Somatic Mutations in BRCA2 BRC Repeat Associated with Outcome in Patients with High Grade Serous Ovarian Cancer

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Research

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Abstract

Background

The interaction between BRCA2 BRC repeats and RAD51 is one of the great important factors affecting the homologous recombination in *DNA* damage *repair* of tumor cells. We investigated the effect of *BRCA2* BRC repeat mutations on outcome in patients with high grade serous ovarian cancer (HGSOC) who received platinum-based chemotherapy.

Methods

We identified the type and location of *BRCA2* BRC repeat mutations by PCR and DNA sequencing in tumor and peripheral blood leukocytes (PBL) samples of 113 patients with stage IIIC/IV high grade serous ovarian cancer (HGSOC), and assessed chemotherapy-free interval (CFI), progression-free survival (PFS) and overall survival (OS).

Results

24 (21.23%) cases with somatic mutation were identified in 113 HGSOC patients. Among them, 8 (7.1%) cases with nonsense mutation resulting in BRCA2 truncation significantly prolonged median CFI (37 *vs* 8 months,*P*=0.000), PFS (43 *vs* 14 months, *p*=0.000) and OS (56 *vs* 31 months, *P*=0.002); Interestingly, 16 (14.13%) cases with missense mutation also prolonged median CFI (15 *vs* 8 months, *P*=0.044), PFS (21 *vs* 14 months, *P*=0.049) and OS (38 *vs* 31 months, *P*=0.037).

Conclusions

Somatic mutations in *BRCA2* BRC5-8 repeat motifs are associated with platinum-based chemotherapy sensitivity and a better outcome in patients with HGSOC.

Background

High-grade serous ovarian cancer (HGSOC) is the most common histological subtype (about 70%) of epithelial ovarian cancer (EOC) [1,2] and is a genetically heterogeneous disease that exhibit highly individual evolution and genomic diversity [3-5]. Approximately 50% of HGSOCs harbour genetic and epigenetic alterations in gene members of the homologous recombination (HR) DNA repair pathway, most commonly in *BRCA1* and *BRCA2* [6]. For a woman with a *BRCA2* mutation, the risk of EOC is 12-20% [7]. Several studies of multidimensional genomics and clinical data have revealed that *BRCA2* mutations are associated with beneficial survival and platinum-based chemotherapy sensitivity in patients with HGSOC[8-10]. However, analysis of two independent cohorts indicated that only HGSOC patients whose germline or somatic mutations of *BRCA2* occur most commonly in exon 11 which codes functionally distinct BRC repeat motifs for RAD51 binding domain (RAD51-BD) prolong platinum-free interval and have better survival[11,12]. These findings suggest that the location and type of *BRCA2* BRC repeat mutations may be associated with highly sensitive to platinum-based therapy in EOC patients.

BRCA2 plays crucial role in regulating the actions of RAD51, a recombinase essential for homology-directed repair of DNA double strand breaks (DSBs) [13-16]. The direct interaction between BRCA2 and RAD51 and their colocalization in nuclear foci after DNA damage was the first evidence for a role for BRCA2 within this DNA repair pathway. BRCA2 is directly involved in RAD51-mediated repair, affecting the choice between gene conversion (GC) and single-strand annealing (SSA). BRCA2 BRC repeats bind RAD51 and are essential for the function of both proteins. BRCA2 binds directly with RAD51 and delivers RAD51 to DNA DSBs through the eight conserved BRC repeats[13,17-19] and promotes RAD51 specific recruitment to DNA damage sites where homologous recombination (HR) process is initiated for cellular response to genotoxic agents by mediating DNA strand exchange during recombination[17,20]. BRCA2 BRC repeats are be provided with functionally distinct effects on RAD51 function. These repeats have been shown to bind distinct regions of RAD51, confirming nonequivalent interactions between the different BRC repeats and RAD51. BRCA2 BRC repeats and their intervening sequences mediate HR repair of DNA damage by two functionally different RAD51-BD *binding modules,* BRC1~4 and BRC5~8 repeat domains. Distinct binding of BRCA2 BRC repeat domains to RAD51 modulates DNA-binding selectivity and confers differential DNA-damage sensitivity [19,21,22]. It is proved that

RAD51-mediated HR repair of DNA damage is dependent on the modular architecture BRC repeats of BRCA2[23-25].

Almost all HGSOC patients in a clinical setting receive platinum-based chemotherapy, inducing inter-strand adducts, and then resulting in DSBs in DNA. In response to DNA DSBs, the interaction between BRCA2 BRC repeat motifs and RAD51 is of great importance in creating a BRC-RAD51 complex for HR repair. Defective binding at a single BRC repeat or a single point mutation within an individual BRC repeat domain can be enough to impair this interaction [14, 26]. Therefore, *BRCA2* BRC repeat mutations may affect platinum sensitivity.

