textit{CXCL11}-positive group (Wilcoxon signed-rank test, $P<0.001$) (Fig. 5B). We then investigate the correlation of immune cell infiltration with the expression of \textit{CXCL11} by spearman correlation coefficients. The results revealed that the expression of \textit{CXCL11} was significantly associated with immune cell infiltration in the TCGA-OSC cohort (Fig. 5C). We also further analyzed the correlation between the immune cell infiltration signal and the expression of \textit{CXCL11} in the TCGA pan-cancer cohorts, and found similar results (Supplementary Fig. 3C).

Furthermore, GSEA on the gene expression profile of the \textit{CXCL11}-positive group against the \textit{CXCL11}-negative group revealed the \textit{CXCL11} expression signature related biological signaling pathway. Genes involved in antigen processing and presentation, autoimmune thyroid and cytokine receptor interaction signaling pathways were the most significantly enriched in the \textit{CXCL11}-positive group (Fig. 5D). However, taste transduction, basal cell carcinoma and hedgehog signaling pathways were enriched in the \textit{CXCL11}-negative group (Fig. 5E).

\textbf{\textit{CXCL11} expression associated with molecules in antigen processing and presentation pathway}

The results from the TIMER and GSEA analysis showed that there were significant differences between the \textit{CXCL11}-positive group and the \textit{CXCL11}-negative group in antigen processing and presentation pathway, hinting that the expression of antigen-related genes might be associated with \textit{CXCL11}
expression. To prove this assumption, we explored the correlation of antigen-related genes with the \( CXCL11 \) expression by using the Pearson correlation coefficient. We found that the expression of MHC class I/II (I: HLA-A, HLA-B, and HLA-C; II: HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR) and antigen binding (B2M, TAP1/2 and so on) molecules were highly correlated with the \( CXCL11 \) expression signature (Fig. 6A). There were significant differences in the expression of HLA-A, HLA-B and other key antigen presenting molecules between the two subgroups (Wilcoxon signed-rank test, \( P < 0.001 \), Fig. 6B). The results were confirmed in the GEO validation cohort (Supplementary Fig. 4). Since antigen processing and presentation pathway plays a crucial role in immune recognition of predict (neo-)antigen produced by cancer cells, we further investigated the relationship between neoantigen load and the \( CXCL11 \) expression signature by Pearson correlation coefficient. Correspondingly, predicted neoantigen load was highly correlated with \( CXCL11 \) expression (Fig. 6C). These results revealed the correlation between \( CXCL11 \) expression and molecules in antigen processing and presentation pathway, suggesting that neoantigen vaccine might be potential therapy for \( CXCL11 \)-positive ovarian cancer treatment.

**CXCL11 expression associated with ICB-related genes**

In recent years, ICB therapy, represented by anti-PD-1/L1, has played an increasingly important role in anti-tumor treatment. The characteristics of TIME and immune checkpoint genes in tumor cells have a profound impact on ICB therapy. Therefore, we collected more than 40 common ICB-related gene signatures and analyzed the relationship between \( CXCL11 \) expression and ICB-related genes. As displayed by heatmap, \( CXCL11 \) expression was significantly correlated with the expression of multiple ICB-related genes (Fig. 7A). Ten of the most relevant ICB-related genes were: LAG3, ICOS, CTLA4, CD48, HAVCR2, PDCD1(PD-1), PDCDILG2(PD-L2), TIGIT, CD274(PD-L1) and CD86, and their expression levels were enriched in the \( CXCL11 \)-positive group (Wilcoxon signed-rank test, \( P < 0.001 \), Fig. 7B). Generally, the key regulatory factors involved in immunity perform similar functions in different tissues. We thus explored \( CXCL11 \) expression and ICB-related gene signatures across cancer types. We found that the co-expression of \( CXCL11 \) and ICB-related genes was not only present in ovarian cancer, but also in 32 other cancer types (Fig. 7C). Taken together, these results show that \( CXCL11 \) expression may be a potential predictor of ICB therapy.

