


The *BRCA2* p.N372 H i.a. I342A>C Could Regulate the Sensitivity of Ovarian Cancer Cells to Platinum-Based Drugs

Technology in Cancer Research & Treatment
Volume 19: 1-8
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1533033820983289
journals.sagepub.com/home/tct


Zhen-Hua Du, PhD¹, Yu Xia, MD¹, Qing Yang, PhD¹ , and Song Gao, PhD¹

Abstract

Background and Objective: We have previously reported that *BRCA2* N372 H i.a. I342A>C heterozygous variation presented in platinum-resistant patients. This study aimed to further investigate the mechanism of *BRCA2* N372 H mutation in the development of platinum resistance in ovarian cancer. **Methods:** The *BRCA2* N372 H i.a. I342A>C was synthesized and used to exchange 1 wildtype allele followed by sequencing to confirm the mutant allele sequence. Plasmids were constructed and transfected into the OVCAR-3 cells after lentiviral packaging. *BRCA2* N372 H mRNA was detected by qPCR. *BRCA2* protein was assessed by immunoblotting. Binding of the *BRCA2* to Rad51 was detected by immunofluorescence staining. Sensitivity of the cells to cisplatin treatment was assessed with CCK-8 assay. **Results:** It was found that expression of *BRCA2* protein in ovarian cancer cells transfected with *BRCA2* N372 H i.a. I342A>C gene (2.177 ± 0.003) was significantly increased compared to that of the cells transfected with lenti-EGFP only (1.227 ± 0.003 , $P < 0.001$). Binding of the *BRCA2* and Rad51 proteins was significantly increased in the cells with *BRCA2* N372 H i.a. I342A>C mutation (3.542 ± 0.24) than that in the cells transfected with lenti-EGFP (1.29 ± 0.32) or empty cells (1.363 ± 0.32 , $P < 0.001$). Cell viability significantly increased in the cells transfected with *BRCA2* N372 H mutant gene. The IC50 value was significantly higher in the cells transfected with *BRCA2* N372 H mutant gene (1.963 ± 0.04) than that of the cells transfected with lenti-EGFP (0.955 ± 0.03 , $P < 0.01$) or empty cells (1.043 ± 0.007 , $P < 0.01$). **Conclusion:** Over expression of mRNA and protein of *BRCA2* was detected in the cells with *BRCA2* N372 H i.a. I342A>C mutation but not in the lentivirus negative control (lenti-EGFP) or the cells without transfection (empty cells), which may lead to resistance to platinum-based drugs in ovarian cancer cells through homologous recombination repair pathway.

Keywords

BRCA2 N372H, drug resistance, ovarian cancer

List of Abbreviations

NCCN, National Comprehensive Cancer Network; HR, homologous recombination; ATM, ataxia-telangiectasia, mutated; ATR, ATM and Rad3-related; FBS, fetal bovine serum

Received: July 16, 2020; Revised: November 1, 2020; Accepted: November 30, 2020.

Introduction

Ovarian cancer is a malignant tumor that poses a serious threat to all women. Platinum-based chemotherapy is the main adjuvant therapy for advanced ovarian cancer. However, even as the most commonly used first-line chemotherapy drug, primary or secondary resistance to platinum therapy remains a major obstacle in the treatment of ovarian cancer, and it is one of the main reasons that contribute to the less than 30% of the five-year survival rate in the patients with advanced ovarian cancer.¹ Therefore, early identification and intervention may

be an effective way to improve the 5-year survival rate of ovarian cancer patients. The most commonly used platinum-

¹ Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, Shenyang, China

Corresponding Authors:

Qing Yang and Song Gao, Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Heping District, Shenyang 110004, China.
Emails: zhenhuadusy@163.com; rsapca@163.com



based drugs in clinical practice are carboplatin and cisplatin. Both carboplatin and cisplatin are cell-cycle nonspecific drugs, mainly acting on the N7 and O6 atoms of guanine, causing DNA interchange and intra-strand crosslinking, preventing helix melting, and interfering with DNA synthesis resulting in cytotoxicity.

The *BRCA2* gene is a tumor suppressor gene located at 13q12-13 and plays an important role in the development, progression, and prognosis of ovarian cancer and breast cancer. *BRCA2* and *RAD51* homologous protein physically interact with each other during homologous recombination (HR), and jointly participate in DNA damage repair and recombination.² In this regard, when DNA double strands are damaged, DNA damage signal activates kinases such as ATM (ataxia-telangiectasia, mutated) and ATR (ATM and Rad3-related) to catalyze the phosphorylation of the *BRCA2/RAD51* protein complex,³ thus converting it from an inactive form to an active form. Double strand breaks of DNA can be repaired by a HR-based error-free mechanism, which is carried out by homology search and strand invasion to complete the repair.^{4,5} *RAD51* is the key protein required for the initial strand invasion step, and this invasion step is dependent on several proteins such as *BRCA1* and *BRCA2*.^{4,6} The *BRCA2* protein carries the *RAD51* protein to the damage site of the double-stranded DNA and participates in the repair process.⁷ There are 3 described genotypes in *BRCA2 N372 H* variant, that is, wild type (AA), heterozygous (AC) and homozygous (CC). In our previous studies, the *BRCA2 N372 H i.a.1342A>C* gene heterozygous variation was specifically found in the plasma of the patients with drug-resistant recurrence of ovarian cancer compared with platinum-sensitive patients.⁸ Therefore, we hypothesized that *BRCA2 N372 H* gene mutation may be associated with platinum resistance in ovarian cancer. The current study was, therefore, designed to explore the effect of *BRCA2 N372 H* mutation gene on platinum-resistance in the ovarian cancer cells. Since OVCAR-3 cell line is a known and widely used ovarian cancer cell line, it was used for the investigation of *BRCA2* gene mutation effect on cell survival and function in the current study.

