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Research Article

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Comprehensive Genetic Features of Serous Ovarian Tumor Patients Revealed *PIK3CA* Mutation and Chromosome Instability as Prognostic Biomarkers

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Abstract

Background: *Ovarian cancer is the seventh most common cancer in women worldwide among which the most frequently occurred histological type is serous ovarian cancer (SOC). Since efficacious treatments for SOC have not advanced beyond platinum-based combination chemotherapy and more than 75% of high-grade SOC will relapse after first-line therapy, it is urgent to observe the genomic abnormalities and identify novel therapeutic targets and prognosis biomarkers.*

Methods: *In order to comprehensively identify molecular features of serous ovarian cancer, we performed targeted sequencing with 425 cancer-related genes on four serous ovarian tumor (SOT) cohorts, classified as ovarian serous adenoma (OSA), ovarian serous borderline tumor (OSBT), low-grade serous cancer (LGSC) and high-grade serous cancer (HGSC). The association between genetic alterations and patients' overall survival (OS) was analyzed.*

Results: *Genomic profiling revealed distinct molecular features among these four cohorts. The frequency of genetic alterations in OSA was relatively low, and in OSBT cohort, the predominantly mutated genes, BRAF and KRAS, were identified at prevalence of 52.6% (10/19) and 36.8% (7/19) respectively with two patients harbored both these two mutations. In LGSC cohort, alterations of KRAS still occupied the highest percentage of patients which was up to 50.0% (5/10) while BRAF was not common (1/10, 10.0%). The most frequently mutated gene was TP53 in HGSC (46/47, 97.9%), whereas BRAF or KRAS mutation was rare. Meanwhile, a higher prevalence of gene copy gains in PTK2 (12/47, 25.5%), MYC (9/47, 19.1%), MDM4 (5/47, 10.6%) and ZNF217 (5/47, 10.6%) were identified only in HGSC group which indicated cancer progression promoted by chromosomal instability in this group. The median tumor mutational burden (TMB) and chromosome instability score (CIS) in cases with LGSC and HGSC higher than that in OSBT. Additionally, analysis of DNA damage repair (DDR) relevant genes showed most altered genes enriched in homologous recombination (HR) pathway in HGSC. Finally, we correlated genomic profiles with overall survival (OS) and found that PIK3CA wildtype or chromosome instability score (CIS) low patients had significantly longer OS in HGSC.*

Conclusion: *In this study, we revealed the comprehensive genomic profiling among four SOT cohorts. Additionally, we correlated PIK3CA status and first associated chromosome instability with clinical outcomes of patients and found them to be useful clinical biomarkers in HGSC prognosis.*

Keywords: Serous Ovarian Tumor (SOT), High-Grade Serous Cancer (HGSC), Low-Grade Serous Cancer (LGSC), *PIK3CA*, Chromosome Instability

Introduction

Ovarian cancer is the seventh most common cancer in women both in China and worldwide among which the most frequently occurred histological type is serous ovarian cancer [1]. Several studies further classified serous ovarian carcinoma into low-grade serous cancer (LGSC) and high-grade serous cancer (HGSC) according to a two-tiered grading system [2, 3]. On the basis of the International Federation of Gynecology and Obstetrics (FIGO) grading system, serous ovarian cancer could also be assorted into grade I, II, III, and IV [4].

The 5-year survival of HGSC is about 25% which is attributable to the lack of successful treatment strategies beyond combination chemotherapy, and more than 75% of HGSC will relapse after first-line therapy [5, 6]. Therefore, it is urgent to observe the genomic abnormalities and identify therapeutic targets on clinically annotated SOC patients.

In order to deeply study the difference of the genomic alterations among serous ovarian tumor cohorts and identify reliable predictive biomarkers, comprehensive genomic mutation profiling analysis with 425 cancer-related genes was performed in ovarian serous adenoma (OSA), ovarian serous borderline tumor (OSBT), LGSC, and HGSC cohorts and revealed distinct genomic features among these four groups. The genomic alteration occurs scarcely in OSA. *KRAS* and *BRAF* mutations frequently occurred in OSBT cohort while only *KRAS* was still so common in LGSC group. Different gene alteration profiling was observed in HGSC cohorts as TP53 mutation was universal presence but *KRAS* and *BRAF* alterations were rare. Most of HGSC patients harbored one or more genetic alterations related with DNA damage repair (DDR) pathway. In addition, we found *PIK3CA* wild-type or chromosome instability score (CIS) low cases had longer overall survival (OS) in HGSC patients.