In the present study, we aimed to determine the effect of *BRCA2* BRC repeat mutations on platinum-free interval (PFI), progression-free survival (PFS) and overall survival (OS) in patients with stage IIIc/IV HGSOC. Here we provide a clinical implication that the location and type of *BRCA2* BRC repeat mutations may disrupt BRCA2 function and be associated with platinum-based chemotherapy sensitivity in HGSOC.

Materials And Methods

Ethics statement

The current study was approved by the Ethics Committee of the Institutional Review Board of Sichuan Cancer Hospital and Institute performed in strict accordance with the *Declaration of Helsinki*. All participants signed informed consent prior to enrollment.

Study subjects

Tumor tissue and peripheral blood leukocytes (PBL) were obtained from 113 HGSOC patients who received primary debulking surgery (PDS) followed by a 6 cycles of platinum-based chemotherapy between March 2015 and December 2017. The tumor specimens and PBL were immediately flash frozen in liquid nitrogen and stored at −80°C in the bio-bank of Oncology Research Laboratory at our institute until extraction of genomic DNA was performed. The amplicons from the obtained genomic DNA were used to identify if mutations in the type and location of *BRCA2* BRC repeat by PCR sequencing. All patients enrolled in this study were histopathologically confirmed as HGSOC with FIGO stage III/IV and showed no family history of ovarian cancer. Clinicopathologic and follow-up data of patients were collected by our Hospital *Patient Information* Reporting System.

DNA Extraction

Genomic DNA with a size of greater than 5Kb was extracted from tumor samples and PBL using TIANamp Genomic DNA Kit (DP304, TIANGEN, China.) according to the manufacturer's protocol. Ethanol precipitated genomic DNA samples were resuspended in sterile distilled water and frozen at -80°C until further use.

Fractional amplification of *BRCA2* BRC repeat

Fractional amplification of the BRC1~8 repeat region spanning c.4549-c.6015 was performed by PCR. All primers (Supplementary Table 1) were designed using Primer Express 5.0 software (Applied Biosystems) according to human *BRCA2* exon 11 coding sequences obtained from GenBank (U43746.1, Accession Number: NM_000059) and synthesized by TsingKe Biotech Co. Ltd. Beijing, China. The thermal cycling procedure for PCR was performed as described in Supplementary Table 2.

DNA sequencing and sequence alignment

PCR amplified fragments (Fig 1 C) were bi-directionally sequenced using 5 primer pairs (Supplementary Table 1) with ABI-3730XL Sequencer. All the nucleotide changes identified were confirmed by repeating the PCR and sequencing reaction using the corresponding forward and reverse primers and by aligning with a homologous sequence of human *BRCA2* exon 11 from NCBI sequence database using BLAST (www.ncbi.nlm.nih.gov/Blast.cgi).

Detection of the BRCA2 N- and C-terminus by immunohistochemistry

Immunostaining for BRCA2 N-terminus (aa.188-563) and C-terminus (aa.3245-3418) using Anti-BRCA2 antibody ([3E6], ab97, Abcam) and anti-BRCA2 Ab-2 (Clone: CA1033, Millipore) was performed as described in the manufacturer's protocol. Briefly, 4 μm thick sections were cut from the paraffin-embedded tumor biopsies. These sections were mounted on amino-propyl-ethoxy-silan (APES) coated glass slides. Sections were deparaffinized in xylene and rehydrated in ethanol. Endogenous peroxidase was blocked by incubation with 0.3% hydrogen peroxidase for 30 minutes. The area around the tissue sections was scored with a Pap pen to limit the amount of antibodies and reagents used. Staining was visualized by

3’3-diaminobenzidine tetrahydrochloride and counterstaining was performed with hematoxylin. PBS without the primary antibody served as negative control.

Outcome evaluation

The primary end-point was progression-free survival (PFS). Secondary endpoints were chemotherapy-free interval (CFI) and overall survival (OS). Date of first relapse was defined as the first instance of disease progression based on computed tomography imaging by Response Evaluation Criteria In Solid Tumors (RECIST ,version 1.1) or clinical progression [27]. Chemotherapy-free interval (CFI) was defined as the interval between the time of completion of platinum-based chemotherapy and the date of first progression/relapse or death; PFS was defined as the interval between histologic diagnosis and first relapse, death or the last follow-up (censored); OS was defined as the interval between histologic diagnosis and the date of death from any cause or last follow-up (censored). CFI of <6, 6-12 and ≥12 months was classified as platinum-resistant, partially platinum-sensitive and platinum-sensitive, respectively by Gynecologic Oncology Group (GOG)[28].

Statistical Analysis

Standard statistical tests were used to analyze the clinical and mutation data, including the chi-square test, Fisher’s exact test, log-rank test, and Cox proportional hazard analysis. Significance was defined as two-sided *P* value less than 0.05. All statistical analyses were performed using SPSS software (version 18.0).

