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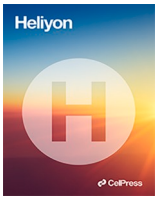

Next-generation sequencing-based analysis of homologous recombination repair gene variant in ovarian cancer

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ABSTRACT

Background: Ovarian cancer is the leading cause of death from gynecological malignancies. Investigating the HRR-related gene status, notably *BRCA1/2* in different regions and populations is of great significance for formulating accurate target therapy.
Methods: We collected 124 ovarian cancer cases from the Affiliated Hospital of.
Qingdao University, detected the genomic alteration of 32 genes by NGS, including.
19 HRR-related genes, 9 proto-oncogenes and 4 tumor suppressor genes. Clinicopathological characteristics, variants, clinical significance, and correlation with prognosis were analyzed.
Results: The incidence of HRR-related gene mutation was 59.68 % and no statistical significance was found with multiple clinicopathological characteristics. *BRCA1/2* (27.42 %) were the most frequent mutated HRR genes. 23 (18.55 %) cases harbored *gBRCA1/2* mutation, with all *BRCA1* mutations were pathogenic/likely pathogenic and 2 cases of *BRCA2* mutation was variant of uncertain significance. Somatic *BRCA1/2* mutations were found in 12 (9.68 %) cases, and *sBRCA1/2* had a higher frequency in less common ovarian cancer than high-grade serous carcinoma. HRR-related gene mutation status was associated with better prognosis than HRR wild-type.
Conclusions: Somatic *BRCA1/2* mutation has higher incidence in less common ovarian cancer. HRR gene mutation status is an independent prognosis factor in ovarian cancer. Clarifying the HRR gene status is important for the selection of target therapy as well as the evaluation of prognosis.

1. Introduction

Ovarian cancer is the third most common cancer type and the second leading cause of cancer-related death in female reproductive system worldwide [1]. In China, ovarian cancer is the second leading cause of incidence and mortality in gynecologic cancer, there were 36,900 new cases and 27,200 deaths from this malignant tumor according to the latest statistics data [2]. Ovarian cancer consists of several histologic subtypes, with epithelial ovarian cancer accounts for 90 % of this disease [3,4], which contains five principal pathological subtypes, including high-grade serous ovarian cancer (HGSOC, 70 %), low-grade serous ovarian cancer (LGSOC, <5 %), endometrioid carcinoma (EC, 10 %), clear cell carcinoma (CCC, 10 %) and mucinous carcinoma (MC, 3 %) [5,6], the biological

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properties and molecular characters of high-grade tumors are distinct from other subtypes [5,7]. Non-epithelial tumor inlcudes germ cell tumor such as dysgerminoma, immature teratoma, and malignant sex cord-stromal tumor like Sertoli-Leydig cell tumor [3]. Despite the advances in the early diagnosis, surgery and treatment therapy in cancer, survival rate of ovarian tumor has remained modest in decades [7,8].

Primary treatment of ovarian cancer including appropriate debulking surgery with or without systemic platinum-based chemotherapy [9]. Recently, as the advanced development of next-generation sequencing (NGS), the management of OC has entered the era of precision medicine. Homologous recombination repair (HRR) is an important double-stranded DNA (dsDNA) damage repair mechanism, it uses sister chromatids as a template to repair dsDNA damage by recruiting BRCA1, BRCA2 and PARP (Poly ADP-ribosepolymerase) proteins in the signaling pathway. Failure of HRR could be caused by the loss of function mutations such as *BRCA1*, *BRCA2*, *RAD51C*, and *RAD51D*, which will lead to the failure of dsDNA damage repair and make the tumor more sensitive to platinum-based chemotherapy and PARP inhibitors (PARPi) [10–12].

PARP protein plays a key role in the repair of single-stranded DNA (ssDNA) damage, PARPi could prevent this process by capturing PARP protein at the site of single-stranded DNA damage, leading to the accumulation of damaged DNA and eventually cause the death of cell on the basis of HRR gene mutation [13]. As the most well-studied HRR-related gene, several clinical trails and studies have showed that patient with germline and/or somatic *BRCA1/2* mutation is the greatest beneficiary of PARPi, with significantly improved PFS [14–16]. Based on these studies, national comprehensive cancer network (NCCN) has recommended PARPi a maintenance option in patients with germline and/or somatic *BRCA1* and *BRCA2* status [17].

The advent of next-generation sequencing (NGS) technology gives the possibility to better understand the genomic landscape of ovarian cancer and offers the guidance to precision treatment. Compared with other prior sequencing method, NGS provides an affordable, high throughput, high resolution, and more comprehensive genomic testing in multiple cancer types, as well as a reliable method for discovering novel genetic mutations to help understand the pathogenesis of disease [18,19]. The current NCCN guideline recommends molecular testing both in the upfront setting and upon recurrence in ovarian cancer. For upfront setting, to determine the molecular alterations including *BRCA1/2* status, loss of heterozygosity (LOH), homologous recombination gene status in the absence of germline *BRCA* mutation to help the selection of interventions with well-known benefits such as PARPi. Other tumor specific markers including (but not limited to) MSI (microsatellite instability), TMB (tumor mutational burden), BRAF, and NTRK were suggest to be detected in the relapse setting to provide possible treatment selection [17].

In this study, we collected 124 ovarian cancer cases and performed a comprehensive analysis including clinicopathological characteristics, genome landscape, treatments and prognosis. Our study provides valuable guidance and reference for the precision medical treatment of ovarian cancer patient in China.

2. Materials and methods

2.1. Patients

We collected retrospective data from 129 cases of various ovarian cancer histological types that were diagnosed and received systemic treatment in the Affiliated Hospital of Qingdao University from January 2019 to February 2023. Patients who refused to enroll in this research (4 cases) and developed more than one malignant tumor (1 case) were excluded. All hematoxylin-eosin (HE) stained slides of the specimen were cross-checked by two pathologists. All patients provided informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (Project identification code: QYFY WZLL 27873).