Materials and Methods

Tissue Collection

Tumor and matched normal tissue biopsies were collected from 86 patients in Zunyi Medical University between 2013 and 2019, fixed in 10% neutral buffered formalin and embedded in paraffin. These specimens were stained with hematoxylin and eosin (HE), evaluated by a pathologist and further classified as OSA (n=10), OSBT (n=19), LGSC (n=10) and HGSC (n=47) according to the newest edition of WHO classification. The study was approved by the Ethical Review Board of the Zunyi Medical University.

DNA extraction, Library Preparation and Targeted Sequencing

Genomic DNA were extracted with QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) from FFPE tissues de-paraffinized with xylene, according to manufacturer's instructions. Then DNA concentrations were determined using a Qubit DNA HS Assay Kit with Qubit 3.0 fluorometer (Life Technologies). For each sample, 1000 ng of genomic DNA was sheared into 350 bp fragments using Covaris M220 instrument (Covaris) and processed into library construction with KAPA HyperPrep Library Preparation Kit (KAPA Biosystems, Wilmington, MA) according to the manufacturer's protocol. Libraries with different indexes were pooled and enriched with probes targeted 425 cancer-related genes with a customized xGen Lockdown panel (IDT). Prior to sequencing, the captured libraries were examined for quality and quantity using the KAPA Library Quantification Kit (KAPA Biosystems) by qRT-PCR (CFX384 real time system, Bio-Rad Laboratories). The final libraries were then sequenced on a HiSeq 4000 platform (Illumina) to a mean coverage of 1000x following the manufacturer's instructions.

Bioinformatics Analysis

Quality control for fastq data and subsequent removal of low quality (quality reading below 15) adapters was performed with the trimmomatic software, which is a flexible trimer for Illumina sequence data [7]. Paired-end sequencing reads were then aligned to the reference human genome (build hg19) using the Burrows-Wheeler Aligner (BWA) with the parameters and further processed to PCR deduplication using the Picard suite (http://picard.sourceforge.net/) [8]. The Genome Analysis Toolkit (GATK) was used to base quality score recalibration of local realignment around indels [9]. In order to identify the somatic single nucleotide variants (SNVs), MuTect software was applied to tumor and paired normal BAM files [10]. The small insertions and deletions were detected with SCALPEL (http://scalpel.sourceforge.net/). For the copy number variation (CNV) pipeline, a ≥1.6-fold change in DNA copy number was set as the cutoff for amplification, while a ≤ 0.6-fold change was the cutoff for deletion. Tumor mutational burden (TMB) and chromosome instability score (CIS) was calculated as previously described [11].

Statistical Analysis

The Kaplan-Meier method was used to calculate survival rates, and the log-rank test was used to analyzed difference between cohorts. A significant threshold was set at P-value < 0.05.

Results

Demographic and Clinical Characteristics of Patients Diagnosed With SOT

A total of 86 patients were classified into four SOT cohorts termed as OSA (Figure 1A, n=10), OSBT (Figure 1B, n=19), LGSC (Figure 1C, n=10) and HGSC (Figure 1D, n=47) according to pathological features (Figure 1, Table 1), and the median age of patients in these four groups was 62 years (range 48-88), 38 years (range 20-74), 41 years (range 19-66) and 53 years (range 40-70), respectively (Table 1). Most patients in OSBT cohort were diagnosed at relatively early stage (stage I-II, 18/19, 94.7%), whereas for HGSC cases, most patients were diagnosed at stage III-IV (37/47, 77.7%). In our cohorts, all of OSA patients only received surgery. In 19 OSBT cases, the majority of patients (12/19, 63.2%) only received surgery and additional patients (6/19, 31.6%) except one case with unknown treatment status, received adjuvant chemotherapy after surgery. In LGSC cohort, all patients received adjuvant chemotherapy after surgery. 37.7% patients (13/47) underwent neoadjuvant chemotherapy before surgery, 34.0% (16/47) suffered adjuvant chemotherapy after surgery and 38.3% (18/47) only received chemotherapy in HGSC patients.