Materials and Methods

Experiment Design

We first synthesized the *BRCA2 N372 H i.a.1342A>C* (rs144848) heterozygous mutant gene using gene synthesis technology and sequenced the *BRCA2 N372 H* gene fragment. Then constructed OVCAR-3 ovarian cancer cells with *BRCA2 N372 H i.a.1342A>C* mutation, and cell clones carrying the target gene were screened out by monoclonal culture. Identification of the mutant gene was performed by digital PCR and *BRCA2* protein expression was determined by immunoblotting. The OVCAR-3 cells without transfection were used as positive cells that constitutively expressed *BRCA2*. Finally, the binding of the *RAD51* protein to *BRCA2* protein in the target cells was detected by immunofluorescence.

Commercially available CCK-8 assay kit, which uses a water-soluble tetrazolium salt to quantify the number of live cells, was used to detect the resistance of *BRCA2 N372 H* mutant cells to cisplatin, and the mechanism by which *BRCA2 N372 H* mutation affecting the sensitivity of cells to platinum-based drugs was determined.

Cell Strain and Culture

The human OVCAR-3 cell line was purchased from a commercial source (Shanghai Suer Biotech, Shanghai, China). Cells were cultured with RPMI-1640 (GIBCO, Invitrogen Corporation, Shanghai, China) containing 10% fetal bovine serum (FBS) in 5% CO₂, 95% humidified air, and 37°C incubator.

BRCA2 N372H Mutant Gene Synthesis and Sequencing

The *BRCA2 N372 H* mutant gene was obtained by gene synthesis and amplification (Generay, Shanghai, China) using PCR specific for the *BRCA2n372 h* gene fragment. Sequence of *BRCA2* has been provided as supplemental material. The *BRCA2 N372H-F1* primer sequence was GCCAAATGTCC-TAGAAGATGAAG and the *BRCA2 N372H-R1* primer sequence was CTGTTTCCTCATTTAATGGCTTC. Genomic DNA was extracted from samples using a genomic DNA extraction kit (AxyPrep Mini DNA extraction kit, Baisai Biology Technology, Shanghai, China) and subjected to electrophoretic identification. Using genomic DNA as a template, the target fragment was amplified with a high-fidelity enzyme. The PCR cycle was as following: 95°C 5 min, 1 cycle, 95°C 30 sec, 58°C 30 sec, 72°C 45 sec, 30 cycles, 72°C 10 min, 1 cycle, after which PCR products were recovered (AxyGen PCR purification kit, Baisai Biology Technology, Shanghai, China) and sequenced.

Construction of *BRCA2 N372 H* Mutant Gene Vector

Primers were designed based on the sequence of the target gene, and the restriction sites *AscI* and *PmeI* were added upstream and downstream of the target fragment, respectively, with *BRCA2 N372H-F* primer sequence TCAAGTTCTT-TAGCTACACCACCC and *BRCA2 N372H-R* primer sequence TTCTTTTTTAAAGTTTGGATCAGTCA. The gene was used as a template to amplify the target fragment by PCR. As shown in the Supplement Figure 1, appropriate restriction sites were added to both ends of the target fragment. The PCR cycle was as following: 95°C 3 min, 1 cycle, 94°C 30 sec, 58°C 30 sec, 68°C 60 sec, 30 cycles, 68°C 10 min, 1 cycle. The PCR product was separated by agarose gel electrophoresis, and the target fragment was recovered using the AxyGen Gel Recovery Kit. The target gene PCR product and the target vector were digested with *AscI* and *PmeI*, respectively. After being digested at 37°C for 4-5 h, the fragments were electrophoretically separated and the target fragment was excised and recovered from the gel. The T4 DNA ligase ligated the digested PCR product and target vector.