2.2. Sample preparation

For formalin-fixed paraffin-embedded (FFPE) samples, tissues were sectioned in 5 μm and enriched as previous described [20]. Genomic DNA was extracted using a Tiangen paraffin-embedded tissue DNA extraction kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Genomic DNA of blood sample was extracted by a Fresh blood/marrow DNA extraction kit following the manufacturer's instructions (Amoy Diagnostics, Xiamen, China). DNA library was prepared by the HRR gene combination detection library preparation kit (Amoy Diagnostics, Xiamen, China) and applied to an HRR 32 gene panel detection, including HRR-related genes (*ATM, ATR, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCA, FANCL, MRE11A, NBN, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L*), proto-oncogenes (*AR, BRAF, ERBB2, ESR1, HDAC2, HOXB13, KRAS, NRAS, PIK3CA*), and tumor suppressor genes (*CDH1,PTEN, STK11, TP53*).

2.3. Next-generation sequencing

DNA sequencing was performed on the NextSeq CN500 Illumina platform (Illumina, San Diego, CA, USA) using a NextSeq Mid Output Reagent Cartridge (Illumina) following the manufacturer's instructions. The average sequencing depth was 1,000X and the effective sequencing depth was over 300X, variants with variant allele fraction (VAF) greater than 3 % were retained. Variant types including single nucleotide polymorphism (SNP), indels, hot spot mutation were analyzed. Patients with *BRCA1/2* mutations were further checked with blood sample to confirm germline or somatic mutations.

2.4. Statistical analysis

All data was processed with SPSS 26.0.0 statistical analysis software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, USA). Statistical significance was defined at $P < 0.05$. Correlation factors of ovarian cancer prognosis were analyzed by the multivariate logistic regression and cox regression models with 95 % confidence intervals (CI). Disease free survival (DFS) was confirmed as the interval between the date of diagnosis and the date of recurrence/metastasis or last follow-up. Survival analysis was performed by the Kaplan–Meier survival curves using log-rank test.

3. Results

3.1. Clinical pathological characteristics of ovarian cancer patients

All the clinical pathological characteristics of 124 OC patients were summarized in Table 1. The median age of 124 OC patients was 57 years old (range from 25 to 79), 116 (93.54 %) of the patients undergo debulking surgery and the average tumor size was 6.3 cm. Serous carcinoma present a majority of pathological subtype (94/124, 75.81 %), other subtypes including clear cell carcinoma (11/124, 8.87 %), endometrioid carcinoma (10/124, 8.05 %), mucinous carcinoma (6/124, 4.84 %), dysgerminoma (1/124, 0.81 %),

Table 1
Clinicopathological characteristics and HRR gene status of ovarian cancer.

Clinicopathological characteristics	Overall (N = 124, %)	HRR-m (N = 74, %)	HRR-w (N = 50, %)	*P* value
Age/years				
Median/range	57/25-79	57/25-79	58/28-79	0.910[a]
Mean ± SD	55.6 ± 11.6	55.5 ± 1.4	55.7 ± 11.9	
Tumor size/cm				
Median/range	6.3/0.5–22	6/0.5–18	6.5/1.1–22	0.851[a]
Mean ± SD	6.7 ± 4.2	6.7 ± 4.0	6.8 ± 4.4	
Unknown	13	9	4	
Pathological type				
Serous carcinoma	94	55	39	0.261[a]
Clear cell carcinoma	11	9	2	
Other	19	10	9	
FIGO				
I	23	16	7	0.298[a]
II	18	8	10	
III	65	41	24	
IV	18	9	9	
Lymph node status				
Positive	4	3	1	0.223[a]
Negative	13	5	8	
Unknown	107	66	41	
LVSI				
Positive	7	5	2	0.798[a]
Negative	117	69	48	
Hormone receptor				
Positive	89	50	39	0.168[a]
Negative	19	11	8	
Unknown	16	13	3	
PD-L1				
Positive	54	30	24	0.611[a]
Negative	24	14	10	
Unknown	46	30	16	
Ki67				
Median/range	45.0/3–90	50/3–90	37.5/5–80	0.332[a]
Mean ± SD	45.1 ± 22.7	47 ± 22.4	42.6 ± 23.1	
Unknown	22	16	6	
Type of surgery				
Neoadjuvant chemotherapy surgery	+49	25	24	0.170[a]
Radical surgery	73	47	26	
Other	2	2	0	
Postoperative				
chemotherapy Yes	117	69	48	0.798[a]
No	7	5	2	
Postoperative targeted therapy				
Yes	62	34	28	0.227[a]
No	61	40	21	
Unknown	1	0	1	

[a] Student's *t*-test; a Pearson's chi-squared test; LVSI, Lymphovascular space invasion; m, mutant; w, wildtype; Hormone receptor including estrogen receptor and progesterone receptor.

