The effects of BRCA1 expression on the chemosensitivity of gastric cancer cells to platinum agents

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Abstract. Breast cancer type 1 susceptibility protein (BRCA1) is a tumor suppressor gene that encodes a nuclear phosphoprotein, which is involved in homologous recombination to repair DNA double strand breaks and maintain genome stability. When BRCA1 is mutated or altered, DNA damage may not be effectively repaired, which leads to DNA replication errors and cancer growth. Accordingly, people carrying a mutation in the BRCA1 gene possess an increased risk of several types of cancer, including breast and ovarian cancer. Previous clinical studies have reported an association between BRCA1 expression level and the incidence of gastric cancer; however, to the best of our knowledge, an *in vitro* study has not been performed to support these clinical observations. Therefore, the present study evaluated BRCA1 expression levels in gastric cancer cell lines. In addition, the IC_{50} values of cisplatin and oxaliplatin in each cell line were determined to investigate a potential correlation between BRCA1 expression level and chemosensitivity to platinum agents. The present results revealed that the BRCA1 expression level in gastric cancer is variable and associated with the treatment response to platinum-based chemotherapy. This suggests that BRCA1 may serve as a therapeutic marker for platinum-based chemotherapy in gastric cancer.

Introduction

The breast cancer type 1 susceptibility protein (BRCA1) gene is a tumor suppressor gene that is ~100 kb in length (1). BRCA1 contains 24 exons that encode a large multi-domain protein, which consists of 1,863 amino acids and is 220 kDa (1). The BRCA1 protein is predominantly present in the nucleus and is phosphorylated by various kinases, including the DNA damage sensor proteins ataxia-telangiectasia mutated (ATM), ataxia telangiectasia, Rad3-related protein and checkpoint kinase 2 (2). As a tumor suppressor, BRCA1 serves an important role in the response to hazardous DNA damage, including DNA double strand breaks, which are repaired by error-free homologous recombination (2). In addition, BRCA1 interacts with a number of proteins involved in chromatin remodeling, transcriptional regulation and the cell cycle to maintain genome integrity (3). In total, >500 different BRCA1 mutations have been identified throughout the coding region and untranslated region (4). A mutation or alteration in BRCA1 results in DNA replication errors and mutations, which induce tumor growth (2). Germline mutations in BRCA1 and breast cancer type 2 susceptibility protein are responsible for hereditary breast-ovarian cancer syndromes (HBOCs). Patients with a HBOC are at an increased risk of breast, ovarian and fallopian tube cancer, and, to a lesser extent, other cancer types, including pancreatic, stomach, laryngeal and prostate cancer (5). In addition, decreased expression or loss of BRCA1 has been reported in sporadic breast cancer and ovarian cancer (6,7). The decrease or loss of BRCA1 expression can be explained by a mutation of the BRCA1 gene, BRCA1 promoter hypermethylation, or overexpression of microRNAs that target BRCA1 mRNA (8-12).

Previously, clinical studies reported an association between a low BRCA1 expression level or BRCA1 mutation and the incidence and prognosis of gastric cancer (13,14). Patients with a high BRCA1 expression level demonstrate a longer overall survival time (14-16). By contrast, patients with a BRCA1-negative status are more likely to have a high tumor grade according to The American Joint Committee on Cancer, a high Tumor-Node-Metastasis (TNM) stage, or a poorly differentiated tumor (14-17). In addition, patients with a BRCA1 single nucleotide polymorphism (SNP) were identified to possess a predisposition for gastric cancer. In the BRCA1 coding sequence, a rs799917 T>C SNP increases the risk of gastric cancer and this SNP is associated with shorter overall survival and progression-free survival times (18,19).