**Olaparib-treated ovarian cancer cells up-regulate CXCL11 expression in vivo and in vitro**

It has been reported that PARPi triggers robust local and systemic antitumor immunity involving both adaptive and innate immune responses through a STING-dependent antitumor immune response in mice bearing ovarian tumors\[27, 28\]. To explore the involvement of \( CXCL11 \) expression responses to PARPi, we re-analyzed the RNA seq data of high-grade serous ovarian cancer tumor tissues harvested from tumor-bearing mice after 18 days of treatment with olaparib or vehicle (GSE120500). Strikingly, boxplots showed markedly upregulated expression of \( CXCL11 \) in tumors treated with olaparib compared with vehicle control (Fig. 8A). We next compared the expression levels of ICB-related genes with the highest correlation with \( CXCL11 \) between the olaparib treatment group and the control group. As shown in Fig. 8B, the expression of these genes in the olaparib treatment group was significantly higher than that
in the vehicle control group (Wilcoxon signed-rank test, \(P< 0.01\)). To further validate that olaparib-treated ovarian cancer cells activate the \textit{CXCL11} expression signature, we conducted \textit{in vitro} experiments. As measured by RT-qPCR (Fig. 8C and D), olaparib treatment caused significant upregulation of \textit{CXCL11} mRNA expression in multiple ovarian cancer cell lines. Together, these data indicate that olaparib could upregulate the expression of \textit{CXCL11} and its related immune checkpoint genes in ovarian cancer cells \textit{in vivo} and \textit{in vitro}.

**Discussion**

Over the years, many efforts have been made to investigate the initiation, development and treatment of ovarian cancer. Postoperative histopathological characteristics of patients such as tumor size, stage and grade, and residual lesions are still used as the most important prognostic factors for ovarian cancer. However, the 5-year relative survival rate of ovarian cancer patients is still unsatisfactory. HRD has been reported to be not only the ubiquitous feature of breast cancer but is also one of the influence factors for ovarian cancer prognosis. However, HRD is present in less than 50% of serous ovarian tumors, so new biomarkers need to be developed for molecular typing of ovarian cancer patients. In this study, we deeply analyzed the molecular characteristics of OSC patients with different HRD scores and identified biomarkers that could be complementary to the HRD score, our contributions are as follows:

(1) A comprehensive analysis of the genomic landscape of the HRD-negative group showed a high proportion of actionable gene mutations. (2) We found that \textit{CXCL11} expression, the gene most related to HRD, displayed a strong ability to predict the prognosis of OSC patients. (3) Our study confirmed that there was a strong correlation between \textit{CXCL11} expression and TIMER in ovarian cancer.

The basket study design is noteworthy because it allows for the possibility that different tumor types with the same molecular biomarker might differ in their sensitivity to therapy targeted at that biomarker[29]. Potentially actionable mutations were seen in 13% of HRD-negative patients: a) loss-of-function mutations in \textit{NF1} were found in 8% of the patients; b) loss-of-function mutations in \textit{CDK12} were found in 5% of the patients. The \textit{NF1} gene encoding neurofibromin works as a negative regulator of RAS activity. Patients with \textit{NF1} gene loss-of-function mutations will progress to RAS hyperactivity and tumorigenesis[30, 31]. The availability of small molecule compounds (such as selumetinib and imatinib) that target RAS signaling implied in the pathogenesis of plexiform neurofibromas [15, 16] has led to multiple clinical trials, and FDA has approved Koselugo (selumetinib) for the treatment of pediatric patients with \textit{NF1} mutations[32]. \textit{CDK12} (Cyclin-dependent kinase 12) is a kinase involved in the regulation of the cell cycle and the regulation of transcriptional elongation of many DNA-damage-response genes. Loss of the \textit{CDK12}/cyclin K complex renders triple-negative breast cancer and HEK293 cells sensitive to various DNA damaging agents, including camptothecin, etoposide and mitomycin C[33]. Comprehensive genomic analysis of HRD-negative ovarian cancer has broadened our knowledge of the molecular events relevant to patients who cannot receive Olaparib plus bevacizumab treatment, and provides a direction for targeted therapy of these patients.
Through the whole transcriptome analysis of the patients with high and low HRD scores, we identified a reliable prognostic risk gene in the TCGA-OSC RNA-seq dataset and proved its efficacy in the GEO microarray datasets. CXCL11 is a small cytokine belonging to the CXC chemokine family that is also called Interferon-inducible T-cell alpha chemoattractant and Interferon-gamma-inducible protein [34]. It is chemotactic for activated T cells. The CXCL11 expression signature stratified the patients into the CXCL11-positive group and the CXCL11-negative group, representing distinct tumor prognosis and survival. Compared with the conventional clinical characteristics and the HRD score for the prognostic ability of the OSC patients, we found that the CXCL11 expression signature has better predictive ability (Fig. 8E). Moreover, about half of the CXCL11-positive patients had low HRD scores, so in ovarian cancer, CXCL11 expression can be used as a biomarker that complemented the HRD score to predict the prognosis of the patients.