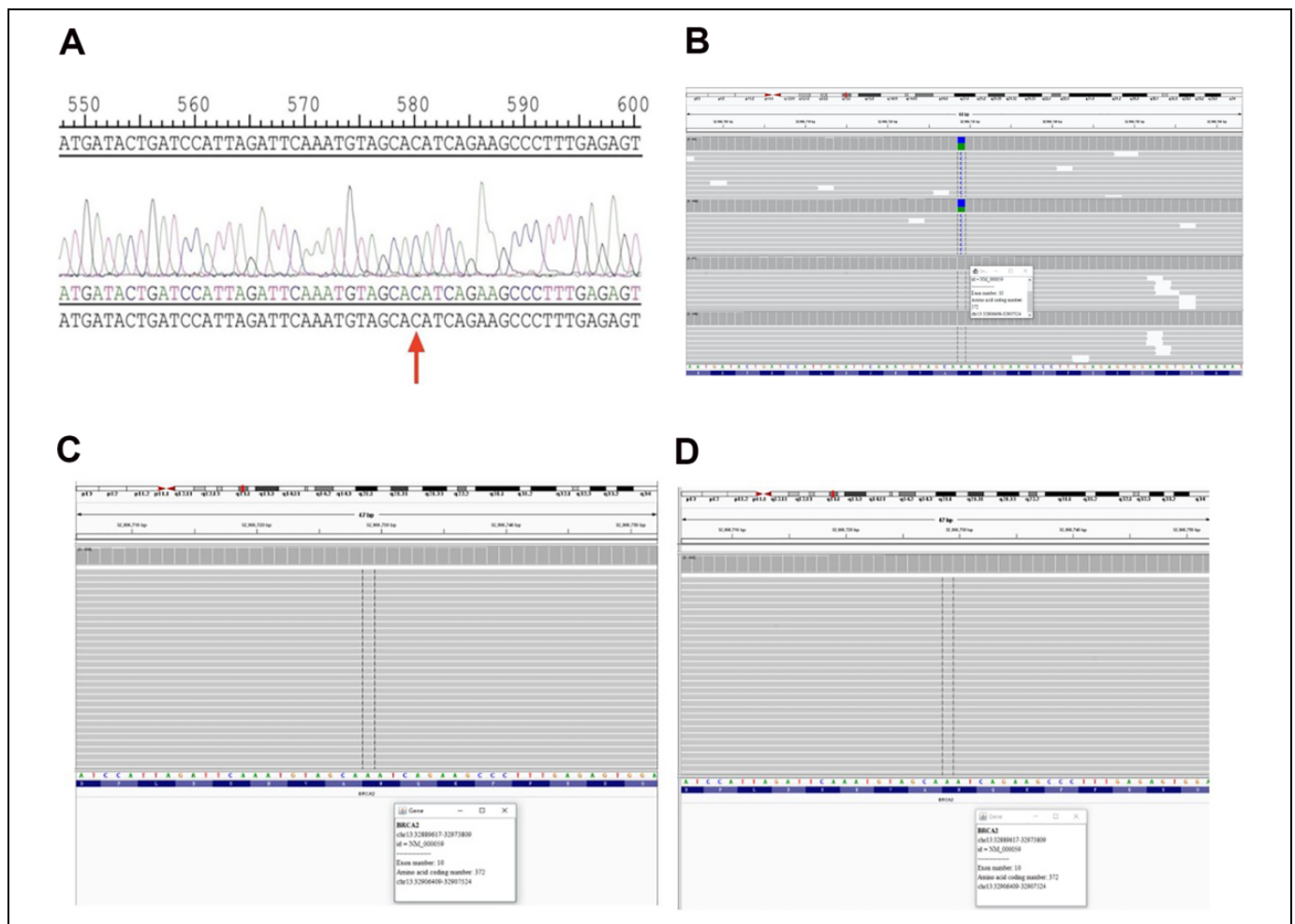


Figure 1. Gene sequences of wild and mutation in the OVCAR3 cell strain. Panel A: Confirmation of BRCA2 N372 H i.a.1342A>C mutant gene by sequencing. Arrow indicated the mutant gene: from normal “A” to mutant “C.” **Panel B:** Variant type of BRCA2 N372 H gene locus in the wild type OVCAR3 cells (Illumina HiSeq 4000 high-throughput sequencing platform). **Panel C:** Sequence of Lenti-EGFP. **Panel D:** Sequence of empty control.

Infection of OVCAR-3 Cells With Lentivirus Carrying BRCA2 N372 H Mutant Gene

The bacteria solution containing target gene plasmids was used for streak culture. Plasmids were extracted using the AxyGen Plasmid Extraction Kit (Baisai Biology Technology, Shanghai, China) and were packaged in lentivirus. OVCAR-3 cells at the logarithmic growth phase were trypsinized, plated in 6-well cell culture plates, and cultured at 37°C with 5% CO₂ until cell fusion reached 40%-50%. Then, 55 µl of the target virus solution (lenti-BRCA2-EGFP, titration of 2.54 x 10⁷ TU/mL) or 25 µl of negative control virus solution (lenti-EGFP, titration of 10⁸ TU/mL), and Polybrene was added at a final concentration of 8 µg/ml for transfection. The cells were incubated with the viral solution for 48 h.

Detection of BRCA2 N372 H Mutant Gene With RT-qPCR in OVCAR-3 Cells Following Transfection

Quantification of BRCA2 N372 H mutant gene in the OVCAR-3 cells was performed following the MIQE guidelines.⁹ A total of

0.5 ml Trizol was added to each well. Total RNA was extracted and reverse-transcribed into cDNA after treatment with DNase I. Quantitative PCR (qPCR) was used to detect expression of BRCA2 gene, with BRAC2 N372H-F2 primer sequence TGGAGCCCAGATGGAGAAA and BRAC2 N372H-R2 primer sequence AGTTCAGATATTGCCTGCTTT. ACTB was used as reference gene and used for data normalization with the following primer sets: hACTB-2F: TCCTTCCTGGGCATGGAGT and hACTB-2 R: CAGGAGGAGCAATGATCTTGAT. The PCR condition was as following: 95°C, 2 sec, 1 cycle (1X); 95°C, 15 sec, 60°C, 2 sec, 72°C, 2 sec, 2 cycles (40X), 60°C-95°C, 3 sec, 3 cycles (39X).

Identification of the BRCA2 N372 H i.a.1342 A>C Gene Mutation in OVCAR-3 Ovarian Cancer Cell Strains

The BRCA2 N372 H mutant gene was obtained by gene synthesis and the 1342 “A” to “C” mutation (N372 H, rs144848) was confirmed by sequencing (Figure 1A), which was compared

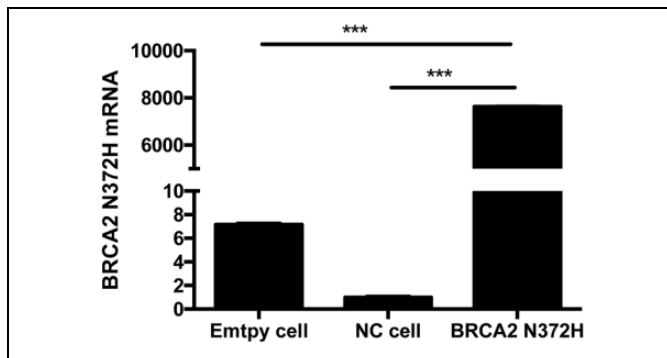


Figure 2. Comparison of BRCA2 N372 H mRNA expression in the 3 OVCAR3 ovarian cell strains. BRCA2 N372 H mRNA expression was quantified by qPCR as described in the methods. Data presented was an average of 3 measurements. Empty cells: OVCAR3 ovarian cell without transfection; NC cells: OVCAR3 ovarian cells transfected with negative control virus; BRCA2 N372 H: cells transfected with *BRCA2 N372 H i.a.1342A>C* mutant gene. *** $P < 0.0001$.

with variant type of *BRCA2 N372 H* gene locus in the wild type cells (Figure 1B, Illumina HiSeq 4000 high-throughput sequencing platform), cells transfected with negative virus control (lenti-EGFP, Figure 1C), or cells without transfection (empty cells, Figure 1D).