Sertoli-Leydig cell tumor (1/124, 0.81 %) and undifferentiated carcinoma (1/124, 0.81 %), the HE stained pathological images of each subtype were shown in Fig. 1. Over half of the patients (65/124, 52.42 %) were in FIGO (International Federation of Obstetricians and Gynecologists, FIGO) III stage at diagnosis, others were in FIGO I (23/124, 18.54 %), FIGO II (18/124, 14.52 %), FIGO IV (18/124, 14.52 %). 49 (39.52 %) patients received neoadjuvant chemotherapy before debulking surgery, with 117 (94.35 %) patients underwent systemic chemotherapy after surgery. Moreover, nearly half (62/123, 50.41 %) of the patients received postoperative targeted

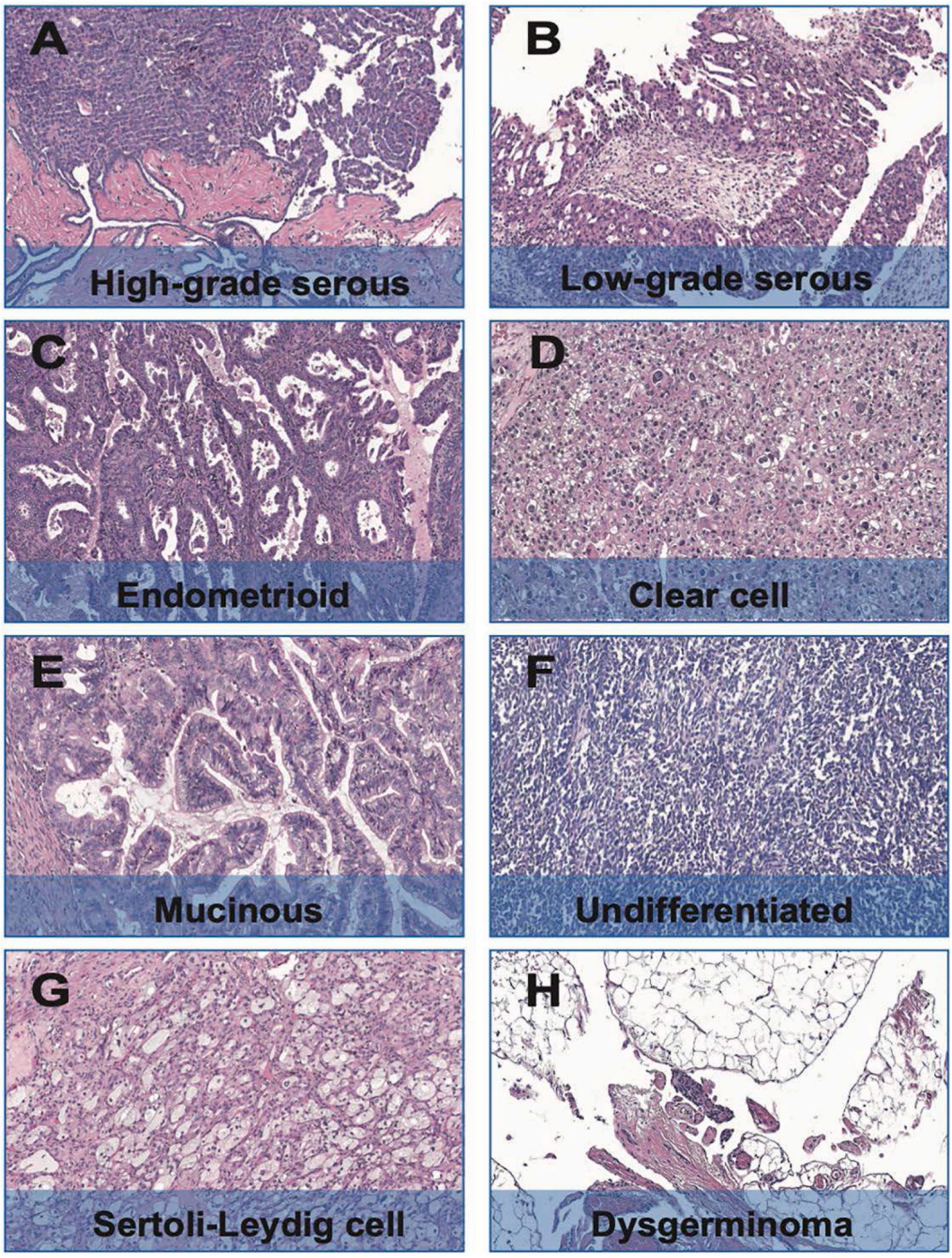

Fig. 1. Photomicrographs (original magnification, 200×; hematoxylin-eosin stain) of the pathological subtypes of ovarian cancer in this study. (A) High-grade serous ovarian cancer (HGSOC); (B) Low-grade serous ovarian cancer (LGSOC); (C) Endometrioid carcinoma (EC); (D) Clear cell carcinoma (CCC); (E) Mucinous carcinoma; (F) Undifferentiated carcinoma; (G) Sertoli-Leydig cell carcinoma; (H) Dysgerminoma.