Platinum agents, including cisplatin and oxaliplatin, are popular anticancer drugs in clinical practice (20). Cisplatin exerts cytotoxic effects by forming DNA adducts and inducing DNA lesions (20,21). The predominant mechanism that repairs DNA adducts is the nucleotide excision repair pathway; however, the mismatch repair pathway can also serve

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a role (20). Each repair pathway typically arrests the cell cycle and resolves the DNA lesion; however, if the damage is excessive the cell will transduce signals to initiate apoptosis (22). In addition, cisplatin has the ability to deplete methionine and cysteine-containing peptides, including glutathione, which depletes antioxidant molecules and induces oxidative stress (23). Reactive oxygen species and nitric oxide induce cytotoxicity via mitochondrial outer membrane permeabilization, which promotes apoptosis via the intrinsic pathway (24). The mechanism of action of oxaliplatin is similar to that of cisplatin; however, it produces fewer adducts and demonstrates a higher cytotoxicity (25). For the treatment of gastric cancer, platinum agents can be used as a monotherapy or in the following combinations: Cisplatin and 5-fluorouracil (5-FU); epirubicin, cisplatin and 5-FU; epirubicin, cisplatin and capecitabine; mitomycin, cisplatin and 5-FU; docetaxel, cisplatin and 5-FU; and 5-FU, leucovorin and oxaliplatin (26). A number of studies have revealed that BRCA1-negative gastric cancer is associated with a poor prognosis and is more sensitive to platinum-based adjuvant chemotherapy compared with BRCA1-positive gastric cancer (15,16). These findings indicate that patients with BRCA1-negative gastric cancer have a longer overall survival time and improved prognosis, which suggests an important association between BRCA1 expression and platinum-based chemotherapy.

In summary, clinical studies have revealed an association between BRCA1 expression and gastric cancer; however, to the best of our knowledge, a comprehensive *in vitro* study has not been performed to support this clinical observation. Therefore, the present study investigated whether BRCA1 expression is correlated with chemosensitivity to platinum agents, including cisplatin and oxaliplatin, in a number of gastric cancer cell lines. The current study revealed that the BRCA1 expression level is variable in different types of gastric cancer and is positively correlated with the treatment response to platinum-based chemotherapy. This suggests that BRCA1 may serve as a therapeutic marker to predict the effectiveness of platinum-based chemotherapy in gastric cancer.

Materials and methods

Cell culture. The human suspension gastric cancer cell lines SNU1, SNU5, SNU16 and SNU620 were purchased from the Korean Cell Line Bank (Seoul, Korea) and cultured in RPMI-1640 (Welgene, Inc., Gyeongsan, South Korea) supplemented with 20% fetal bovine serum (Welgene, Inc.), 1 mM sodium pyruvate (Welgene, Inc.), minimal essential medium non-essential amino acids (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 10 mM HEPES (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/m streptomycin (Gibco; Thermo Fisher Scientific, Inc.). The human adherent gastric cancer cell lines SNU216, SNU484, SNU601, AGS and NCI-N87, and the human mixed type gastric cancer cell line KATO III (all from the Korean Cell Line Bank) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The human gastric adherent gastric cancer cell line Hs746T was obtained from the Korean Cell Line Bank. The human normal gastric cell line HFE-145 was kindly provided by Professor Won Sang Park (Catholic University, Seoul, Korea) with permission from Professor Hassan Ashktorab (Howard University, Washington, DC, USA) who had originally established the cell line. Hs746T and HFE-145 cells were cultured in Dulbecco's modified Eagle's medium (Welgene, Inc.) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. All cells were cultured at 37˚C and 5% CO_2.

Cell viability assay. All gastric cancer cell lines and HFE-145 cells were seeded at a density of $5x10^3$ cells/75 μl per well in a 96-well plate. Following incubation overnight, the cells were treated with 0.000, 0.025, 0.076, 0.228, 0.685, 2.060, 6.170, 18.500, 55.500 and 167.000 μM cisplatin (Selleck Chemicals, Houston, TX, USA) or 0.000, 0.019, 0.058, 0.173, 0.518, 1.550, 4.660, 14.000, 42.000 and 126 μM oxaliplatin (Selleck Chemicals). Cisplatin and oxaliplatin powders were obtained, and 167 μM cisplatin and 126 μM oxaliplatin stock solutions were prepared in dimethyl sulfoxide (Sigma-Aldrich; Merck KGaA) and subsequently three-fold diluted in culture medium as aforementioned. Following 48 h of treatment at 37˚C, 20 μl MTT (5 mg/ml; Sigma-Aldrich; Merck KGaA) was added. Cells were incubated for 6 h and then 150 μl acidic isopropanol (0.04 N HCl final concentration) was added to dissolve the formazan crystals. To quantify the viable cells, the optical density was measured at 540 nm using an EMax microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA).