Results

Genomic DNA and amplified fragments are qualified for DNA *sequencing*

The genomic DNA samples with a size of >5Kb (Fig 1B)isolated from all HGSOC tissues and PBL were fractionally amplified by PCR. First-*round* PCR amplicons of *BRCA2* exon 11 (c.2803-c.6728) (Fig 1A) were amplified from genomic DNA using the 8 pairs of primers; the *second-round* PCR amplicons covering BRCA2 BRC1~8 repeats and their spacing sequences (c.2915-c.6534) were amplified from the first-*round* PCR amplicons using the other 8 pairs of specific primers and available for DNA sequencing (Fig 1B and C).

The mutation sites in *BRCA2* BRC repeat

No mutation in *BRCA2* BRC repeat was identified in PBL of all patients with HGSOC. 44 different nucleotide mutation sites in *BRCA2* BRC repeat were identified in tumor tissues of 27 HGSOC patients, inclunding 10 of them were silent mutation, 26 missense mutation and 8 nonsense mutation. Most of them were situated in the spacing sequences between the evolutionary conserved BRC domains. Only 2 (4.5%) missense mutations (c.5076G>A and c.5587A>T) and 2 (4.5%) nonsense mutations (c.5038T>C and c.5608T>C) were located within BRC5 and BRC6 domain, respectively, others (91%) were occurred in

the spacing sequences between the BRC4~8 repeat domains(Fig.2A and Table 1). These results showed that all the patients identified were somatic but not germline mutations.

Type and frequencyof *BRCA2* BRC repeat mutations

Among 113 patients with HGSOC, silent mutations were identified in 66 (58.4%) cases (c.3623A>G in BRC1~2), 47 (41.6%) cases (c.4034T>C in BRC2~3), 2 (17.7%) cases (c.5430T>C and c.5133A>G in BRC5~6), 1 (0.9%) case (c.4953T>C in BRC4~5), 1 (0.9%) case (c.5562T>C in BRC6) and 4 (3.5%) cases (c.6363A>G, c.6513G>C, and c.6462T>C in BRC8~); A relatively high ratio (21.2%, 24/113) of missense and nonsense mutations was found in the BRC4~8 repeats but not in the BRC1~3 repeats. 8 (7.1%) cases with nonsense mutation and 16(14.2%) cases with missense mutation were identified in 113 cases, respectively (Fig. 2B); Among the 24 cases with these mutations, 11 (45.8%)cases were multi-site mutations and 13 (54.2%) cases were single-site mutations (Table 1). Accordingly, the cases having a single nonsense or missense mutation were respectively included in analyses comparing with those without mutation for assessment of clinical outcome.

Nonsense mutation leads to C-terminal truncation of BRCA2 protein

Immunohistochemistry results showed that all patients were positive immunostaining in the N-terminus of BRCA2 protein, but 8 cases with nonsense/frameshift mutation were negative immunostaining in the C-terminus (Fig. 2C), proving that nonsense/frameshift mutation leads to C-terminal truncation of BRCA2 protein.

BRCA2 BRC repeat mutations prolong CFI

HGSOC patients with nonsense or missense in *BRCA2* BRC repeat (21.2%, 24/113) had significantly longer CFI and higher sensitivity to platinum-based chemotherapy than non-mutated ones. As shown in Fig. 3A, The median CFI for nonsense (7.1%) and missense (14.13%) mutation were 37 months (95%CI,30.56-43.44 months) and 15 months (95%CI, 12.294-23.972 months), respectively, compared to 8 months (95%CI, 4.657-11.343) for non-mutation (P=0.000 and P=0.044).

BRCA2 BRC repeat mutations contribute to better survival

The median PFS for nonsense and missense mutations were 43 months (95%CI, 36.74-49.26 months) and 21 months (95% CI, 17.573-29.818 months), respectively, compared to 14 months (95%CI,11.55-16.45 months) for non-mutation (P=0.000 and P=0.049) (Fig. 3B). The median OS for nonsense and missense mutation were 56 months (95%CI,38.36-73.64 months) and 38 months (95%CI,33.29-42.71 months), respectively, compared to 31 months (95%CI,28.72-33.28 months) for non-mutation (P=0.002 and P=0.037)(Fig. 3C). The patients with nonsense and missense mutation exhibited significantly higher PFS and OS compared to those with non-mutation. In multivariate analysis, *BRCA2* nonsense mutation is an independent factor for longer PFS (HR=0.079, 95%CI, 0.023-0.266, P<0.001) and OS (HR=0.121, 95%CI, 0.029-0.497, P=0.003), and *BRCA2* missense mutation is an independent factor only for longer PFS (HR=0.429, 95%CI, 0.206-0.892, P=0.023) (Fig. 3B and C).