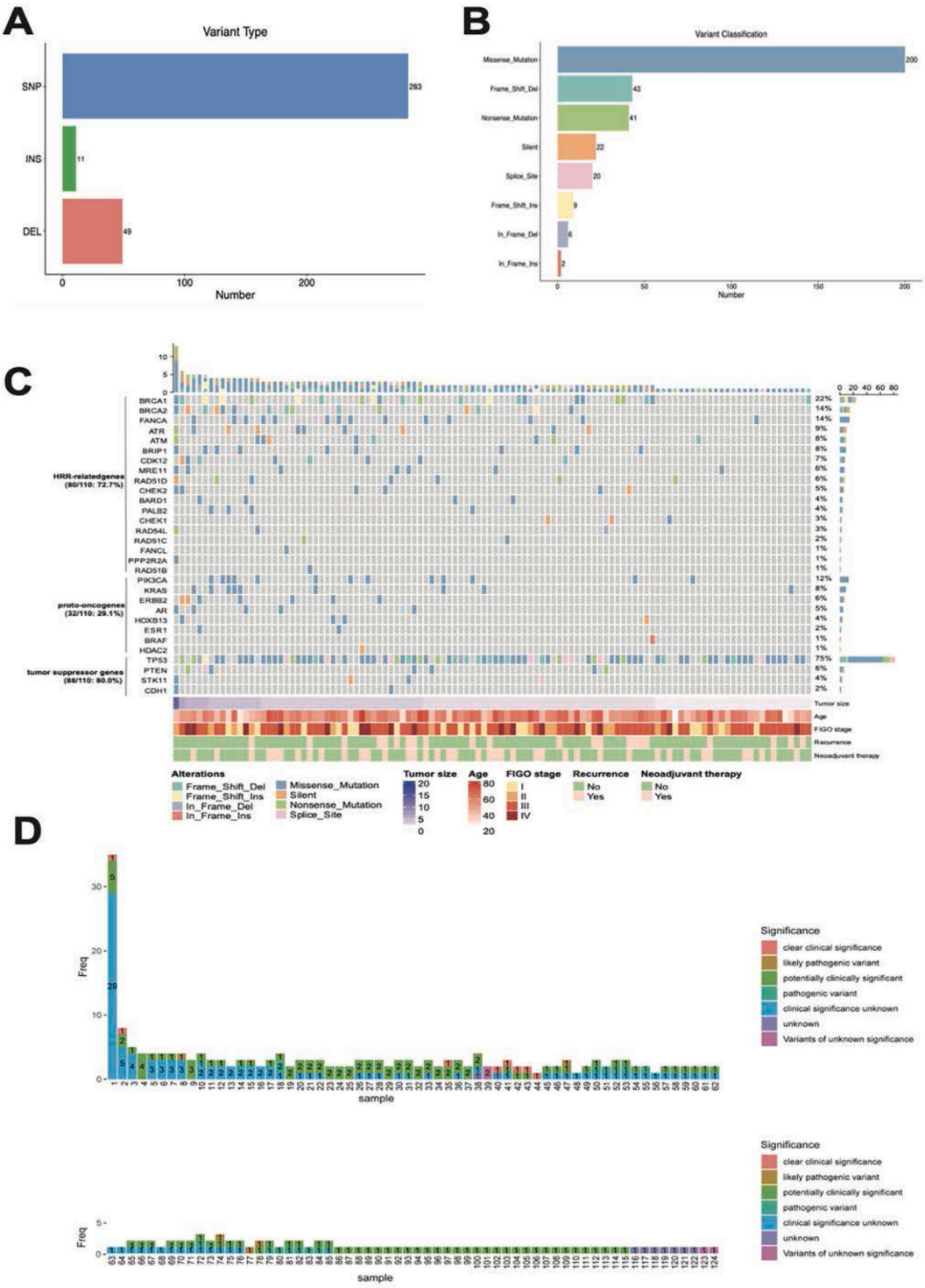

Fig. 2. Variants of the HRR-related gene, proto-oncogene and tumor suppressor gene in 124 cases of ovarian cancer. (A) The number of mutations in different variant type; (B) The number of mutations in different variant classifications; (C) The Oncoplot of multiple gene variants in 110 ovarian cases. Each row represents one gene and each column indicates one patient. The right panel shows the mutation frequeny of the individual gene. Classification of the alteration was indicated by different colors. Clinical pathological characteriatics including age, tumor size, FIGO stage, recurrence and neoadjuvant therapy were listed under the plot; (D) The clinical significance and frequency of each mutation in 124 ovarian cancer patients were delineated, significance including both germline and somatic mutations were indicated by different colors. SNP: single nucleotide polymorphism; INS: insertion; DEL: deletion; Freq: frequency.

therapy including PARP inhibitors, Bevacizumab, and Anlotinib. The pathological characteristics listed in Table 1 showed no statistical significance with HRR-related gene mutation status.

3.2. Variants of HRR-related genes and other genes

All the 124 OC cases were tested the genomic alterations by NGS and the variant files were summarized in Fig. 2. For all of the variants, SNP was the most common type (Fig. 2 A) and missense mutation was the leading subtype of variant classfication (Fig. 2 B). HRR-related gene variants were found in 74 (59.68 %) cases, with *BRCA1* (19.35 %), *BRCA2* (11.29 %) as the most frequent mutated genes, other genes including *FANCA* (14, 11.29 %), *ATM* (9, 7.26 %), *ATR* (7, 5.65 %), *CDK12* (7, 5.65 %), *MRE11* (6, 4.84 %), *RAD51D* (6, 4.84 %), *BARD1* (4, 3.23 %), *RAD54L* (4, 3.23 %), *RAD51C* (4, 3.23 %), *BRIP1* (3, 2.42 %), *CHEK2* (3, 2.42 %), *PALB2* (2, 1.61 %), *FANCL* (2, 1.61 %), *PPP2R2A* (2, 1.61 %), *CHEK1* (1, 0.81 %) and *RAD51B* (1, 0.81 %). This NGS panel also detected the alterations of proto-oncogene including *PIK3CA, KRAS, ERBB2, AR, HOXB13, ESR1, BRAF* and *HADC2*, and 29 (23.39 %) cases were found with proto-oncogene variants. Furthermore, variants of tumor suppressor gene including *TP53, PTEN, STK11, CDH1* were found in 89 (71.77 %) patients, with *TP53* (83/89, 93.26 %) the most frequent mutated gene. To better represent the genomic variability in these ovarian cancer patients, we removed the genomic polymorphism alterations in all samples, as well as the germline mutations in blood samples, retained variants specified in the Clinvar database, and generated a waterfall plot of the mutations in 110 patents (Fig. 2 C). In addition, we reported the clinical significance of each mutation in all cases (Fig. 2 D).