It has been reported that interferon-γ signaling is associated with BRCA1 loss-of function mutations in high grade serous ovarian cancer[35]. Key interferon-γ-induced genes upregulated at baseline in BRCA1-null vs. BRCA1+ ovarian and breast cancer cells included CXCL10, CXCL11, and IFI16[35]. Combining previous research results and our study, we speculate that the factors that can cause HRD can also lead to the activation of INF-γ signaling, leading to the up-regulation of its downstream target genes, and CXCL11 is one of them.

Although immunotherapy has made a breakthrough in the field of oncology, but generally speaking, the efficacy of immunotherapy in epithelial ovarian cancer is not so good[36]. PD-L1 expression was not a clear predictive factor for anti-PD-1 and anti-PD-L1 therapy in patients with ovarian cancer[37].

Basically, two kinds of biomarkers for response to ICB therapy have been identified: 1) those associated with the number of neoantigens derived from tumors, such as the tumor mutational burden[38, 39] and MSI[40]; and 2) those indicative of infiltrating immune cells in tumors, including PD-1/PD-L1/CTLA4 expression on the tumor and immune cells and gene signatures of activated T cells. This study first identified that the CXCL11 expression signature was significantly associated with neoantigen load and infiltrating immune cells in the ovarian cancer tumor samples. Considering the importance of the two types of biomarkers in predicting the response to ICB therapy, we speculated that patients with the high expression level of CXCL11 may be more sensitive to ICB therapy.

The antitumor activity of PARPi has been observed in patients regardless of BRCA1/2 mutation status or the presence of HRD[41]. PARPi leads to activation of the stimulator of interferon genes innate immune pathway in ovarian cancer, which is relevant to trigger proper anti-tumor immune responses[28]. Our analysis revealed that PARPi induced high expression level of CXCL11 and ICB-related genes in vivo and in vitro. Since the CXCL11 expression signature was significantly associated with TIME, including neoantigen load, infiltrating immune cells and the expression of ICB-related genes, we thus hypothesized that CXCL11 expression may be a novel molecular biomarker to reflect the immunogenic responses induced by PARPi.
However, there were several limitations of our study. The main limitation of this study was the use of retrospective data sets to identify and validate the predictive effect of \textit{CXCL11} expression in OSC. Therefore, the expression of \textit{CXCL11} combined with clinical validation in the patients from the OSC prospective cohort is needed to prove its efficacy. Additionally, the genomic landscape of the HRD-negative group derived from the TCGA-OSC cohort was not validated in independent datasets owing to the unavailability of SNP array data and mutation data. Finally, due to a lack of OSC cohorts being treated with anti-PD-1 or anti-PD-L1 therapy, we are unable to validate the association between the expression of \textit{CXCL11} and the immunotherapeutic responsiveness and believe further investigation is needed.