Discussion

Up to 50% of patients with HGSOC are described as having identifiable defects in the HR pathway, with the archetypal defects being germline or somatic *BRCA2* inactivation that results in a distinct clinical phenotype comprising hypersensitivity to platinum and prolonged survival**[2,7,9,29]**. DNA DSBs are more problematic than SSBs since the complementary strand is not available as a template for repair. DSBs may arise as a result of either exogenous insults, such as exposure to ionizing radiation (IR) and platinums. Three DSB repair pathways have been identified within eukaryotic cells: nonhomologous end-joining (NHEJ), GC and SSA. Both GC and SSA rely on sequence homology for repair while NHEJ utilizes no, or little, homology. BRCA2 is the major recombination mediator and regulator in mammalian cells and involved in HR repair of DNA DSBs and maintaining *genome stability****[30,31]***. BRCA2 can interact with RAD51 through multiple sites of BRCA2 and control recombination and/or of genomic integrity through binding to RAD51. Cells deficient for functional BRCA2 show various cellular abnormalities including increased sensitivity to genotoxic agents, accumulation of DNA damage, changes in cell cycle checkpoint and apoptotic responses[*32,33*]. Surprisingly, not all BRCA2 mutations are highly sensitive to DNA damage agents. Location and type of mutation in *BRCA2* has been shown to be associated with inactivate BRCA2 [11,12]. Mutations in *BRCA2* at other locations (either exons 1~10 or exons 12~27) than the BRC repeats (exon 11) do not impact the outcome in patients with HGSOC compared to those with non-mutation, suggesting that only germline or somatic mutations in the BRC repeats or RAD51-BD but not other domains are highly sensitive to DNA damage agents such as platinums.

Interaction between BRCA2 and RAD51 mediated by BRC repeats is critical for the cellular response to DNA damage[34]. The *BRCA2* encodes the eight conserved BRC repeats (residues 1009~2082) with approximately 35 amino acids that are located well spaced from one another amidst the long and divergent exon 11 region, and the spacing sequences between individual repeats varies from 60 to 300 amino acids [21,25]. The role of the BRC repeats in DNA repair has been well characterized in the induction of ionizing radiation- or platinum-induced assembly of RAD51 complex, which is independent of the BRCA2 C-terminal domain [20,35].

Although the BRC repeats are highly conserved between mammalian species, the individual repeats differ greatly from one another within a species [18],suggesting a specific role for each BRC in RAD51 binding. In fact, the BRC repeats has been shown to work in two classes of regulatory elements (BRC1~4 and BRC5~8) that, via distinct mechanisms, display unique functional characteristics to ultimately facilitate loading of RAD51 onto sites of DNA damage. BRCA2 binds to monomeric RAD51 via its BRC repeats and the BRC repeats and isolated domains of BRCA2 contribute to RAD51 binding revealed that two distinct clusters of residues in the BRC repeats can differentially regulate DNA-binding selectivity and sensitivity of RAD51 in targeting active RAD51 to single-stranded DNA and prohibiting RAD51 nucleation onto double- stranded DNA (dsDNA)*[19,24,25]***.** The BRC1~4 repeats share the common property of inhibiting

the binding of RAD51 to dsDNA, the BRC5~8 repeat domain can efficiently repair nuclease induced DNA DSBs and accelerate the assembly of RAD51 repair complexes upon DNA damage*[23-25].*

While the BRC repeats are themselves well conserved, the intervening sequences between consecutive BRC repeats are remarkably poorly preserved, suggesting that the intervening sequences are indispensable for preservation of the functional structure of BCR repeats[25,36] and that disruption of a single RAD51 interaction site, one of several simultaneous interactions occurring throughout the BRC repeats of BRCA2, might modulate the ability of RAD51 to promote recombinational repair[37]. Therefore, mutation at sites crucial for the interaction between BRC repeats and RAD51 disrupts the BRCA2-RAD51 complex formation and impair the ability of BRCA2 to recruit RAD51 to DNA DSBs. It is striking that the principal role of BRCA2 in HR is dependent on its interaction with RAD51 through the BRC repeats and yet, no deleterious missense mutations have been located in that region. One reason for this might be that the other BRC repeats can compensate for the mutated one. However, this explanation is at odds with the prediction from the structural analysis of mutations affecting this region by which the BRC repeats would form a Velcro-strip like structure where the mutation of one BRC repeat would affect the interaction of the other BRC repeats with RAD51[14]. Consistent with the role of the BRC repeats in contacting RAD51, several mutations affecting the structure of the BRC repeats have shown that the weakening of RAD51 affinity in the case of even one repeat is sufficient to affect BRCA2-RAD51 complex-mediated HR repair of DNA damage[21]. This provides insight into why mutation in just one of the BRC repeats of *BRCA2* affects the way that RAD51-mediated HR of DNA damage.

Nonsense or missense mutation in *BRCA2* BRC repeats, which results in an impaired function of BRCA2 protein, disrupts a RAD51-binding domain-mediated HR of DNA damage, then increasing the sensitivity to DNA crosslinking agents such as platinums.