3.3. Pathogenic/likely pathogenic (P/LP) alterations and variants of uncertain significance (VUS) in BRCA1/2 genes

In 124 ovarian cancer patients, 8 cases were tested with blood, 116 cases used paraffin tissues for sequencing, and only 20 cases using paraffin tissues with *BRCA* gene alteration were further confirmed for germline mutation. HGSOC accounted for most of the g*BRCA1/2* mutations (95.65 %). For *BRCA1/2* gene, 23 out of 124 (18.55 %) cases were found with germline mutations, including 14 (11.29 %) *BRCA1* mutation, 8 (6.45 %) *BRCA2* mutation, and 1 (0.81 %) case with both *BRCA1/2* P/LP mutation. All *BRCA1* mutations were P/LP and 2 cases of *BRCA2* mutations were VUS. For 8 cases using blood samples, 32 genes listed in panel were all analyzed for germline mutation, and 2 cases were totally negative. P/LP mutations were found in 3 cases, 2 cases were *BRCA1* and 1 case was *RAD51D*, 4 VUS were found in 3 cases, including *BRIP1, FANCA*, and *RAD51C*. All of the germline variants of 26 cases were concluded in Table 2. Classification of germline mutation was determined on the guidelines of ACMG/AMP [21].

Table 2
Germline mutations of *BRCA1/2* gene in ovarian cancer.

Case	Histologic NO. type	Tissue type	Variant		P/LP	VUS
1	HGSOC	P	*BRCA1*	exon11 c.3841C > T p.Gln 1281[a]	Y	
2	HGSOC	P	*BRCA1*	intron 21 c.5332+1 del	Y	
3	HGSOC	P	*BRCA1*	exon11 c.3770_3771 del p.Glu1257Glyfs[a]9	Y	
4	HGSOC	P	*BRCA1*	exon11 c.2138C > G p.Ser 713[a]	Y	
5	HGSOC	P	*BRCA1*	exon11 c.3748G > T p.Glu 1250[a]	Y	
6	HGSOC	P	*BRCA1*	exon11 c.3352C > T p.Gln 1118[a]	Y	
7	HGSOC	P	*BRCA1*	exon 24 c.5470_5477del p.Ile1824Aspfs[a]3	Y	
8	HGSOC	P	*BRCA2*	exon11 c.5645C > A p.Ser 1882[a]	Y	
9	HGSOC	P	*BRCA1*	exon11 c.1961 dup p.Tyr655Valfs[a]18	Y	
10	HGSOC	P	*BRCA2*	exon11 c.6447_6448dup p.Lys2150Ilefs[a]19	Y	
11	HGSOC	P	*BRCA1*	exon 2 c.66 dup p.Glu23ArgfsTer18	Y	
12	HGSOC	P	*BRCA2*	exon19 c.8481T > G p.Pro 2827 =		Y
13	HGSOC	P	*BRCA2*	exon10 c.1905T > A p.Asp635Glu		Y
14	HGSOC	P	*BRCA1*	exon 22 c.5386 dup p.Ser1796PhefsTer34(S1796Ffs[a]34)	Y	
15	EC	P	*BRCA2*	exon11 c.6235_6245del p.Val2079IlefsTer2(V2079Ifs[a]2)	Y	
16	HGSOC	P	*BRCA2*	exon11 c.4415_4418del p.Lys1472ThrfsTer6(K1472Tfs[a]6)	Y	
17	HGSOC	P	*BRCA2*	exon11 c.5789 del p.Leu1930TyrfsTer33 (L1930Yfs[a]33)	Y	
18	HGSOC	P	*BRCA1*	exon11 c.2788_2795del p.Pro930TrpfsTer5(P930Wfs[a]5)	Y	
19	HGSOC	P	*BRCA1*	exon11 c.1016 dup p.Val340GlyfsTer6(V340Gfs[a]6)	Y	
	HGSOC	P	*BRCA2*	exon8 c.658_659del p.Val220IlefsTer4(V220Ifs[a]4)	Y	
20	HGSOC	P	*BRCA1*	exon19 c.5162A > C p.Gln1721Pro(Q1721P)	Y	
21	HGSOC	P	*BRCA1*	exon11 c.2747 dup p.Asn916LysfsTer9(N916Kfs[a]9)	Y	
22	HGSOC	B	*RAD51D*	exon 6 c.562C > T p.Arg 188[a]	Y	
23	HGSOC	B	*BRIP1*	exon 16 c.2324A > G p. (Asn775Ser)		Y
24	HGSOC	B	*BRCA1*	exon 24 c.5521 del p.Ser1841Valfs[a]2	Y	
25	HGSOC	B	*FANCA*	exon 42 c.4225C > T p.Arg1409Trp		Y
		B	*RAD51C*	exon 4 c.664_681 del p.Gln222_Pro227del		Y
26	HGSOC	B	*BRCA1*	exon11 c.1016 del p.Lys339Argfs[a]2	Y	
27	MC	B	*FANCA*	exon 42 c.4225C > T p.Arg1409Trp		Y

[a] HGSOC: High-grade serous ovarian cancer; EC: Endometrioid carcinoma; MC: Mucinous carcinoma; P: Paraffin-embedded; B: Blood; Y:Yes.

3.4. Somatic mutations in BRCA genes

In 124 ovarian patients, we found 12 (9.68 %) cases harbored 23 *BRCA1/2* gene alterations, including 9 (39.13 %) *BRCA1* mutation and 14 (60.87 %) *BRCA2* mutation, 1 case harbored both g*BRCA1* and s*BRCA2* mutation. Of the 12 cases with s*BRCA1/2* mutations, HGSOC accounted for 58.33 % and LCOC had a proportion of 41.67 %. 73.91 % of the mutations were tier III (variants of unknown clinical significance), others were tier I (variants of strong clinical significance). Most of the variants were SNPs (Single Nucleotide Polymorphism, SNP), the others were introns. All of the somatic variants of 12 cases were summarized in Table 3. Clinical significance was identified according to the AMP/ASCO/CAP 2017 guidelines [22].