Immunoblotting

After washing once, boiled protein loading buffer (120 μ l) was added to each well to lyse the cells. The sample was boiled at 100°C for 10 min, centrifuged at 12000 rpm (14 cm diameter of the centrifuge) for 1 min, and stored at -20°C after dispensing. Proteins were loaded and electrophoresis was performed at constant pressure 80 V for 30 min followed by constant pressure 120 V for 1 h. Distance between the 2 electrodes was 12 cm. The protein was then transferred to the PVDF membrane and blocked with the blocking solution (containing 5% skim milk powder in TBST) for 1 h at room temperature. The membrane was then incubated with the primary antibody against BRCA2 (1:200, Abcam, Cat#: ab27976) at 4°C overnight. Next day, the secondary antibody (Anti-mouse second antibody, Jackson, Cat #: 315-035-003; or anti rabbit secondary antibody, Jackson, Cat#: 111-035-008) was applied at room temperature for 1 h. After washing, protein band was visualized, and densitometry analysis was performed.

Detection of RAD51 Protein by Confocal Microscopy

The cells were fixed with 4% paraformaldehyde for 15 min followed by rinsing twice with PBS. Cells were then permeabilized with 0.5% Triton-100 in PBS for 10 min followed by blocking with 4% BSA in PBS at room temperature for 1 h. Primary anti-RAD51-antibody (1:100, Abcam, Cat#: ab133534) was allowed to react at 4°C overnight. After washing 5 times with PBS, goat anti-rabbit Alexa-594 secondary antibody (Abcam, Cat#: ab150080) were applied for 1 h at

room temperature. After washing with PBS, nuclei were stained with DAPI, and the slides were sealed and observed using a confocal microscope (ZEISS, LSM710). Fluorescence intensity (IntDen value) was obtained using the Image J software following the manufacturer's instruction (NIH, Public Domain, USA).

Assessment of Cellular Sensitivity to Cisplatin by CCK-8 Assay Kit

Sensitivity of the cells to cisplatin was assessed using CCK-8 kit following the manufacturer's instruction (Dojindo, Cat#: CK 04). The transfected cells and non-transfected cells were trypsinized after 72 h culture. The cell suspension was seeded in 96-well culture plates (8000 cells/well) and incubated at 37°C with 5% CO₂. Cisplatin was added to the cell culture plates at concentrations of 0, 0.05, 0.1, 0.5, 1, 2, 4, and 8 μ g/ml. After 24 h and 48 h treatment, respectively, 10 μ l of CCK-8 was added to each well and incubated for 2 h followed by measuring the absorbance at 450 nm with a microplate reader (Thermo, MuLTiSKAN MK3).

Statistical Analysis

Image J software was used to analyze the immunofluorescence intensity for the binding of RAD51 protein expression. An unpaired *t* test in the Prism 7.0 software package was used to compare the differences of mRNA expression and semi-quantification of protein levels. SPSS23.0 was used to analyze the IC50 of each group in the CCK-8 experiment.

Results

Quantification of BRCA2 N372 H Mutant Gene mRNA in the 3 Strains of OVCAR-3 Cells

Following the co-transfection of *BRCA2 N372 H* mutant gene expression vectors and the packaged plasmids into the OVCAR-3 cells, expression of BRCA2 N372 H mRNA in OVCAR-3 cells was quantified by RT-qPCR. As shown in the Figure 2, mRNA expression of *BRCA2 N372 H* mutant gene was 7000 fold higher in the cells transfected with *BRCA2 N372 H* mutant gene (7625.8 ± 30.5) compared to that in the cells either non-transfected (empty cells, 1.00 ± 0.12 , $P < 0.0001$) or cells transfected with Lenti-EGFP (7.16 ± 0.15 , $P < 0.0001$).

Comparison of BRCA2 Protein Expression Following Transfection of BRCA2 N372 H Mutant Genes in the OVCAR-3 Cells

Protein level of BRCA2 was detected by immunoblotting in the cells transfected with the *BRCA2 N372 H* mutant gene, the lentivirus negative control (Lenti-EGFP), or the OVCAR-3 cells without any transfection (empty cells). As shown in Figure 3, the OVCAR-3 cells transfected with the *BRCA2*

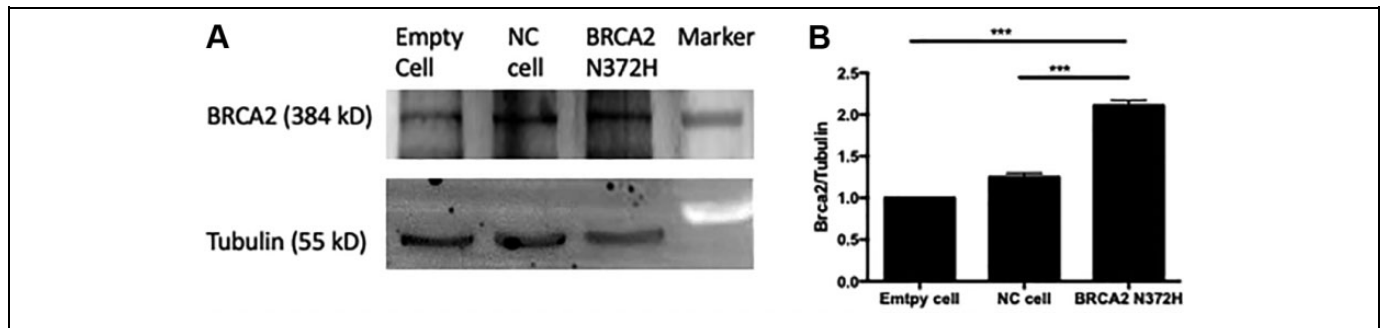


Figure 3. Comparison of BRCA2 protein expression in the 3 cell strains. Immunoblotting of BRCA2 protein was performed as described in the methods. **Panel A:** Representative immunoblotting image. **Panel B:** Semi-quantitative comparison of BRCA2 expression in the 3 cell strains. Data were compared as ratio of BRCA2 band density over tubulin band density and were an average of 3 separate experiments. Empty cells: OVCAR3 ovarian cell without transfection; NC cells: OVCAR3 ovarian cells transfected with negative control virus; BRCA2 N372 H: cells transfected with *BRCA2 N372 H i.a.1342A>C* mutant gene. *** $P < 0.0001$.