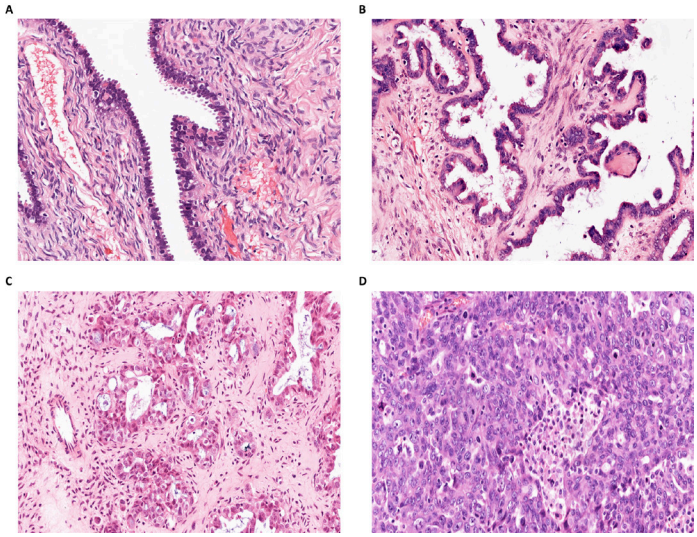


Figure 1: Pathological features of four serous ovarian tumor (SOT) cohorts. (A) ovarian serous adenoma (OSA), cyst wall lining monolayer cubic or low columnar ciliated epithelial cells and cell without atypia can be seen. (B) ovarian serous borderline tumor (OBST), tumor cells form gradually branched nipples, epithelium appear stratified and budding, single cells or cell clusters can be seen in the glandular cavity, spike-like cells are visible and rare mitotic. (C) low-grade serous carcinoma (LGSC), the invasive micropapillary pattern, consistent cell size, higher nuclear plasma ratio and obvious nuclear atypia were observed in this cohort. (D) high-grade serous carcinoma (HGSC), which display typical morphology of papillary and/or solid areas, hyperplasia of fibrous tissue around tumor, locally visible necrosis and highly atypical nuclei.

Table 1: Clinical characteristics of patients diagnosed with SOT.

	All (N=86)	OSA (N=10)	OSBT (N=19)	LGSC (N=10)	HGSC (N=47)
Age, median (range), y	50 (19-88)	62 (48-88)	38 (20-74)	41 (19-66)	53 (40-70)
FIGO Stage, n (%)					
I	20 (23.3)	/	14 (73.7)	1 (10.0)	5 (10.6)
II	10 (11.6)	/	4 (21.1)	2 (20.0)	5 (10.6)
III	31 (36.0)	/	1 (5.3)	7 (70.0)	22 (46.8)
IV	15 (17.4)	/	0	0	15 (32.0)
TMB, average (range), mutations per Mb	3.7 (0-39.1)	0.2 (0-1.1)	1.3 (0-2.3)	2.3 (0-5.7)	5.7 (1.1-39.1)
Microsatellite status	MSI: 1	MSI: 0	MSI: 0	MSI: 0	MSI: 1
	MSS: 85	MSS: 10	MSS: 19	MSS: 10	MSS: 46
Treatment, n (%)					
Neoadjuvant chemotherapy	13 (15.1)	0	0	0	13 (27.7)
Adjuvant chemotherapy	32 (37.2)	0	6 (31.6)	10 (100)	16 (34.0)
Only surgery	40 (46.5)	10 (100)	12 (63.2)	0	18 (38.3)
Unknown	1 (1.2)	0	1 (5.3)	0	0