\section*{Conclusions}

To summarize, this work provided a new perspective on the molecular characteristics of the genomic and transcriptome of the patients with OSC. Our results were the first to find that the HRD-negative patients had the opportunity for targeted therapy, laying the foundation for molecular typing of OSC. Furthermore, this work identified the \textit{CXCL11} expression signature that could not only predict OSC patients’ survival outcomes but also reflect the TIME status. Our study showed high clinical application value and provided new clues for enrolling OSC patients in precision medicine. Through further prospective validation and mechanism research, biomarkers derived from this work may become important molecules for molecular typing of OSC.

\section*{Abbreviations}

HRD, homologous recombination deficiency; PAPRi, PAPR inhibitor; TIME, tumor immune microenvironment; ICB, immune checkpoint blockade; OSC, Ovarian serous cystadenocarcinoma; HR, Homologous recombination repair; TCGA, the Cancer Genome Atlas; GEO, Gene Expression Omnibus; \textit{CXCL11}, C-X-C motif chemokine 11; TPM, transcripts per million; CNV, copy number variation; LOH, Loss of heterozygosity; LST, Large-scale State Transitions; TAI, Telomeric Allelic Imbalance; GSEA, Gene set enrichment analysis; DEGs, differentially expressed genes; MSI, microsatellite instability;

\section*{Declarations}

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\section*{Author contributions}

Designing research studies: SZ and HK. Conducting experiments: QJ, ZQ and LB. Analyzing data: SZ and WH. Preparing the manuscript: SZ and QJ. Grammar Check: QX and HX. Supervision: WH, QJ and HK. Funding Acquisition: HK and QJ; The authors read and approved the final manuscript.
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Availability of data and materials

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

Ethics approval and consent to participate

No Applicable.

Consent for publication

Not applicable.

Competing interests

No financial and non-financial competing interests exist in this study.

References

1. Cabasag CJ, Arnold M, Butler J, Inoue M, Trabert B, Webb PM, Bray F, Soerjomataram I: The influence of birth cohort and calendar period on global trends in ovarian cancer incidence. International Journal of Cancer 2020, 146(3):749-758.
2. Zhao S, Shi Y, Zhang X, Cheng L, Song T, Wu B, Li J, Yang H: MIEF2 over-expression promotes tumor growth and metastasis through reprogramming of glucose metabolism in ovarian cancer. 2020.
3. Qi X, Yu C, Wang Y, Lin Y, Shen B: Network vulnerability-based and knowledge-guided identification of microRNA biomarkers indicating platinum resistance in high-grade serous ovarian cancer. Clinical and translational medicine 2019, 8(1):1-11.
4. Matsuda A, Katanoda K: Five-year relative survival rate of ovarian cancer in the USA, Europe and Japan. Japanese journal of clinical oncology 2014, 44(2):196-196.
5. Li X, Heyer W-D: Homologous recombination in DNA repair and DNA damage tolerance. Cell research 2008, 18(1):99-113.
6. Ray-Coquard I, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, Fujiwara K, Vergote I, Colombo N, Mäenpää J: Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. *New England Journal of Medicine* 2019, **381**(25):2416-2428.

7. Heeke A, Lynce F, Baker T, Pishvaian M, Isaacs C: Prevalence of homologous recombination deficiency (HRD) among all tumor types. *JCO Precis Oncol* 2018, 2018:10.1200.

8. Wang T, Wang G, Zhang X, Wu D, Yang L, Wang G, Hao D: The expression of miRNAs is associated with tumour genome instability and predicts the outcome of ovarian cancer patients treated with platinum agents. *Scientific reports* 2017, **7**(1):1-11.

9. Bao S, Zhao H, Yuan J, Fan D, Zhang Z, Su J, Zhou M: Computational identification of mutator-derived lncRNA signatures of genome instability for improving the clinical outcome of cancers: a case study in breast cancer. *Briefings in bioinformatics* 2020, **21**(5):1742-1755.