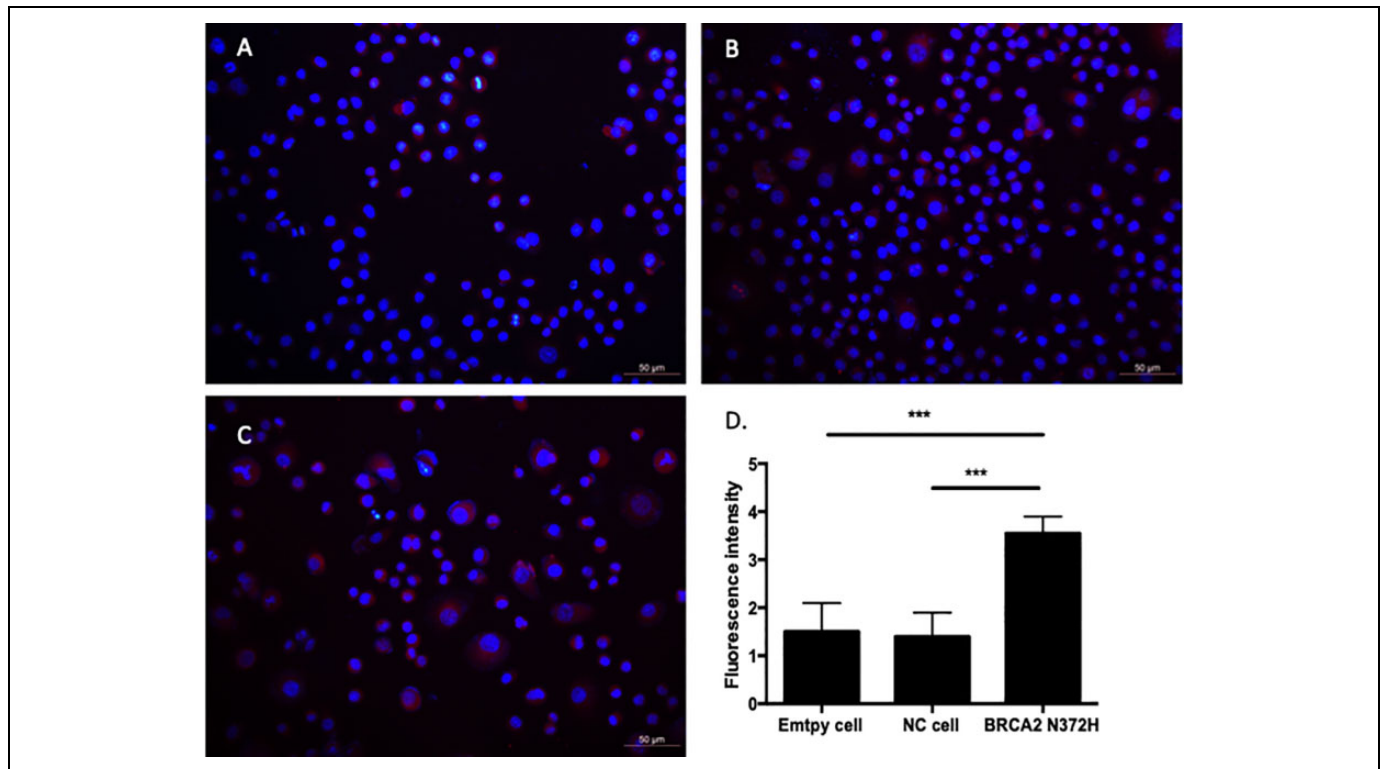


Figure 4. Detection of Rad51 protein by immunofluorescence staining in the 3 cell strains. Immunofluorescence staining was performed as described in the methods. **Panel A:** Empty OVCAR3 cells. Scale bar: 50 μm . **Panel B:** Negative control cells. Scale bar: 50 μm . **Panel C:** Cells with *BRCA2 N372 H* mutation. Scale bar: 50 μm . **Panel D:** Quantitative comparison of fluorescence intensity in the 3 cell strains. *** $P < 0.001$.

N372 H mutant gene showed significantly increased BRCA2 protein expression (Ratio of BRCA2/tubulin: 2.177 ± 0.003) compared to that of the cells transfected with Lenti-EGFP (Ratio of BRCA2/tubulin: 1.227 ± 0.003 , $P < 0.001$).

Comparison of RAD51 Protein Expression

In order to assess the effect of *BRAC2 N372 H* gene mutation on the physical binding of BRCA2 protein and RAD51 protein in

OVCAR-3 cells, expression of RAD51 protein in the OVCAR-3 cells transfected with virus expressing the *BRAC2 N372 H* mutant gene and Lenti-EGFP was examined by immunofluorescence staining. As shown in the Figure 4, the immunofluorescent intensity of RAD51 protein in OVCAR-3 cells transfected with expressing the *BRAC2 N372 H* mutant gene (3.542 ± 0.24) was significantly stronger than that in the cells either transfected with Lenti-EGFP (1.29 ± 0.32) or the cells without transfection (empty cell) groups (1.363 ± 0.32 , $P < 0.001$).