3.5. BRCA1/2 variants in high-grade serous ovarian cancer (HGSOC)

In our cohort, 92 (74.19 %) patients were diagnosed with HGSOC. 22 (23.91 %) were detected with g*BRCA1/2* mutations, including 15 (16.30 %) with g*BRCA1*, 6 (6.52 %) with g*BRCA2* and 1 (1.09 %) with both g*BRCA1*/2. 7 (7.61 %) cases were with s*BRCA1/2* mutations, including 4 (4.35 %) with s*BRCA1*, 2 (2.17 %) with s*BRCA2* and 1 (1.09 %) with both s*BRCA1* and 2. *TP53* mutation was found in 74 (80.43 %) patients and 23 (31.08 %) of them were combined with *BRCA1/2* mutations.

3.6. BRCA1/2 variants in less common ovarian cancer (LCOC)

In our study, we investigated the *BRCA1/2* variants in less common ovarian cancer, including low-grade serous ovarian cancer, clear cell carcinoma, mucinous carcinoma, dysgerminoma, Sertoli-Leydig cell tumor and undifferentiated carcinoma [23]. 32 out of 124 OC cases were identified with LCOC. *BRCA2* germline mutation was found in 1 case of endometrioid carcinoma, and 23 s*BRCA1/2* mutations were detected in 5 cases, including 2 cases of endometrioid carcinoma, 1 case of clear cell carcinoma, 1 case of mucinous carcinoma and 1 case of undifferentiated carcinoma. LCOC seemed to habor more somatic mutations than HGSOC and over half of the somatic mutations were variants of unknown clinical significance (17/23, 73.91 %). Detailed mutations were summarized in tables 2 and 3.

3.7. Genomic alterations and prognosis

We followed up the patients from 1 month to 98 months, and the median follow-up time was 14 months. 27 cases were found with recurrence or new distant metastases, 1 case was dead of disease, and the median DFS was 12.5 months. Survival analysis indicated that patients with HRR-related gene mutations ($P = 0.037$) or *TP53* mutations ($P = 0.048$) had better prognosis than HRR or *TP53* wild-type patients (Fig. 3). Univariate cox analysis showed that patient age ($P = 0.008$), tumor size ($P = 0.032$), pathological subtype ($P = 0.035$), HRR-related gene status ($P = 0.045$), lymphovascular space invasion (LVSI, $P = 0.020$) and postoperative targeted therapy ($P = 0.005$) were related with prognosis of ovarian cancer. In addition, multivariate cox analysis showed that HRR-related gene status ($P = 0.043$) and postoperative targeted therapy ($P = 0.020$) were independent prognosis factors of ovarian cancer (Table 4).

Table 3
Somatic mutations of *BRCA1/2* gene in ovarian cancer.

Case NO.	Histologic Tissue Variant type type				Tier
1	CCC	P	*BRCA2*	exon11 c.4189G > T p.Glu 1397[a]	I
			BRCA1	exon11 c.1740C > A p.F580L	III
			BRCA1	exon11 c.1576C > A p.Q526K	III
			BRCA2	exon10 c.953A > C p.K318T	III
			BRCA2	exon11 c.3069C > A p.N1023K	III
			BRCA2	exon11 c.3341T > G p.L1114R	III
			BRCA2	exon11 c.3800A > G p.D1267G	III
			BRCA2	exon11 c.4477G > A p.E1493K	III
			BRCA2	exon11 c.5705A > C p.D1902A	III
			BRCA2	exon11 c.6115T > G p.L2039V	III
			BRCA2	exon11 c.6772G > A p.E2258K	III
2	HGSOC	P	*BRCA1*	exon11 c.3756_3759del p.Ser1253Argfs[a]10 I	
3	EC	P	*BRCA2*	exon 24 c.9127G > T p.Glu 3043[a] I	
			BRCA1	intron 2 c.80 + 15G > T III	
4	EC	P	*BRCA1*	exon 17 c.5066T > C p.Met1689Thr III	
5	HGSOC	P	*BRCA1*	exon8 c.446A > C p.Glu149Ala III	
6	HGSOC	P	*BRCA1*	exon11 c.1687C > T p.Gln563Ter I	
7	HGSOC	P	*BRCA2*	intron 5 c.475+5G > C III	
8	MC	P	*BRCA2*	exon11 c.5683G > A p.Glu1895Lys (E1895K) III	
9	HGSOC	P	*BRCA2*	exon11 c.6591_6592del p.Glu2198AsnfsTer4 (E2198Nfs[a]4) I	
10	UC	P	*BRCA1*	exon 7 c.343C > A p.Pro115Thr (P115T) III	
11	HGSOC	P	*BRCA1*	exon11 c.2129 del p.Thr710IlefsTer26 (T710Ifs[a]26) I	
12	HGSOC	P	*BRCA2*	exon 3 c.266C > T p.Pro89Leu (P89L) III	

[a] HGSOC: High-grade serous ovarian cancer; EC: Endometrioid carcinoma; MC: Mucinous carcinoma; UC: Undifferentiated carcinoma; P: Paraffin-embedded.