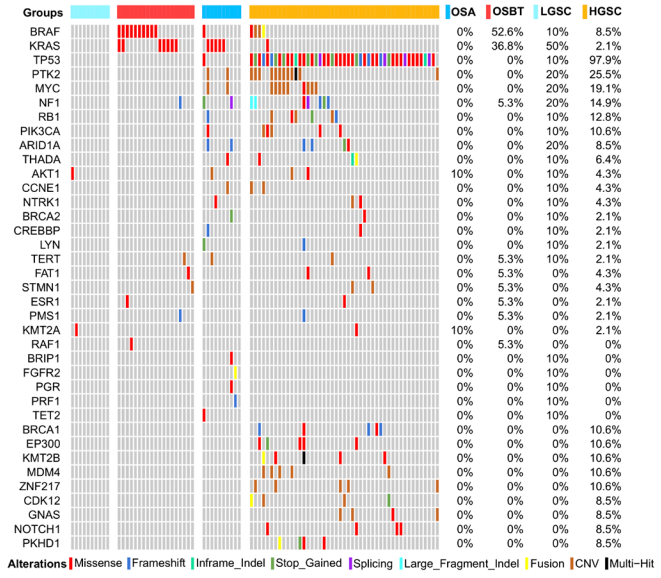
Note: TMB, tumor mutational burden; OSA, ovarian serous adenoma; OSBT, ovarian serous borderline tumor; LGSC, low-grade serous carcinoma; HGSC, high-grade serous carcinoma. /: not applicable.

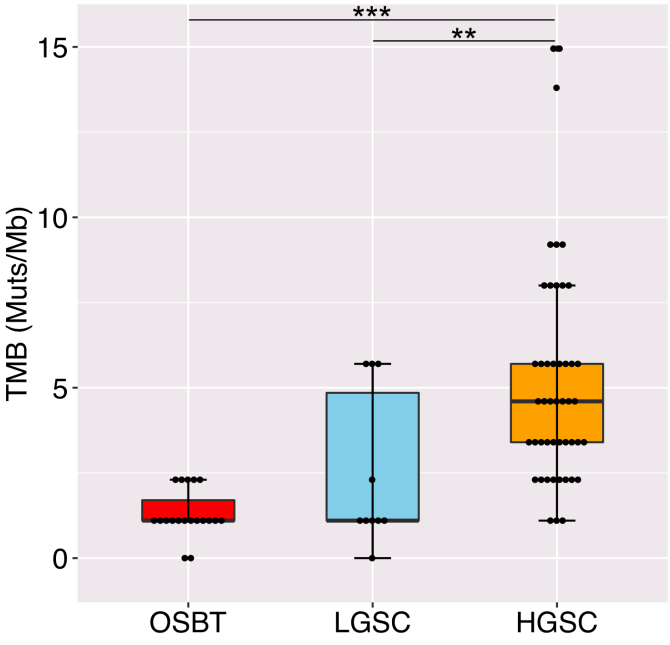
The Genomic Features of Chinese SOT Cohorts

Genomic profiling revealed distinct molecular features among these four cohorts (Figure 2A). As expected, the frequency of genetic alterations in OSA was relatively low with only missense mutations in AKT1 and KMT2A were observed (Figure 2A). For OSBT cohort, the predominantly mutated genes, *BRAF* and *KRAS*, were identified at prevalence of 52.6% (10/19) and 36.8% (7/19) respectively with two patients harbored both *BRAF* and *KRAS* mutations. In LGSC group, alterations of *KRAS* still occupied the highest percentage of patients which was up to 50.0% (5/10) while *BRAF* was not common (1/10, 10.0%). In HGSC patients, different genetic alteration profile was observed as the most frequently mutated gene was TP53 (46/47, 97.6%), whereas the mutation of *BRAF* or *KRAS* mutation was rare. Several genomic alterations including PTK2 (2/10), MYC (2/10), NF1 (2/10) and ARID1A (2/10) were detected in LGSC.

Interestingly, a higher prevalence of gene copy gains in PTK2 (12/47, 25.5%), MYC (9/47, 19.1%), MDM4 (5/47, 10.6%) and ZNF217 (5/47, 10.6%) were identified in HGSC group which indicated cancer progression promoted by chromosomal instability in this group.

Then, we further analyzed MSI, TMB and CIN which were correlated with instability at the genome-wide level in OSBT, LGSC and HGSC groups. Within 86 patients, only one case was tested to be microsatellite instability-high (MSI-H) while the other 85 cases remained to be microsatellite stability (MSS) (Table 1). The average tumor mutational burden (TMB) in cases with LGSC and HGSC were 2.3 and 5.7 mutations per Mb, higher than that in OSBT (1.6) (Table 1) which indicated an increasement of gene mutations during tumor progression (Figure 2B). The result of CIS was consistent with TMB as the levels of CIS in LGSC and HGSC were significantly higher than that in OSTB. Although CIS values in HGSC were high, no significant difference was observed in LGSC and HGSC cohorts (Figure 2C).