In this study, we showed a relatively high ratio (21.23%) of *BRCA2BRC* repeat mutations in patients with HGSOC, including 7.1% nonsense mutation and 14.13% missenes mutation (Fig 2B and Table 1), this may be involved in mutation predisposition to stage IIIc/IV HGSOC that exhibits highly individual evolutionary trajectories prior to therapy[38-40]. Interestingly, our results showed that missense mutation in *BRCA2BRC* repeats significantly prolonged CFI, PFS and OS in patients with HGSOC compared to those with non-mutation (Fig 3). Surprisingly, 91% of the mutation sites identified in the BCR repeat region occurred in the intervening sequences between consecutive BRC4~8 repeat, proving that the BRC repeats are highly conserved across while most of the intervening sequences are not, suggesting that the BRC repeats are important for BRCA2 function mutation in the poorly-

conserved intervening sequences contributes to inactivation of BRCA2 and disrupts the interaction of the BRC repeat domain with RAD51 for *repair* of DNA *DSBs*[24,25,35,41]. This can be explained by the absence of analysis of BRCA2 based on the two modules of BRC repeats that display unique functional characteristics of BRCA2. The BRC5~8 repeat domain of BRCA2 is responsible for repair of DNA DSBs induced by inter-strand crosslinks. DNA DSBs caused by platinum can be deemed as acting as a “targeted chemotherapy” in *BRCA2* mutated HGSOC. Therefore, *BRCA2* BRC repeat mutations can be

used to identify HGSOC cells with an impaired function of BRCA2 protein that confers higher sensitivity to platinum, thus extending platinum-free interval that is a strong predictor of survival and relates to the response to subsequent platinum treatment in HGSOC. Additionally, the hyperactivation of PARP-1 due to the functional defects of BRCA2 protein contributes to an effective maintenance therapy of PARP inhibitors for patients with HGSOC[42].

Conclusion

In summary, our study demonstrates that somatic mutations in *BRCA2* BRC5-8 repeat motifs could impact on BRCA2 function for DNA damage repair and confer a higher sensitivity to platinum-based therapy and are associated with a favourable outcome in patients with HGSOC.

Our findings highlight the importance of location and type of somatic mutations in *BRCA2* BRC repeat in the context of HGSOC.

List Of Abbreviations

Abbreviation	Definition
BRCA1	breast cancer susceptibility gene 1
BRCA2	breast cancer susceptibility gene 2
BRC	BRC repeat
HGSOC	high grade serous ovarian cancer
PBL	peripheral blood leukocytes
CFI	chemotherapy-free interval
PFS	progression-free survival
OS	overall survival
EOC	epithelial ovarian cancer
HR	homologous recombination
RAD51-BD	RAD51 binding domain
DSBs	DNA double strand breaks
GC	gene conversion
SSA	single-strand annealing
PFI	platinum-free interval
PDS	primary debulking surgery
APES	amino-propyl-ethoxy-silan
GOG	Gynecologic Oncology Group
SSBs	Single-stranded breaks
IR	ionizing radiation
NHEJ	nonhomologous end-joining
dsDNA	double-stranded DNA

Declarations

Author's Contributions: Profs. GN Zhang and JM Huang had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design*: Profs. GN Zhang and JM Huang. *Acquisition of clinical data*: Drs. J Zhang, Y Zhu, H Liu and Y Shi. *Quality control of data and algorithms*: Profs. K Mi, MY Li. *Experimentalwork*: J Zhang and Q

Zhao. *Statistical and bioinformatical analysis*: Dr.Y Zhu and ZY Huang. *Drafting of the manuscript*: J Zhang. *Approval of the manuscript*: GN Zhang and JM Huang.

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Ethics approval and consent to participate

The current study was approved by the Ethics Committee the Institutional Review Board of Sichuan Cancer Hospital and Institute performed in strict accordance with the *Declaration of Helsinki*. All participants signed informed consent prior to enrollment.

Competing interests

The author declares no competing interest exists.

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Tables

Table 1. *BRCA2* BCR Repeat Mutations in 27 Patients with HGSOC

Patient No.	Age *yr*	FIGO stage	Nucleotide change	Amino acid variation	Type of mutation	BRC repeat
1	58	IIIc	c.5155A>G TAA>TGA	p.N1719D Asn>Asp	M	B5-6
2	60	IIIc	c.6363A>G GAA>GAG	p.E2121E Gly>Gly	S	B8~
			c.4976C>G TCC>TGC	p.S1659C Ser>Cys	M	B4-5
3	59	IV	c.4906A>T AAA>TAA	p.K1635* Lys>Stop	N	B4-5
			c.4975T>C GTC>TCC	p.S1659P Ser>Pro	M	B4-5
4	60	IIIc	c.5587A>T GAA>GTA	p.K1862* Lys>Stop	N	B6
			c.5398T>C TAC>CAC	p.Y1800H Tyr>His	M	B5-6
			c.5562T>C GTT>GTC	p.V1855V Val>Val	S	B6
5	47	IIIc	c.5608T>C TTC>CTC	p.F1870L Phe>Leu	M	B6
			c.6322C>A CGT>AGT	p.H2108S His>Ser	M	B8~
6	48	IIIc	c.5292delA TCA>TCA^{del}	p.L1776fs >Stop	F	B5-6
7	41	IIIc	c.4951C>T CCT>TCT	p.P1651S Pro>Ser	M	B4-5
			c.5021G>A AGT>AAT	p.S1647N Ser>Asn	M	B4-5
			c.6092C>T ACT>ATT	p.T2031I Thr>Iie	M	B7-8
8	42	IIIc	c.6124C>T CAA>TAA	p.Q2401* Gln>Stop	N	B7-8
			c.6199T>C TCC>CCC	p.S2067P Ser>Pro	M	B-8
			c.6428C>T TCA>TTA	p.S2143L Ser>Leu	M	B8~
9	60	IIIc	c.6070C>T CAG>TAC	p.Q2024* Gln>Stop	N	B7-8
10	62	IIIc	c.6462T>C TAT>TAC	p.Y2154Y Tyr>Tyr	S	B8~
			c.4931A>G GAA>GGA	p.E1644G Glu>Gly	M	B4-5
11	48	IIIc	c.6100C>T CGT>TGT	p.R2034C Arg>Cys	M	B7-8
12	47	IIIc	c.6298C>T CAA>TAA	p.Q2100* Gln>Stop	N	B8~
13	49	IIIc	c.5404C>T CAA>TAA	p.Q1801* Gln>Stop	N	B5-6
			c.5430T>C GTT>GTC	p.V1810V Val>Val	S	B5-6
			c.6513G>C TGT>TCT	p.V2171V Val>Val	S	B8~
			c.6319C>A CCT>ACT	p.P2107T Pro>Thr	M	B8~
14	37	IIIc	c.5443A>G ACT>GCT	p.T1815A Thr>Ala	M	B5-6