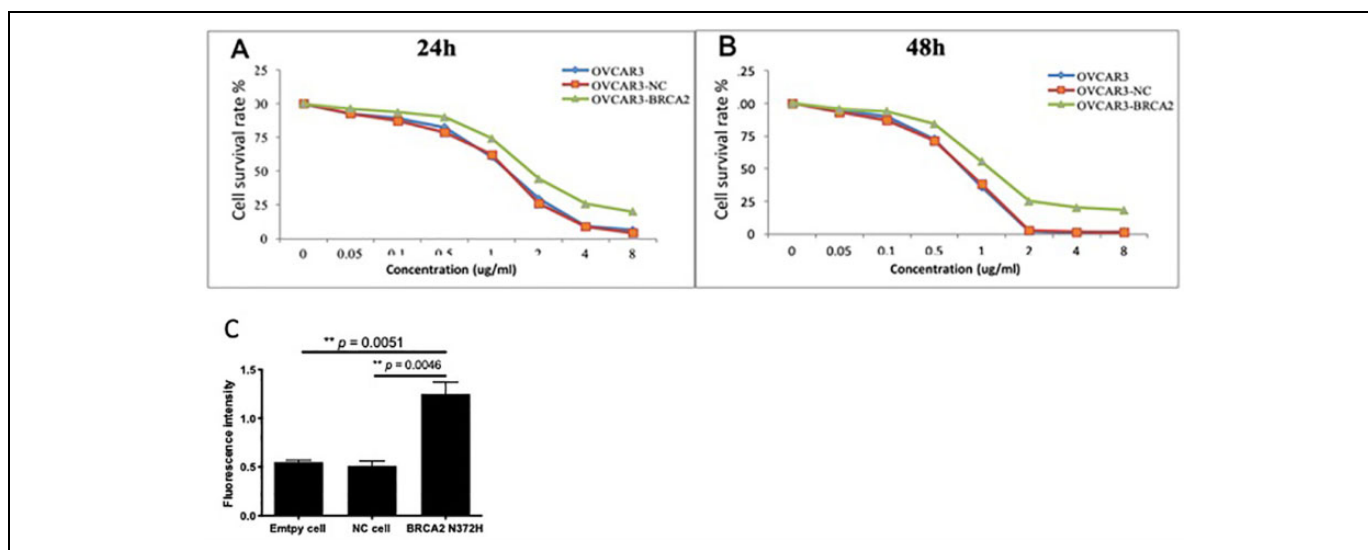


Figure 5. Comparison of cell viability in the 3 cell strains. Cell viability in response to cisplatin was assessed in the 3 strains as described in the methods. **Panel A:** Cell survival rate at 24 h. OVCAR3: wild type ovarian cancer cell strain without transfection; OVCAR3-NC: OVCARD cells transfected with negative virus control, lenti-EGFP; OVCAR3-BRCA2: OVCAR3 cells transfected with mutated *N372 H i.a.1342A>C*. **Panel B:** Cell survival rate at 48 h. OVCAR3: wild type ovarian cancer cell strain without transfection; OVCAR3-NC: OVCARD cells transfected with negative virus control, lenti-EGFP; OVCAR3-BRCA2: OVCAR3 cells transfected with mutated *N372 H i.a.1342A>C*. **Panel C:** Comparison of IC50 at 48 h in the 3 strains.

Cell Viability in Response to Cisplatin and IC50 in 3 Cell Strains

Following transfection of *BRCA2 N372 H* mutant gene and negative control Lenti-EGFP vectors, the OVCAR-3 cells were treated with different concentrations of cisplatin (0.05, 0.1, 0.5, 1, 2, 4, and 8 µg/ml) for 24 and 48 h, respectively. Cell viability and the IC50 of the cells in response to cisplatin were assessed by CCK-8 kit. As shown in Figure 5, cell viability was significantly higher in the cells that transfected with *BRCA2 N372 H* mutant gene compared to that of the cells transfected with Lenti-EGFP or empty cell groups when they were treated with different concentrations of cisplatin at 24 h (Figure 5A) or 48 h (Figure 5B). After 24 h treatment, the difference was especially significant at cisplatin concentrations of 2, 4, and 8 µg/ml, and after 48 h, the difference was significant at concentrations of 1, 2, 4, and 8 µg/ml. The IC50 value was significantly higher in the cells transfected with *BRCA2 N372 H* mutant gene (1.963 ± 0.04 at 24 h and 1.299 ± 0.06 at 48 h) than that of the cells transfected with Lenti-EGFP (0.955 ± 0.03 at 24 h and 0.575 ± 0.02 at 48 h, $P < 0.01$) or non-transfected control (empty) cells (1.043 ± 0.007 at 24 h and 0.593 ± 0.01 at 48 h, $P < 0.01$, Figure 5C).

Discussion and Conclusion

In the TCGA database, rs144848 (GG) homozygotes and (GT) heterozygotes cause a significantly increased risk of ovarian cancer, breast cancer, and prostate cancer. For this special single nucleotide polymorphism (SNP), the allele with risk is "G."¹⁰ For the *BRCA2 N372 H* (rs144848), there are 3 genotypes of

variation: wild type (AA), heterozygous (AC), and homozygous (CC). Correspondences of AA is TT, AC is TG, and CC is GG, respectively. The heterozygous mutation (AC) of *N372 H* in the *BRCA2* gene is a common recessive genetic polymorphism (rs144848). The nonsynonymous mutation of *1342A>C* causes a substitution from asparagine (Asp) to histidine (His) in the N-terminus of exon 10. Research on the *BRCA2 N372 H* gene was initially focused on aspects related to the risk of morbidity. For example, CC homozygous mutations increase the risk of breast cancer by approximately 1.3-fold, and AC heterozygous mutations may slightly increase the risk of breast cancer.¹¹ Studies have also shown that the *BRCA2 N372 H* gene polymorphism is associated with the risk of ovarian cancer.¹² Recently, Woo Sun Kwon et al. showed that the *BRCA2 N372 H* mutation leads to drug resistance in breast cancer cell strains by regulating HAT activity.³ In our previous studies, we found that 4 patients with platinum-resistant recurrence carried the *BRCA2 N372 H i.a.1342A>C* gene mutation, and none of the 10 patients with platinum-sensitive recurrence carried this gene mutation. Therefore, we speculated that the mutation of *BRCA2 N372 H i.a.1342A>C* gene may be associated with the risk of drug-resistant recurrence of ovarian cancer.

We used gene synthesis technology to synthesize the *BRCA2 N372 H i.a.1342 A>C* mutant gene, which was confirmed by sequencing. Then, *BRCA2 N372 H* mutant gene vectors were constructed and packaged with lentivirus to transfect OVCAR-3 cells. Monoclonal culture of *BRCA2 N372 H i.a.1342 A>C* mutant OVCAR-3 cell lines was performed and the expression of large amounts of *BRCA2 N372 H* mRNA was detected using the RT-qPCR method, demonstrating the

BRCA2 N372 H i.a.1342 A>C mutant OVCAR-3 cell line was successfully constructed. In addition, expression of BRCA2 protein in OVCAR-3 cells with *BRCA2 N372 H i.a.1342 A>C* mutation was significantly higher than that in the cells transfected with Lenti-EGFP or the cells without transfection, indicating that *BRCA2 N372 H i.a.1342 A>C* mutation can increase the expression of BRCA2 protein.