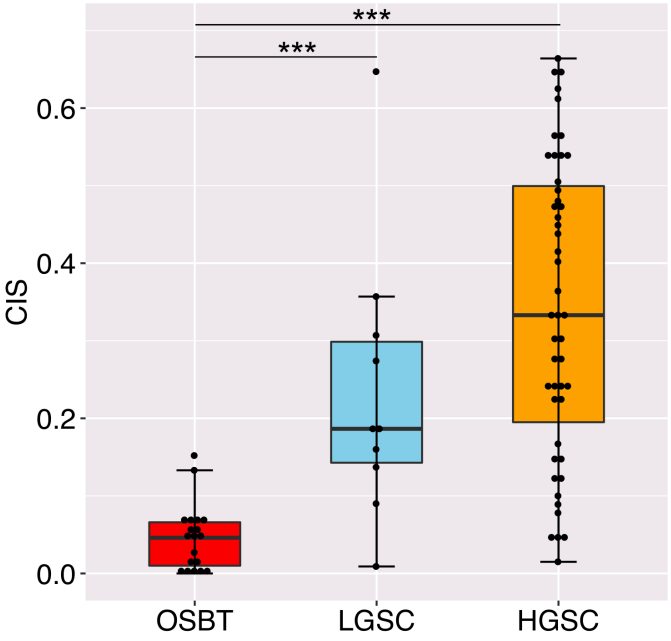
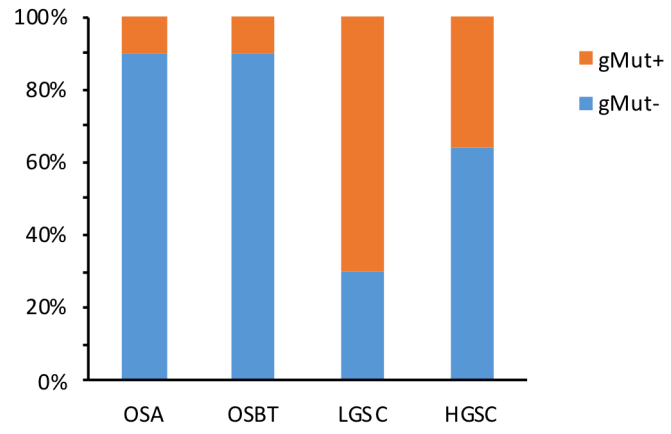


Figure 2: The molecular feature of serous ovarian tumor cohorts. (A) Co-mutation plot of the most frequently altered genes identified by next-generation sequencing of OSA, OSBT, LGSC and HGSC cohorts. Box plots comparing genomic features of TMB (B) and CIS (C) among three serous ovarian tumor cohorts apart from OSA group. The top and bottom of the boxes are the lower and upper quartiles, the middle line in the box is median. Wilcoxon's rank-sum test was used for inter-group comparison and P value calculation with two-side. * indicates a significant threshold of P-value <0.05. ** and*** represent the P-value were <0.01 and <0.001, respectively.

Additionally, germline mutation in these four groups was analyzed. The frequency of germline mutations was relatively low in both OSA and OSBT cohorts, whereas higher in LGSC (70%, 7/10) and HGSC (36%, 17/47) cohorts (Figure 3A). For LGSC cohort, BRCA2 mutations were detected, accompanied with DYPD and APC mutations. The most frequent germline mutation we detected in HGSC cohort was BRCA1 (21.3%, 10/47), followed by BRCA2 (6.4%, 3/47) and WRN (4.26%, 2/47).