15	42	IIIc	c.5076G>A TGG>TGA	p.W1692* Trp>Stop	N	B5
16	44	IV	c.6286C>T CCT>TCT	p.P2096S Pro>Ser	M	B8~
			c.5440G>A GTG>AGT	p.V1814M Val>Met	M	B5-6
17	69	IIIc	c.5354C>T ACT>ATT	p.T1785I Thr>Ile	M	B5-6
18	56	IIIc	c.4915G>A GTA>ATA	p.V1639I Val>Ile	M	B4-5
19	42	IV	c.6326T>C GTT>GCT	p.V2109A Val>Ala	M	B8~
			c.5038T>C TCT>CCT	p.S1680P Ser>Pro	M	B5
20	60	IIIc	c.5291C>T TCA>TTA	p.S1764L Ser>Leu	M	B5-6
21	62	IIIc	c.6376T>C TGC>CGC	p.C2126R Cys>Arg	M	B8~
22	42	IIIc	c.5443A>G ACT>GCT	p.T1815A Thr>Ala	M	B5-6
23	47	IIIc	c.5133A>G GTA>GTG	p.V1711V Val>Val	S	B5-6
			c.5501G>A AGT>AAT	p.S1834N Ser>Thr	M	B5-6
24	54	IIIc	c.4953T>C CCT>CCC	p.P1651P Pro>Pro	S	B4-5
25	57	IV	c.5479A>T ATT>TTT	p.I1823F Ile>Phe	M	B5-6
26	62	IIIc	c.6363A>G GAA>GAG c.6462T>C TAT>TAC	p.T2154T Tyr>Tyr	S	B8~
27	54	IIIc		p.E2121E Glu>Glu p.Q2121Q Gln>Gln	S S	B8~ B8~

Pateint No.: patient number; yr: year; M: missense; F: frameshift; N :nonsense; S: silent; *: stop codon mutation; HGSOC: high-grade serous epithelial ovarian cancer