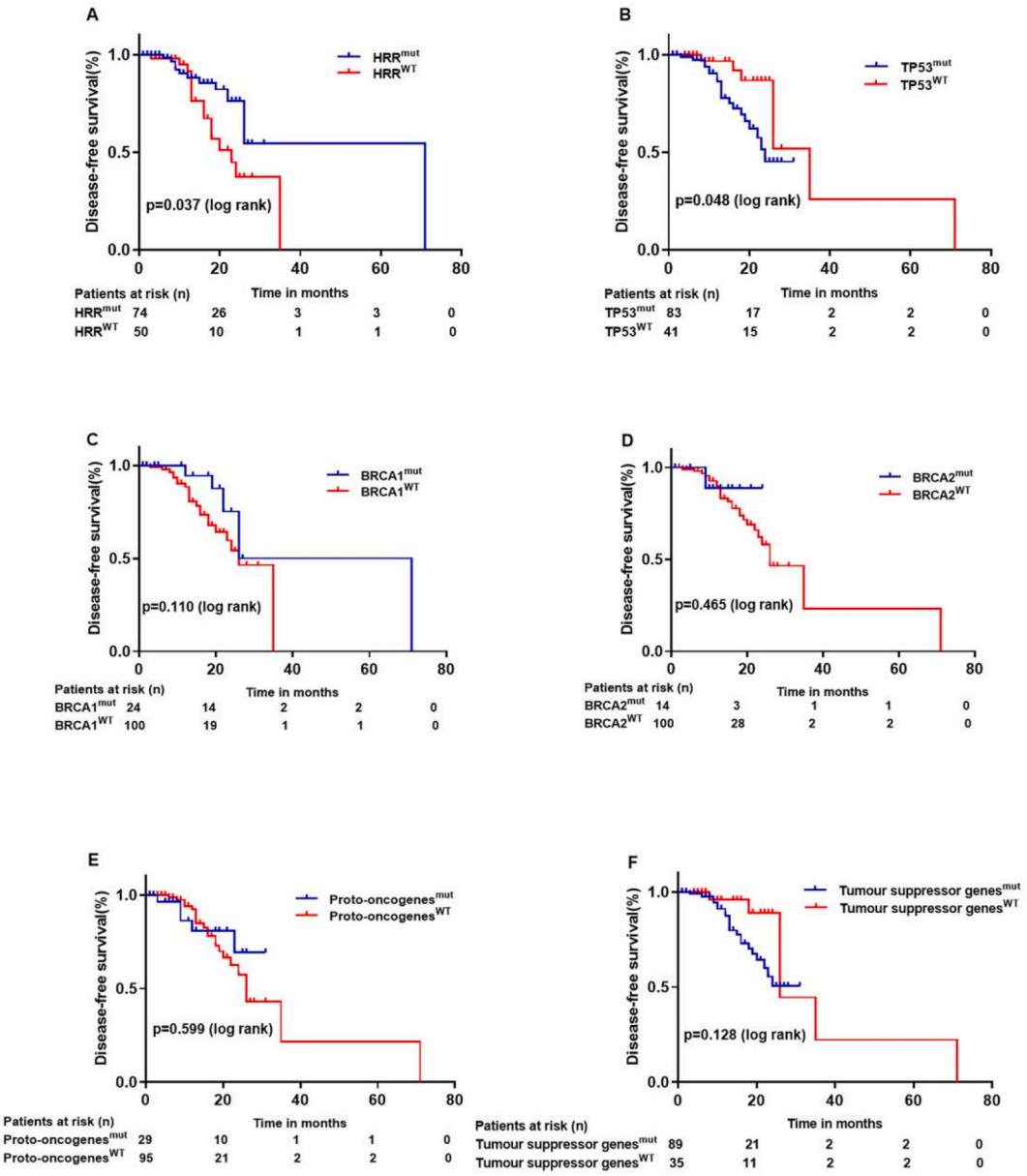

Fig. 3. Kaplan–Meier survival curves based on different gene mutations. (A) Patients with HRR-related gene mutation status had better DFS (P = 0.037); (B) Patients with TP53 gene mutation status had poor DFS (P = 0.048). (C–F) The gene status of (C) *BRCA1* (P = 0.110), (D) *BRCA2* (P = 0.465); (E) Proto-oncogenes (P = 0.599) and (F) Tumour suppressor genes (P = 0.128) had no statistical relationship with DFS. *P* values are calculated using the log-rank test.

Table 4
Cox regression analysis for the identification of factors associated with the prognosis of ovarian cancer.

	Univariate HR (95%CI)	Multivariate *P* value HR (95%CI) *P* value		
Age/years (25–79)	1.053 (1.013–1.094)	0.008	ns	ns
Tumor size/cm (0.5–22)	0.869 (0.765–0.988)	0.032	ns	ns
Pathological type (Serous vs other)	0.116 (0.016–0.856)	0.035	ns	Ns
Tumor size/cm (0.5–22)	0.869 (0.765–0.988)	0.032	ns	Ns
HRR gene status (m vs w)	2.230 (1.019–4.880)	0.045	2.979 (1.037–8.561)	0.043
LVSI (- vs +)	3.702 (1.230–11.136)	0.020	ns 4.085	ns
Postoperative targeted therapy (- vs +)	2.406 (1.306–4.433)	0.005	0.020	(1.249–13.361)

* LVSI, Lymphovascular space invasion; m, mutant; w, wildtype.

4. Discussion

Ovarian cancer is composed of several malignant tumors and has been a deadly gynecologic tumor for decades. 70 % patients were in advanced stage at first diagnosed [24] and about 70–80 % advanced cases were found relapsed in 5 years and developed platinum resistance, which indicated worse prognosis and higher mortality [25]. Earlier diagnosis and more precise targeted treatment can help patients with ovarian cancer to achieve longer survival. In recent years, several PARP inhibitors such as Olaparib were proved to be effective in recurrence ovarian cancer and have been approved by FDA for multiple indications, especially for those with *BRCA* gene mutations or homologous recombination deficiency (HRD) [26–28]. Therefore, identifying the genomic landscape of ovarian cancer patients, notably HRR-related genes such as *BRCA1/2*, is extremely critical for the selection of treatment and targeted drug.