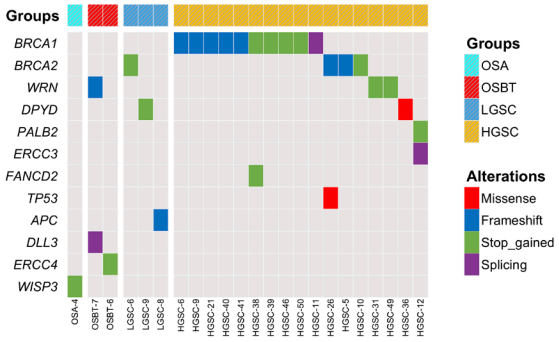
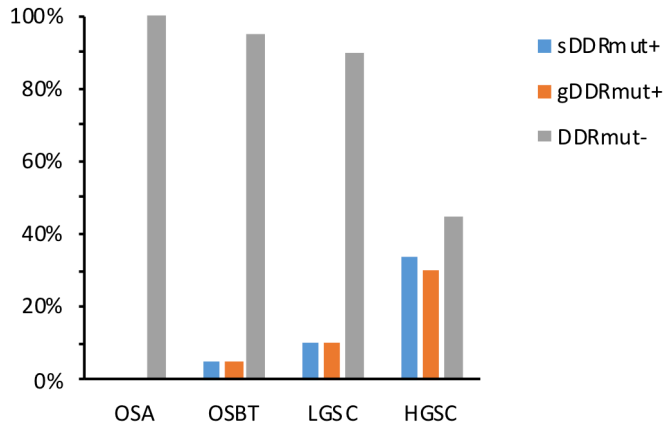


Figure 3: Analysis of germline mutation in serous ovarian tumor (SOT). (A) Frequency of germline mutation in SOT. (B) Germline mutation profiling of serous

Comprehensive Molecular Profiles Revealed Many Mutations Associated with DNA Damage Repair (DDR) System

Since the deficiency of DNA damage repair (DDR) system significantly affect genomic stability and finally lead to occurrence of cancer in multiple cancer types, we further studied the genetic alterations related to DDR signaling. As expected, low frequency of DDR-relevant gene mutation was observed both in OSA (0%, 0/10) and OSBT (10.5%, 2/19) cohorts (Figure 4A). On the contrary, 10.0% (1/10) of LGSC patients and 55.3% (26/47) of HGSC patients harbored at least one gene alteration associated with DDR signaling and enriched in homologous recombination (HR) pathway (Figure 4A). Further analysis in 26 HGSC patients with DDR-relevant mutations showed that 61.5% (16/26) cases had somatic mutations among which the most frequently mutated were BRCA1 (17%), followed by ATM (10%) and PARP1 (10%). Additionally, in the other 53.8% (14/26) cases with germline mutation, BRCA1 (67%) and BRCA2 (20%) were the most frequently altered (Figure 4B, right panel).



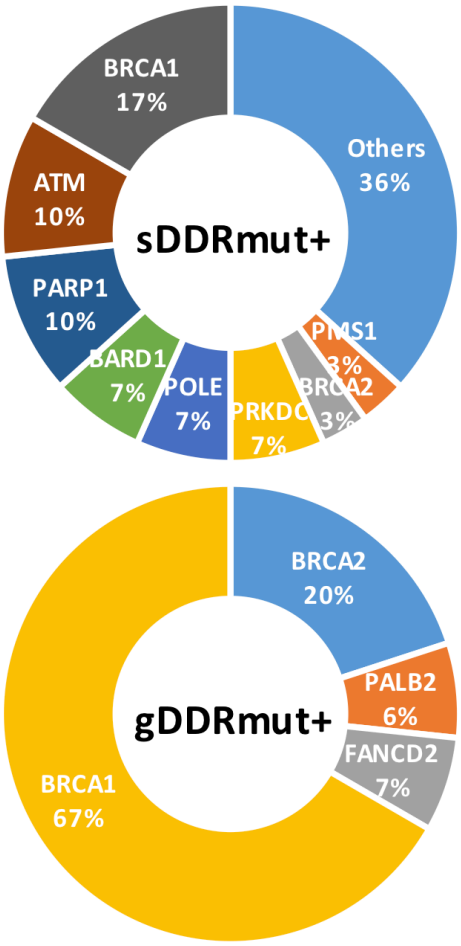


Figure 4: Mutation analysis of gene included in 425 Panel associated with DNA damage repair (DDR) pathway in serous ovarian tumor. (A) Mutation frequency of gene associated with DDR pathway in four serous ovarian tumor cohorts. High frequency of somatic mutation gene (B, left panel) and germline mutation gene (B, right panel) associated with DDR pathway in HGSC cohort. sDDRmut+, gDDRmut+ represents somatic mutation and germline mutation associated with DNA damage repair pathway, respectively. Whereas DDRmut- indicates without mutated events associated with DDR pathway. The total number of somatic alteration and germline alteration incidents were 30 and 16, respectively.