Homologous recombination (HR) is one of the major mechanisms in DNA double-strand break repair pathways. The crucial step of HR is homologous strand exchange directed by the protein RAD51, which is recruited by BRCA2.^{5,13} BRCA2 regulates both the intracellular localization and DNA binding ability of RAD51, and loss of these controls following BRCA2 inactivation may be a key event leading to genomic instability and tumorigenesis.¹⁴ In the current study, therefore, expression of RAD51 was examined by immunofluorescence staining in the 3 cell strains. The purpose of this design was to test the function of the mutant BRCA2 protein in OVCAR-3 cells and verify whether these BRCA2 proteins can effectively bind to RAD51 protein and exert DNA damage repair function. The results showed that the fluorescence intensity of RAD51 protein in OVCAR-3 cells with *BRCA2 N372 H i.a.1342 A>C* mutation was higher than that in the cells transfected with Lenti-EGFP or the cells without transfection (empty cells), demonstrating that the mutated BRCA2 protein could bind to the RAD51 protein.

Since recent studies also indicated that the *BRCA2 N372 H* mutation lead to drug resistance in breast cancer cell strains,^{3,15} survival of the 3 cell strains in response to cisplatin was examined by CCK8 assay. It was found that cell viability of the OVCAR-3 cells transfected with the BRCA2 mutant was significantly higher than that of the cells transfected with Lenti-EGFP or the cells without transfection (empty cells) at 24 or 48 h, with a more pronounced difference as the concentration of cisplatin was increased. Furthermore, in the OVCAR-3 cells with *BRCA2 N372 H i.a.1342 A>C* mutation, the IC50 of cisplatin was significantly higher than that of the cells transfected with Lenti-EGFP or the cells without transfection (empty cells). These findings demonstrated that *BRCA2 N372 H i.a.1342A>C* mutation in the OVCAR-3 cells resulted in significant increase of resistance to cisplatin.

Consistent with our findings, many researchers suggested that secondary mutation of *BRCA2* may be an important mechanism for platinum resistance in *BRCA2* mutations.¹⁶⁻¹⁹ This interaction occurs at the *BRCA2* exon 11 binding site or the so-called BRCA2 DBD (DNA/DSS1-binding domain) domain on the carboxy terminus of *BRCA2*.²⁰ In *BRCA1* and *BRCA2* ovarian cancers that carry frameshift mutations, acquired platinum resistance is generated in the gene by secondary mutations, by which the open reading frames (ORF) of mutated *BRCA1/2* are corrected, and the wild-type *BRCA1/2* expression and DNA homologous recombination repair function are recovered. In the pancreatic or ovarian cancer cell lines with *BRCA2* mutations, secondary mutations in *BRCA2* are induced by cisplatin or poly ADP-ribose polymerase (PARP) inhibitors.¹⁹ As a result of these secondary mutations, the truncated BRCA2 protein is reconnected and carries out the function of homologous recombination.¹⁸ In addition to

the regulation of homologous recombination repair genes, it has also been reported that the development of platinum resistance may be attributed to other mechanisms since BRCA2 mRNA expression is not detected in 13% of ovarian cancer cases.²¹ In this regard, we found no truncating mutations in the *BRCA2* gene in the first tumor specimen of 4 patients who had drug resistant ovarian cancer recurrence and the *BRCA2 N372 H i.a.1342 A>C* mutation. This discrepancy with results reported by other researchers suggested that the *N372 H i.a.1342 A>C* mutation may increase the resistance to platinum-based drugs in tumor cells through different pathways.

Therefore, we have further explored the potential mechanisms and found that increased physical binding of BRCA2 protein and RAD51 protein may contribute to the regulation of *BRCA2 N372 H i.a.1342 A>C* mutation on platinum sensitivity in the cells. Specifically, binding of the aforementioned 2 proteins may increase the homologous recombination repair function of the cells, and lead to the development of platinum resistance in ovarian cancer cells, which was confirmed by the CCK-8 experiment.

In addition, studies have also suggested that expression of ATP-binding cassette (ABC) transporters in cancer cells can confer resistance to cytotoxic and targeted chemotherapy.²²⁻²⁴ ABC transporters regulate cellular transportation of variety types of molecules including hormones, lipids, ions, and other small molecules across cell membranes. On one hand, loss of function of a particular ABC transporter is associated with a number of heritable diseases such as cystic fibrosis.²⁵ On the other hand, ABC transporters are also associated with drug resistance in variety kinds of cancers including ovarian cancer.²³ In this regard, whole-genome sequencing of 92 patients with high-grade serous ovarian carcinoma with primary and matched resistant disease showed that reversion of BRCA1 and/or BRCA2 mutations were potential mechanisms of drug resistance.¹⁵ Whether expression of ABC transporters is altered in the cells with *BRCA2 N372 H i.a.1342 A>C* mutation remains to be determined.

One of the limitations of this study was that a cell line with overexpressing wild type BRCA2 was not applied as control for comparison with BRCA2 N372 H mutation although the “empty cells” OVCAR3 ovarian cell that contains wild type BRCA2 gene was used. However, it has been reported that exogenous expression of wild type BRCA2 in a human pancreatic cancer line (Capan-1 cells) conferred resistance to methyl methanesulfonate treatment,²⁶ and that complex of BRCA2 and RAD51 in breast cancer MCF-7 cells was diminished upon conditional expression of a wild type, but not a mutated, BRC4-repeat.²⁷

The *BRCA2 N372 H i.a.1342 A>C* mutation increased the expression of BRCA2 protein in ovarian cancer cells. This mutation-associated BRCA2 protein up-regulation resulted in enhanced binding of BRCA2 and RAD51 proteins and augmented DNA homologous recombination repair function of ovarian cancer cells, and by which mechanism, it led to the development of resistance to platinum-based drugs. While these *in vitro* experimental results need to be further confirmed *in vivo* with large population screening of the patients with

drug-resistant recurrence of ovarian cancer, findings of this study may provide the theoretical basis for drug development and targeted therapy of ovarian cancer.