Figures

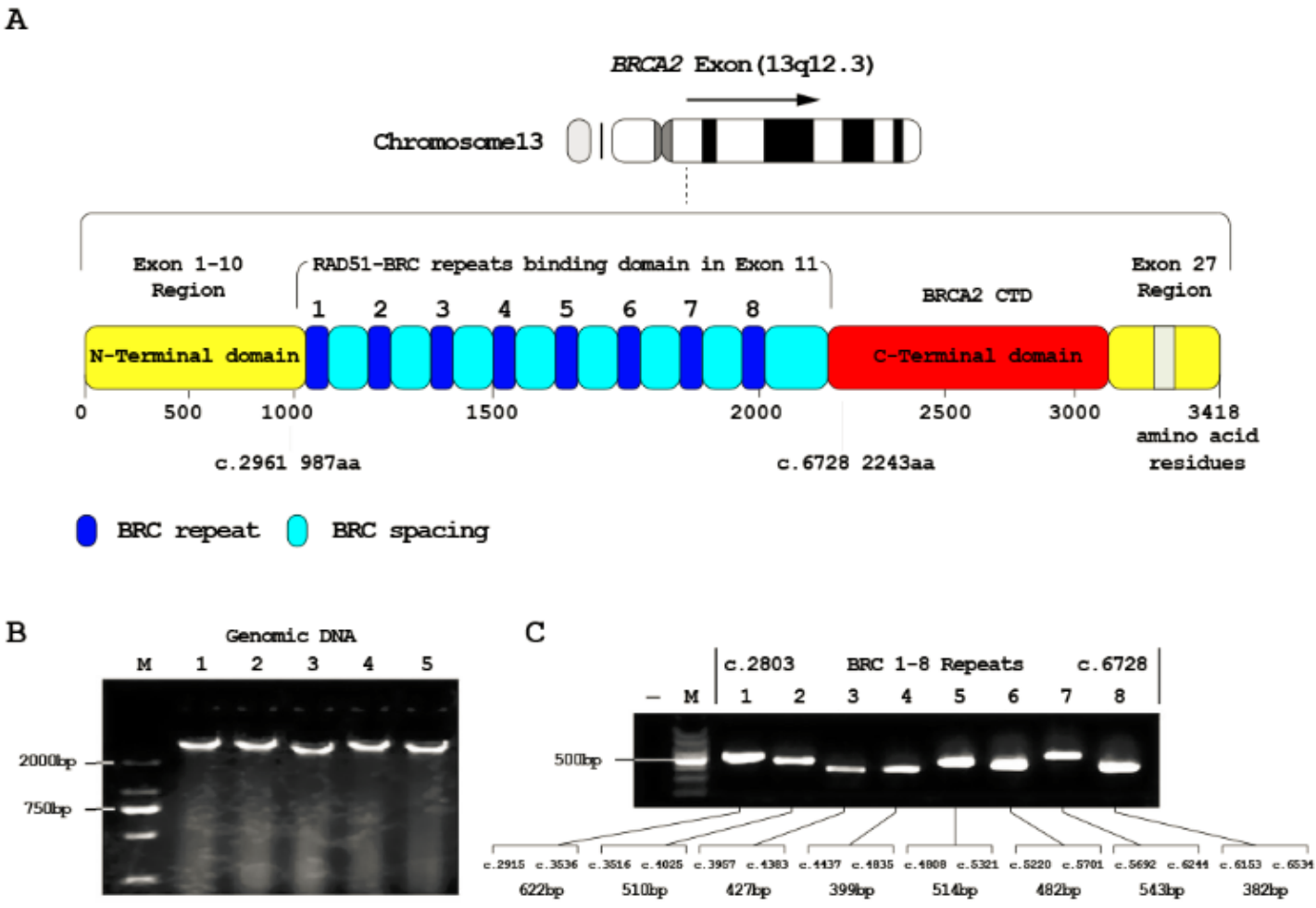

Figure 1

The BRC1~8 repeat region in BRCA2 and amplified fragments from DNA of HGSOC samples for DNA sequencing. A: The human BRCA2 is located on the long arm of chromosome 13 (13q12.3) and is composed of 27 exons that encode for a protein of 3418 amino acids residues. BRCA2 contains 8 BRC repeats located in the central portion of the protein; the eight conserved BRC repeats are primarily involved in binding to RAD51. The BRC1~4 repeat domain is responsible for assembly of RAD51 onto single-stranded DNA (ssDNA); the BRC5~8 repeat domain is responsible for formation of RAD51-dsDNA complexes. B: Agarose gel electrophoresis of genomic DNA extracted from tumor samples and PBL,Lane 1-2: Genomic DNA from PBL, Lane 3-5 genomic DNA from tumor tissue C: Five PCR amplicons of BRCA2 BRC1-8 repeats (c.2803~c.6728) acquired from the first round PCR amplicons for DNA sequencing.