PIK3CA Wildtype and CIS Low are related with Longer Overall Survival in HGSC

Genetic alterations could be potential predictors of prognosis in ovarian cancer therapy. Therefore, to deeply evaluate the relationship between molecular profiling and clinical outcomes, and

identify biomarkers correlated with longer overall survival (OS), we conducted survival analysis in HGSC cohort.

Numerous genetic and functional studies have clearly showed that *PIK3CA* gene played an important role in the PI3K-Akt pathway which associated with development of neoplasia in ovarian tumors. In our results, the data showed that patients without *PIK3CA* mutation had a significantly better OS (median: NA), compared to *PIK3CA* mutated patients (median: 6.4 months) ($P < 0.001$). The 2-year survival probability was also different between these two groups (wt vs. mut: 0 vs 34.3%) (Figure 5A).

Given genomic instability may play crucial role in HGSC progression and have effect on therapeutic approach selection, we further compared OS in CIS low and high subgroups which was divided according to the value of 0.35 because of similar cases between these two groups. Interestingly, patients with lower CIS had a drastically better OS compared to CIS high group ($P < 0.01$), as well as a higher 2-year survival probability between these two groups (lower CIS vs. higher CIS: 40.0% vs 21.1%) (Figure 5B). These results suggested that CIS may function as a novel prognostic biomarker in HGSC.

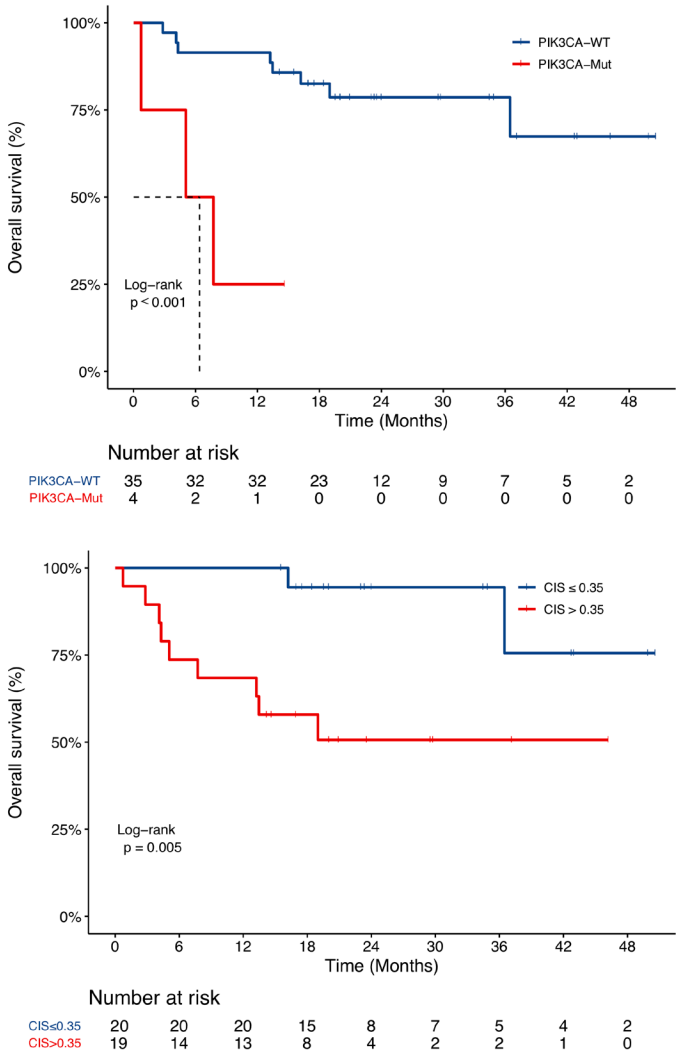


Figure 5: Genetic features affected the clinical response to treatment in HGSC cohort. Kaplan-Meier curves showing different effects of genetic features on patients' OS. The effect of *PIK3CA* status (A) and CIS (B) in HGSC cohort. Patients were divided into two groups with or without *PIK3CA* mutation (A). At the same time, patients were classified into two groups according to their CIS values ordered from small to big, the cutoff is setter as 0.35 because of similar cases between above 0.35 group and the other group (B). Log-rank test was performed to inter-group comparison and p value calculation.