Conclusion

Taken together, the current study revealed that the *BRCA2* N372 H i.a.1342 A>C mutation could regulate the sensitivity of ovarian cancer cells to platinum-based drugs.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Experimental study on the relationship between BRCA2 N372H mutation and platinum resistance in ovarian cancer, Science and Technology Department of Liaoning Province, No: JC2019018.

ORCID iD

Qing Yang  <https://orcid.org/0000-0002-3669-3537>

Supplemental Material

Supplemental material for this article is available online.

References

- Diaz LA, Jr., Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol*. 2014;32(6):579-586.
- Li J, Zou C, Bai Y, Wazer DE, Band V, Gao Q. DSS1 is required for the stability of BRCA2. *Oncogene*. 2006;25(8):1186-1194.
- Kwon WS, Rha SY, Jeung HC, Kim TS, Chung HC. Modulation of HAT activity by the BRCA2 N372 H variation is a novel mechanism of paclitaxel resistance in breast cancer cell lines. *Biochem Pharmacol*. 2017;138:163-173.
- San Filippo J, Sung P, Klein H. Mechanism of eukaryotic homologous recombination. *Annu Rev Biochem*. 2008;77:229-257.
- Zhao W, Steinfeld JB, Liang F, et al. BRCA1-BARD1 promotes RAD51-mediated homologous DNA pairing. *Nature*. 2017;550(7676):360-365.
- Kolinjivadi AM, Sannino V, de Antoni A, Techer H, Baldi G, Costanzo V. Moonlighting at replication forks—a new life for homologous recombination proteins BRCA1, BRCA2 and RAD51. *FEBS Lett*. 2017;591(8):1083-1100.
- Diaz LA, Jr., Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*. 2012;486(7404):537-540.
- Du ZH, Bi FF, Wang L, Yang Q. Next-generation sequencing unravels extensive genetic alteration in recurrent ovarian cancer and unique genetic changes in drug-resistant recurrent ovarian cancer. *Mol Genet Genomic Med*. 2018;6(4):638-647.
- Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009;55(4):611-622.
- Xue WQ, He YQ, Zhu JH, Ma JQ, He J, Jia WH. Association of BRCA2 N372 H polymorphism with cancer susceptibility: a comprehensive review and meta-analysis. *Sci Rep*. 2014;4:6791.
- Auranen A, Song H, Waterfall C, et al. Polymorphisms in DNA repair genes and epithelial ovarian cancer risk. *Int J Cancer*. 2005;117(4):611-618.
- Su L, Wang J, Tao Y, et al. BRCA2 N372 H polymorphism and risk of epithelial ovarian cancer: an updated meta-analysis with 2344 cases and 9672 controls. *Medicine (Baltimore)*. 2015;94(42):e1695.
- Luo K, Li L, Li Y, et al. A phosphorylation-deubiquitination cascade regulates the BRCA2-RAD51 axis in homologous recombination. *Genes Dev*. 2016;30(23):2581-2595.
- Davies AA, Masson JY, McIlwraith MJ, et al. Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. *Mol Cell*. 2001;7(2):273-282.
- Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. *Nature*. 2015;521(7553):489-494.
- Pomerantz MM, Spisak S, Jia L, et al. The association between germline BRCA2 variants and sensitivity to platinum-based chemotherapy among men with metastatic prostate cancer. *Cancer*. 2017;123(18):3532-3539.
- Yan L, Sun F, Yang H, Wang Y. Relationship of BRCA2 N372 H polymorphism and risk of cancer: a systematic meta-analysis under PRISMA guidelines. *Int J Clin Exp Med*. 2018;11(10):10326-10335.
- Sakai W, Swisher EM, Karlan BY, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature*. 2008;451(7182):1116-1120.
- Sakai W, Swisher EM, Jacquemont C, et al. Functional restoration of BRCA2 protein by secondary BRCA2 mutations in BRCA2-mutated ovarian carcinoma. *Cancer Res*. 2009;69(16):6381-6386.
- Rajendra E, Venkitaraman AR. Two modules in the BRC repeats of BRCA2 mediate structural and functional interactions with the RAD51 recombinase. *Nucleic Acids Res*. 2010;38(1):82-96.
- Hilton JL, Geisler JP, Rathe JA, Hattermann-Zogg MA, DeYoung B, Buller RE. Inactivation of BRCA1 and BRCA2 in ovarian cancer. *J Natl Cancer Inst*. 2002;94(18):1396-1406.
- Sharom FJ. ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics*. 2008;9(1):105-127.
- Tamaki A, Ierano C, Szakacs G, Robey RW, Bates SE. The controversial role of ABC transporters in clinical oncology. *Essays Biochem*. 2011;50(1):209-232.
- Cole SP, Bhardwaj G, Gerlach JH, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science*. 1992;258(5088):1650-1654.
- Tarling EJ, de Aguiar Vallim TQ, Edwards PA. Role of ABC transporters in lipid transport and human disease. *Trends Endocrinol Metab*. 2013;24(7):342-350.
- Chen PL, Chen CF, Chen Y, Xiao J, Sharp ZD, Lee WH. The BRC repeats in BRCA2 are critical for RAD51 binding and resistance to methyl methanesulfonate treatment. *Proc Natl Acad Sci U S A*. 1998;95(9):5287-5292.
- Chen CF, Chen PL, Zhong Q, Sharp ZD, Lee WH. Expression of BRC repeats in breast cancer cells disrupts the BRCA2-Rad51 complex and leads to radiation hypersensitivity and loss of G(2)/M checkpoint control. *J Biol Chem*. 1999;274(46):32931-32935.