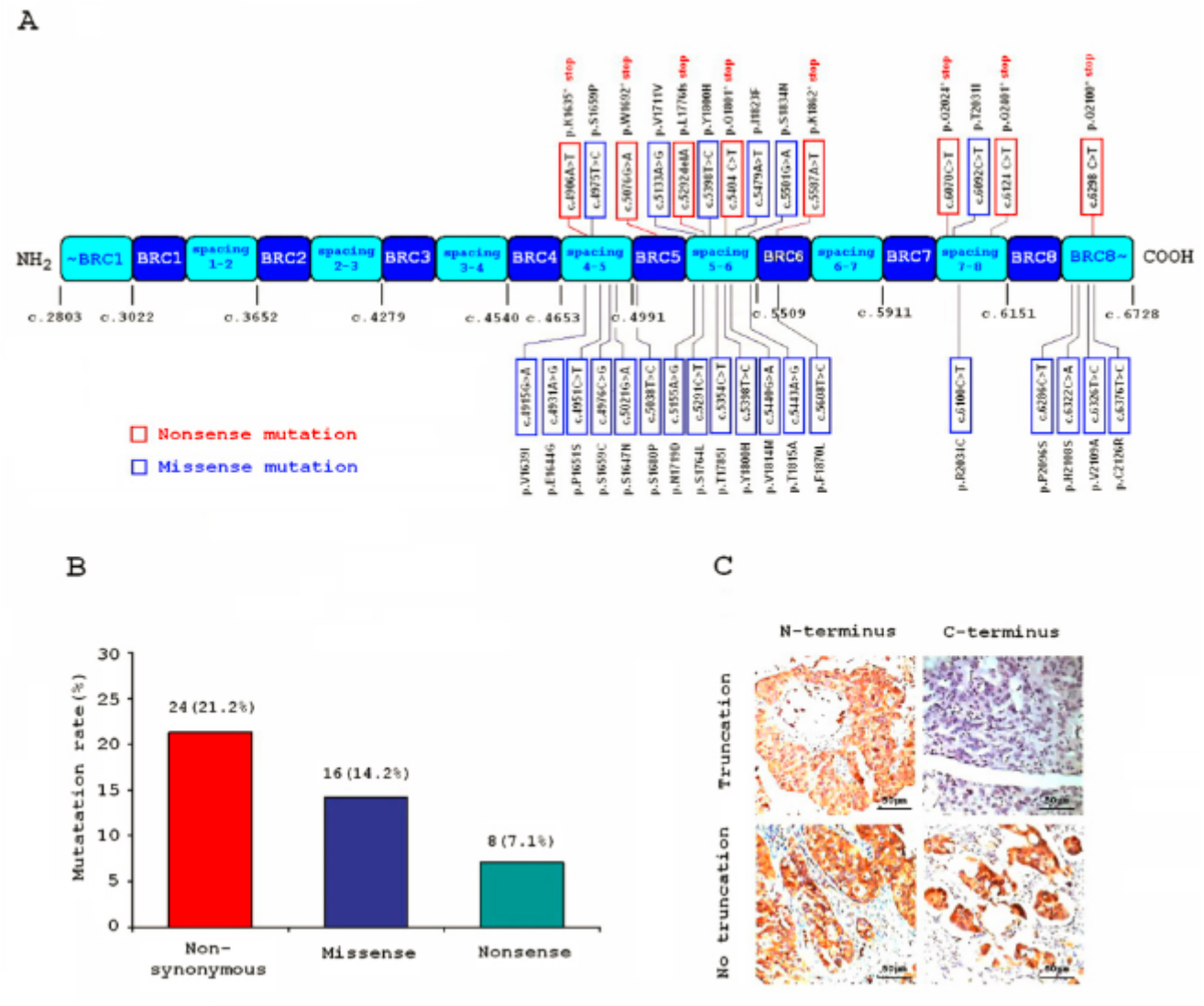

Figure 2

Frequencies of mutations in BRCA2 BRC1~8 repeat region and BRCA2 truncation in 113 HGSOC cases. A: Location and type of missense and nonsense mutations in BRCA2 BRC repeat are shown in blue and red rectangular boxes, respectively; B: The frequencies of missense and nonsense mutations are shown with a histogram; C: Truncation at the C-terminus of BRCA2 protein is represented with negative immunohistochemistry staining.

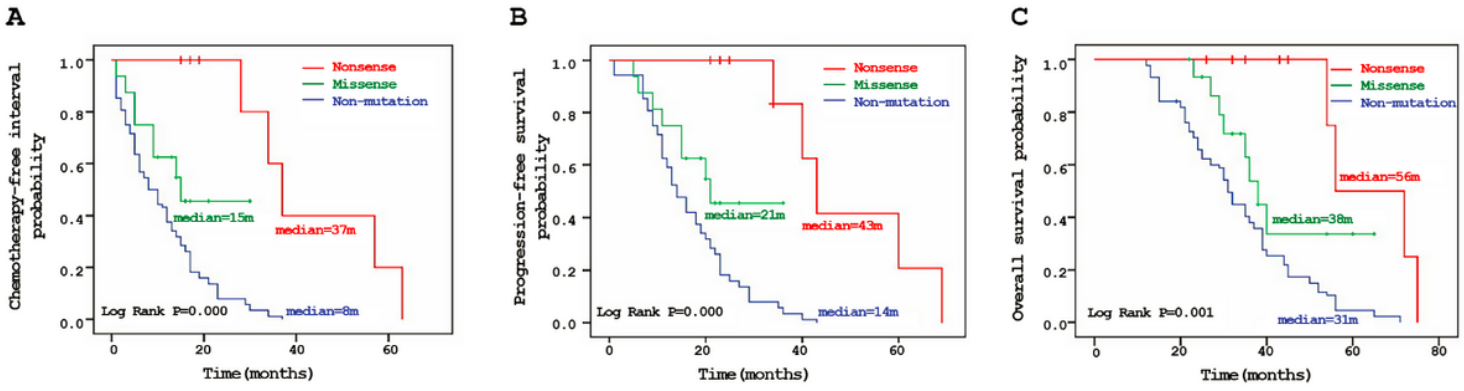

Figure 3

BRCA2 BRC repeat mutations correlates with chemotherapy-free interval, progression-free survival and overall survival in the study. A: Chemotherapy-free interval for HGSOC patients with nonsense and missense mutation in BRCA2 BRC repeat and with non-mutation. B: Progression-free survival for HGSOC patients with nonsense and missense mutation in BRCA2 BRC repeat and with non-mutation; C: Overall survival for HGSOC patients with nonsense and missense mutation in BRCA2 BRC4~8 repeats and with non-mutation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.tif
- SupplementaryTable1.doc
- SupplementaryTable2.doc