Discussion

In this study, comprehensive genomic profiling of four serous ovarian tumor cohorts was generated using targeted sequencing of 425 cancer-related genes. Molecular features showed high frequencies of *BRAF* and *KRAS* mutation in OSBT group, whereas TP53 was the most dominant mutation in HGSC patients which was consistent with previous studies [12-14]. Numerous studies have shown that LGSC originates from OSBT and has a high prevalence of *BRAF* and *KRAS* mutations, but in our study, only *KRAS* was still so common in LGSC group and certain alterations of LGSC were somehow similar with that in HGSC group [15, 16]. We observed that PTK2 amplification occurred in 25.5% (12/47) cases in HGSC cohort among which 41.7% (5/12) also harbored co-amplification with MYC, a gene located close to PTK2 on chromosome 8. PTK2, located at the tip of chromosome 8q24.3 locus which has been confirmed as a susceptibility locus in serous ovarian cancer, encodes focal adhesion kinase (FAK) [12, 17-19]. Excitingly, several clinical trials (NCT01138033, NCT01943292, and NCT00787033) on FAK inhibitors were ongoing and may be benefit to HGSC patients with PTK2 amplification [20].

As we known, certain germline mutations played important roles in cancer occurrence. Thence, an analysis of germline mutation was performed in all four SOT cohorts and revealed that germline mutations were relatively common both in LGSC and HGSC but rare in OSA and OSBT cohorts. As the most frequent germline mutations, BRCA1 and BRCA2 which were known in mediating homologous recombination (HR), were found to be mutated at a prevalence of 21.3% and 6.4% respectively in HGSC patients. Several studies suggested that ovarian cancer patients carried BRCA1/2 germline mutations are sensitive to PARP inhibitors (PARPi) which indicated an important therapeutic approach for HGSC cohort [21-23]. Besides BRCA1 and BRCA2, other mutations in HR pathway such as WRN, PALB2, RAD50, BLM were also identified in our study. Inhibitors targeted HR related proteins also have been exploited and are being tested in clinical trials (NCT02157792, NCT01955668) in cancer therapy.

Finally, we systematically correlated the molecular profile of HGSC patients with their clinical outcomes and found several molecular indexes that could be used as prognostic biomarkers. Numerous genetic and functional studies have clearly showed that *PIK3CA* gene played an important role in the PI3K-Akt pathway associated with development of neoplasia in ovarian tumors [24, 25]. In our study, we identified that patients carrying *PIK3CA* mutation had a shorter OS. In-depth investigation of *PIK3CA* mutation sites showed that mutation sites of *PIK-3CA* occurred in the helical domain (p.E542K and p.E545K) and kinase domain (p.H1047R) in our results. A recent study on non-small-cell lung cancer patients evidenced that *PIK3CA* mutations in helical domain (p.E542K), kinase domain (p.Y1021H and p.H1047R) and C2 domain (p.N345K) were associated with

a worse progressive free survival (PFS) [11]. These findings indicated *PIK3CA* status can be considered as an efficacious biomarker for clinical outcomes prediction. Since the sample size in our study is small, these results are needed to be further confirmed in the future.

Some researches demonstrated that chromosome instability (CIN) was associated with the occurrence of tumors, the acquisition of multi-drug resistance and poor clinical outcome in many cancer types [26-31]. In our study, TMB and CIN analysis showed an increasement of prevalence in OSBT, LGSC and HGSC cohorts, which indicated that genomic instability was exacerbated accompanying tumor progression. In order to further elucidate whether CIN had effect on the clinical outcome, chromosome instability score (CIS) was measured to evaluate the chromosome stability status of three SOT cohorts and linked to OS in HGSC cohort. Obviously, higher CIS indicated a shorter OS. Accordingly, the CIS value of chromosome instability thus may also be a useful clinical biomarker in HGSC cohort.

In conclusion, our study systematically revealed the comprehensive genomic profiling among four SOT cohorts. Additionally, we first correlated chromosome instability with clinical outcomes of patients and found that CIS could be a useful clinical biomarker in HGSC prognosis.

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