Western blotting. All gastric cancer cell lines and HFE-145 cells were harvested and resuspended in lysis buffer containing 0.0625 M Tris-HCl (pH 6.8), 20% glycerol, 2% SDS and 5% b-mercaptoethanol in distilled water. The protein concentration was measured using a Pierce™ BCA Protein assay kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total protein (50 μg) was then loaded onto an 8% SDS-PAGE gel and transferred to an Immune-Blot® polyvinylidene difluoride membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membrane was blocked for 1 h at room temperature with TBS containing 0.1% Tween-20 (TBS-Tween; Amresco, LLC, Solon, OH, USA) and 5% skim milk powder (Bioworld Technology, Inc., St. Louis Park, MN, USA). Following blocking, the membrane was washed and incubated with an anti-BRCA1 mouse monoclonal antibody (catalog no. OP92; 1:1,000; EMD Millipore, Billerica, MA, USA) in TBS-Tween containing 5% bovine serum albumin at 4˚C overnight. Following washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated horse anti-mouse IgG antibody (catalog no. 7076S; 1:3,000; Cell Signaling Technology, Inc., Danvers, MA, USA) for 1 h at room temperature. For the detection of β-tubulin, an anti-β-tubulin rabbit monoclonal antibody (catalog no. 2128S; 1:2,000; Cell Signaling Technology, Inc.) was used at 4˚C overnight, followed by incubation with goat anti-rabbit IgG-HRP-conjugated antibody (catalog no. 1706515; 1:3,000; Bio-Rad Laboratories, Inc.) for 1 h at room temperature. West-Q Pico enhanced chemiluminescent solution (GenDEPOT, Barker, TX, USA) was used to visualize the protein bands on the membrane. A ChemiDoc XRS densitometer (Bio-Rad Laboratories, Inc.) and Quantity One software (version 4.6.3; Bio-Rad Laboratories, Inc.) were used to detect and quantify the protein bands.