10. Network CGAR: Integrated genomic analyses of ovarian carcinoma. *Nature* 2011, **474**(7353):609.

11. Tomczak K, Czerwińska P, Wiznerowicz M: The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemporary oncology* 2015, **19**(1A):A68.

12. Abkevich V, Timms K, Hennessy B, Potter J, Carey M, Meyer LA, Smith-McCune K, Broaddus R, Lu KH, Chen J: Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *British journal of cancer* 2012, **107**(10):1776-1782.

13. Manié E, Popova T, Battistella A, Tarabeux J, Caux-Moncoutier V, Golmard L, Smith NK, Mueller CR, Mariani O, Sigal-Zafrani B: Genomic hallmarks of homologous recombination deficiency in invasive breast carcinomas. *International journal of cancer* 2016, **138**(4):891-900.

14. Birkbak NJ, Wang ZC, Kim J-Y, Eklund AC, Li Q, Tian R, Bowman-Colin C, Li Y, Greene-Colozzi A, Iglehart JD: Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. *Cancer discovery* 2012, **2**(4):366-375.

15. Takaya H, Nakai H, Takamatsu S, Mandai M, Matsumura N: Homologous recombination deficiency status-based classification of high-grade serous ovarian carcinoma. *Scientific reports* 2020, **10**(1):1-8.

16. Ellrott K, Bailey MH, Saksena G, Covington KR, Kandoth C, Stewart C, Hess J, Ma S, Chiotti KE, McLellan M: Scalable open science approach for mutation calling of tumor exomes using multiple genomic pipelines. *Cell systems* 2018, **6**(3):271-281. e277.

17. Mayakonda A, Lin D-C, Assenov Y, Plass C, Koeffler HP: Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome research* 2018, **28**(11):1747-1756.

18. Li X, Wen D, Li X, Yao C, Chong W, Chen H: Identification of an Immune Signature Predicting Prognosis Risk and Lymphocyte Infiltration in Colon Cancer. *Frontiers in immunology* 2020, **11**:1678.

19. Robinson MD, McCarthy DJ, Smyth GK: edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 2010, **26**(1):139-140.

20. Metsalu T, Vilo J: ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic acids research* 2015, **43**(W1):W566-W570.
21. Subramanian A, Kuehn H, Gould J, Tamayo P, Mesirov JP: GSEA-P: a desktop application for Gene Set Enrichment Analysis. Bioinformatics 2007, 23(23):3251-3253.

22. Therneau T, Lumley T: R survival package. In.; 2013.

23. Tibshirani R: Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society: Series B (Methodological) 1996, 58(1):267-288.

24. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS: TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer research 2017, 77(21):e108-e110.

25. Zohar Y, Wildbaum G, Novak R, Salzman AL, Thelen M, Alon R, Barsheshet Y, Karp CL, Karin N: CXCL11-dependent induction of FOXP3-negative regulatory T cells suppresses autoimmune encephalomyelitis. The Journal of clinical investigation 2014, 124(5):2009-2022.

26. Li B, Severson E, Pignon J-C, Zhao H, Li T, Novak J, Jiang P, Shen H, Aster JC, Rodig S: Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome biology 2016, 17(1):1-16.

27. Ding L, Kim HJ, Wang Q, Kearns M, Zhao JJ: PARP Inhibition Elicits STING-Dependent Antitumor Immunity in Brca1-Deficient Ovarian Cancer. Cell Reports 2018, 25(11):2972-2980.e2975.

28. Shen J, Zhao W, Ju Z, Wang L, Peng Y, Labrie M, Yap TA, Mills GB, Peng G: PARPi triggers the STING-dependent immune response and enhances the therapeutic efficacy of immune checkpoint blockade independent of BRCAness. Cancer research 2019, 79(2):311-319.

29. Hyman DM, Puzanov I, Subbiah V, Faris JE, Chau I, Blay J-Y, Wolf J, Raje NS, Diamond EL, Hollebecque A: Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. New England Journal of Medicine 2015, 373(8):726-736.

30. Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, Lange BJ, Freedman MH, McCormick F, Jacks T: Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nature genetics 1996, 12(2):144-148.

31. Nichols RJ, Haderk F, Stahlhut C, Schulze CJ, Hemmati G, Wildes D, Tzitzilonis C, Mordec K, Marquez A, Romero J: RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. Nature cell biology 2018, 20(9):1064-1073.

32. Voelker R: A New Treatment for Children With Neurofibromatosis Type 1. Jama 2020, 323(19):1887-1887.

33. Bösken CA, Farnung L, Hintermair C, Schachter MM, Vogel-Bachmayr K, Blazek D, Anand K, Fisher RP, Eick D, Geyer M: The structure and substrate specificity of human Cdk12/Cyclin K. Nature communications 2014, 5:3505.

34. Tokunaga R, Zhang W, Naseem M, Puccini A, Berger MD, Soni S, McSkane M, Baba H, Lenz H-J: CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation—a target for novel cancer therapy. Cancer treatment reviews 2018, 63:40-47.

35. Cardenas H, Jiang G, Pepin JT, Parker JB, Condello S, Nephew KP, Nakshatri H, Chakravarti D, Liu Y, Matei D: Interferon-γ signaling is associated with BRCA1 loss-of-function mutations in high grade serous ovarian cancer. NPJ precision oncology 2019, 3(1):1-14.
36. Martinez A, Delord J-P, Ayyoub M, Devaud C: Preclinical and clinical immunotherapeutic strategies in epithelial ovarian cancer. Cancers 2020, 12(7):1761.

37. González-Martín A, Sánchez-Lorenzo L: Immunotherapy with checkpoint inhibitors in patients with ovarian cancer: Still promising? Cancer 2019, 125:4616-4622.

38. Yarchoan M, Hopkins A, Jaffee EM: Tumor mutational burden and response rate to PD-1 inhibition. The New England journal of medicine 2017, 377(25):2500.

39. Yang Y, Zhang J, Chen Y, Xu R, Zhao Q, Guo W: MUC4, MUC16, and TTN genes mutation correlated with prognosis, and predicted tumor mutation burden and immunotherapy efficacy in gastric cancer and pan-cancer. Clinical and translational medicine 2020, 10(4).

40. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen H-Z, Reeser JW, Yu L, Roychowdhury S: Landscape of microsatellite instability across 39 cancer types. JCO precision oncology 2017, 1:1-15.

41. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, Fabbro M, Ledermann JA, Lorusso D, Vergote I: Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. New England Journal of Medicine 2016, 375(22):2154-2164.

Figures
Computational overview of HRD-related RNAs detection. The columns reflected ovarian cancer samples, and the rows reflected three biomarkers of the HRD score. The color reflects the scores for each biomarker on each sample. HRD-related RNAs were detected by comparing the RNA expression profile between the top 20% patients with high HRD scores and the bottom 20% patients with low HRD scores.
The HRD Score was significantly correlated with the prognosis and molecular characteristics of TCGA-OSC cohort. (A) Kaplan–Meier estimates of overall survival of patients with the HRD-positive or HRD-negative tumors calculated by the HRD score in the TCGA-OSC cohort. (B) Violin plot of somatic mutations in the HRD-positive group and HRD-negative group. Somatic mutation counts in the HRD-positive group were significantly higher than those in the HRD-negative group (Wilcoxon signed-rank test, p < 0.0001; HR=0.49, 95% CI(0.37, 0.66)).
****P<0.0001). (C) Violin plot of fraction genome altered in the HRD-positive group and the HRD-negative group. (Wilcoxon signed-rank test, *P<0.05). (D) Two-dimensional plan of fraction of the genome and somatic mutation counts in different subgroups.

Figure 3

Mutational landscape of TCGA-OSC cohort stratified by the HRD-positive group and the HRD-negative group. (A) Genetic profile of the HRD-positive OSC patients. (B) Genetic profile of the HRD-negative OSC patients.
patients. The genes in the red box are actionable genes.