In this retrospective study, we collected a cohort that were consist of 124 ovarian cancer patients with several histologic subtypes, grouped them into HRR-mutant and HRR-wildtype. Analysis of the correlation between HRR gene status and clinicopathological characteristics including age, tumor size, FIGO stage, treatments and so on, showed no statistical significance. Next, we analyzed the variants in HRR-related genes in our cohort, about 59.68 % patients were found with multiple types of mutations. Excluding the high mutation frequency in *BRCA1/2*, we also found several non-*BRCA* gene alterations, including *FANCA* (11.29 %), *ATM* (7.26 %), *ATR* (5.65 %), *CDK12* (5.65 %), *MRE11* (4.84 %), *RAD51D* (4.84 %), *BARD1* (3.23 %), *RAD54L* (3.23 %), *RAD51C* (3.23 %), *BRIP1* (2.42 %), *CHEK2* (2.42 %), *PALB2* (1.61 %), *FANCL* (1.61 %), *PPP2R2A* (1.61 %), *CHEK1* (0.81 %) and *RAD51B* (0.81 %), the frequencies of these gene variants were varied from previous studies [29]. Although current treatment options were mostly focused on *BRCA* gene status, studies showed that non-*BRCA1/2* specific HRR gene variants might represent a large number of HRD tumors, as well as a great number of patients that might benefit from targeted treatment [12,30]. Our study indicated that over half of the Chinese patients might be the potential beneficiary from targeted therapy such as PARP inhibitors.

BRCA1/2 gene status is critical for the selection of maintenance therapy in stage II-IV ovarian cancer after primary chemotherapy. Moreover, figuring out the alterations of *BRCA1/2*, especially germline mutation, is an effective way to identify the inherited risk of cancer and take preventive measures. Previous studies showed that about 8.6–24.5 % patients harbored g*BRCA1/2* mutations, while 3.7–7.1 % patients presented s*BRCA1/2* mutations [10,31–35]. Our study excluded the benign mutations and found the frequencies of g/s *BRCA1/2* mutation were 18.55 % and 9.68 %. All the g*BRCA1* mutations were P/LP, while 2 out of 8 g*BRCA2* mutations were VUS, these results might be related to our small sample size, studies with a large number of samples are needed.

One interesting finding in our study was that s*BRCA1/2* presented much higher frequency in LCOC patients than in HGSOC (41.67 % VS 4.35 %), and a majority (73.91 %) of these mutations were tier III (variants of unknown clinical significance). It is well known that the different histologic subtypes of OC had diversified clinical manifestation, outcomes, and responses to treatments, which mainly due to the diverse intrinsic tumor biology. Currently, whether to conduct comprehensive molecular testing for ovarian cancer remains controversial, but our results indicated that more complete molecular analysis might be particularly important for the uncommon histologic subtypes that had limited approved treatment options. Besides, those tier III mutations offer great possibilities for application of target therapies that might be useful.

With the widely application of PARPi maintenance therapy, treatment of ovarian cancer has gradually developed into a "surgery + chemotherapy + maintenance treatment" standard model. PARPi plays its anti-tumor role through the synthetic lethality effect, several clinical trails had proved that OC patients with g*BRCA1/2* mutation had received significant PFS benefit from PARPi maintenance therpay [36–39]. To date, the FDA has approved three PARPi drugs for the maintenance treatment of ovarian cancer, which are Olaparib, Rucaparib and Niraparib [40–42]. Furthermore, germline or somatic mutation in HRR-related genes is usually associated with better prognosis [43]. Beyond PARPi, the monotherpay or combined application of angiogenesis drug Bevacizumab, platinum-based chemotherapty, as well as Pembrolizumab in certain circumstances are also recommended by NCCN [17].

Here in our study, we investigated the correlations between multiple clinical features and prognosis, including the status of HRR-related gene, proto-oncogene, age, tumor size, and the application of postoperative targeted therapy. The postoperative targeted therapy in our study refers to the simultaneous or metachronous use of antiangiogenic therapy, PARPi therapy, and Anlotinib during the patient's care. Univariate cox analysis showed that age, tumor size, histologic type, HRR-related gene status, LVSI and postoperative targeted therapy were associated with OC patient prognosis, while multivariate cox analysis showed that HRR-related gene status and postoperative targeted therapy were independent prognosis factors of ovarian cancer. Survival analysis represented the same conclusion. Our results indicated that the evaluation of HRR-related genes showed clinical significance in the prognosis of ovarian cancer, consistent with previous conclusions [43,44].

There were several limitations to our study: (1) a single medical center study; (2) a small size of samples; (3) limited analysis of germline variants in non-*BRCA1/2* HRR genes; (4) a relatively short following-up period and (5) no explicit classification by the therapeutic regimen of patient.

5. Conclusions

This study reported the accurate incidence of HRR-related genes, particularly *BRCA1/2* in eastern China patients and found that *BRCA1/2* somatic mutation showed a higher incidence in LCOC than HGSOC, HRR-related gene mutation status was significantly associated with the prognosis of ovarian cancer patients. This comprehensive analyze of HRR gene study provides a valuable reference for clinicians to select appropriate treatment plan for OC patient, as well as a better understanding of this malignant tumor.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the affliated hospital of Qingdao University (Project identification code: QYFY WZLL 27873).

Informed consent statement

All patients provided informed consent for inclusion before they participated in the study.

Data availability statement

The data presented in this study are available on request from the corresponding author.

CRediT authorship contribution statement

Yaolin Song: Writing - original draft, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Wenwen Ran:** Methodology. **Huiqing Jia:** Formal analysis. **Qin Yao:** Formal analysis. **Guangqi Li:** Methodology. **Yang Chen:** Formal analysis. **Xiaonan Wang:** Methodology. **Yujing Xiao:** Methodology. **Mengqi Sun:** Formal analysis. **Xiao Lu:** Formal analysis. **Xiaoming Xing:** Writing - review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The manuscript titled “Next-Generation Sequencing-based Analysis of Homologous Recombination Repair Gene Variant in Ovarian Cancer” has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. We have read and understood your journal’s policies, and we believe that neither the manuscript nor the study violates any of these. All authors declare no conflicts of interest.

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