Figure 4

Screening prognosis related RNA based on the HRD score. (A) Unsupervised clustering of 348 OSC patients based on the expression pattern of 124 HRD-related RNAs. (B, C) Lasso coefficient profiles of the 17 prognosis-associated HRD genes. (D) Heatmap of the signature consisting of the HRD score and the CXCL11 expression signature based on the Cox coefficients. Patients were divided into high-risk and low-
risk groups and the median risk score was utilized as the cutoff value. (E) Kaplan–Meier estimates of overall survival of patients with the CXCL11-positive or the CXCL11-negative tumors in the TCGA-OSC cohort (log-rank test).

Figure 5

The CXCL11 expression signature was associated with the immune infiltration. (A). The TIMER analysis identified the relative infiltration of 6 types of immune cell subpopulations with different CXCL11
subgroups. (B) Violin plot of immune cell subpopulations in the CXCL11-positive group and the CXCL11-negative group (Wilcoxon signed-rank test, **P <0.01, ***P <0.001). (C) Correlation between the CXCL11 expression signature and immune cell subpopulations in the TCGA-OSC cohort. (D) GSEA identified that antigen processing and presentation, autoimmune thyroid and cytokine receptor interaction signaling pathways were upregulated in the CXCL11-positive group compared to the CXCL11-negative group. (E) GSEA identified that taste transduction, basal cell carcinoma and hedgehog signaling pathways were upregulated in the CXCL11-negative group compared to the CXCL11-positive group.
Figure 6

The correlation between the expression of CXCL11 and antigen-related genes. (A). Correlation between the CXCL11 expression signature and antigen-related genes in the TCGA-OSC cohort. (B) Violin plot of top ten antigen-related genes in the CXCL11-positive group and the CXCL11-negative group (Wilcoxon signed-rank test, ***P <0.001). (C) Correlation between the CXCL11 expression signature and neoantigen load in the TCGA-OSC cohort.
Figure 7

Correlation among the expression of CXCL11 and ICB-related genes. (A). Correlation between the CXCL11 expression signature and ICB-related genes in the TCGA-pan cancer the CXCL11 expression signature and ICB-related genes in the TCGA-OSC cohort. (B) Violin plot of top ten ICB-related genes in the CXCL11-positive group and the CXCL11-negative group (Wilcoxon signed-rank test, ***P <0.001). (C) Correlation between cohorts.

| Variable   | N   | Estimate | OR (95% CI) | P      |
|------------|-----|----------|-------------|--------|
| Age        |     |          |             |        |
| >60        | 153 |          |             |        |
| ≤60        | 195 |          | Reference   |        |
| Grade      |     |          |             |        |
| G2         | 40  |          | Reference   |        |
| G3         | 296 |          | 1.22(0.81, 1.76) | 0.36 |
| Stage      |     |          |             |        |
| Stage I-II | 22  |          | Reference   |        |
| Stage III  | 272 |          | 2.00(1.10, 3.62) | 0.089 |
| Stage IV   | 51  |          | 2.42(1.25, 4.71) | 0.033 |
| HRD group  |     |          |             |        |
| HRD -      | 166 |          | Reference   |        |
| HRD +      | 182 |          | 0.50(0.38, 0.67) | <0.0001 |
| CXCL11 group |    |          |             |        |
| CXCL11 -   | 239 |          | Reference   |        |
| CXCL11 +   | 109 |          | 0.39(0.29, 0.51) | <0.0001 |
Figure 8

Olaparib elicits the expression of CXCL11 and ICB-related genes in vivo and in vitro. (A and B). Olaparib elicits the expression of CXCL11 and ICB-related genes in vivo (Wilcoxon signed-rank test, **P <0.01). (C and D) Olaparib elicits the expression of CXCL11 in multiple ovarian cancer cell lines (Student t test, *P <0.05, **P <0.01). (E) Forest plot representation of the multivariate Cox regression model delineated the association between the CXCL11 expression signature and survival in the TCGA-OSC cohort.

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