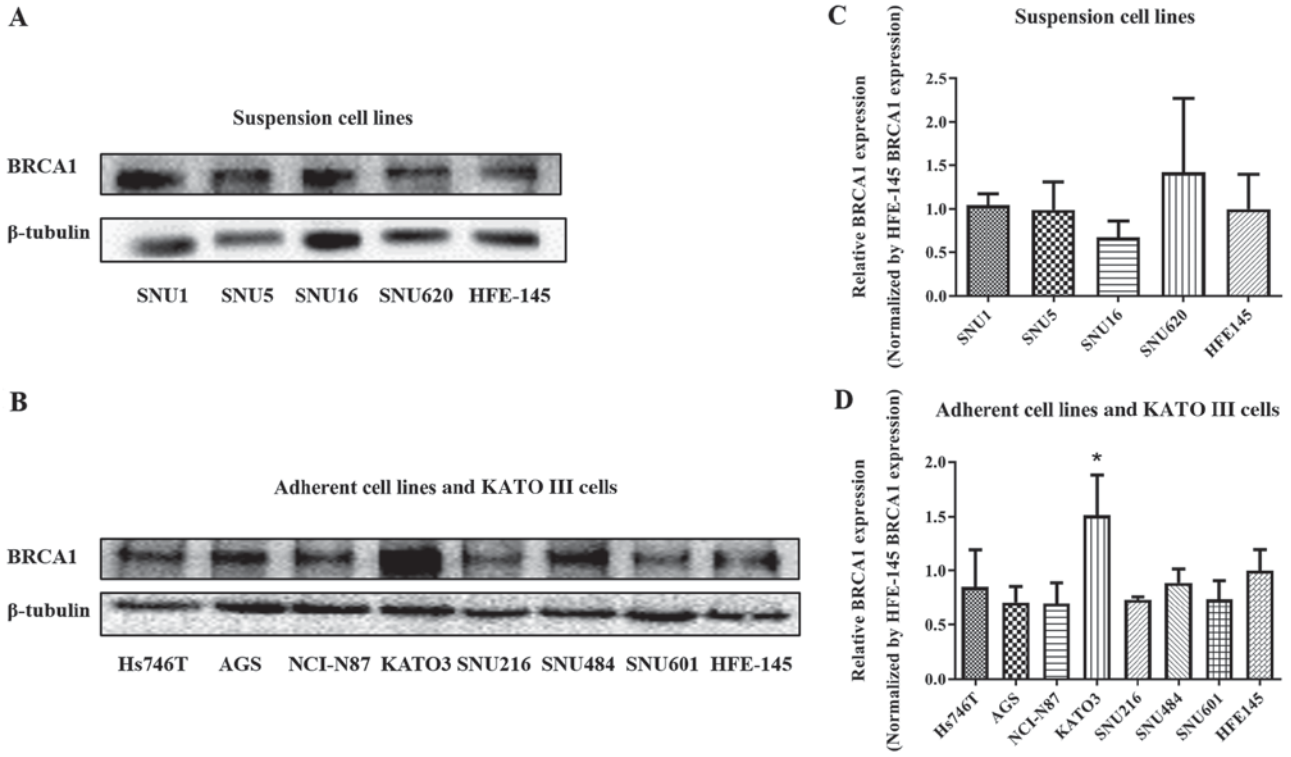

Figure 1. BRCA1 protein expression levels in gastric cancer cell lines. BRCA1 expression levels in the (A) suspension cell lines, (B) adherent cell lines and mixed type KATO III cell line were measured by western blot analysis. The BRCA1 expression levels in the (C) suspension cell lines, (D) adherent cell lines and KATO III cell line were quantified and normalized to that of HFE-145 cells. One-way analysis of variance followed by Dunnett's multiple comparison test was applied to compare the BRCA1 protein levels in the different cell lines. Data are presented as the mean ± standard deviation (n=3). *P<0.05 vs. HFE-145. BRCA1, breast cancer type 1 susceptibility protein.

Statistical analysis. All data are presented as the mean ± standard deviation. Each experiment was performed a minimum of three times and representative data were obtained. Pearson's correlation coefficients were calculated using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Statistical significance was assessed by one-way analysis of variance followed by Dunnett's multiple comparison test. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) or R 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria). P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of BRCA1 at the protein level. To measure BRCA1 protein expression levels in various gastric cancer cell lines western blot analysis was performed. The gastric cancer cell lines were categorized into suspension cell lines and adherent cell lines, and protein expression in these two categories was analyzed separately. KATO III cells are mixed type gastric cancer cells, growing as mixed adherent and suspension cultures. The BRCA1 expression in the mixed type KATO III cells was also analyzed. As presented in Fig. 1, the BRCA1 protein expression level varies depending on the cell type. No significant differences were identified in the BRCA1 expression in the suspension or adherent gastric cancer cell lines compared with the HFE-145 control cell line. However, BRCA1 protein expression was significantly higher in the mixed type gastric cancer KATO III cell line compared with that in the HFE-145 cell line (P<0.05).

Effects of platinum agents against gastric cancer cell lines. To investigate the anticancer effects of platinum agents on gastric cancer cells, the IC_{50} values of cisplatin or oxaliplatin in each cell line were measured using an MTT assay. As presented in Tables I and II, the IC_{50} values of cisplatin or oxaliplatin depend on the type of gastric cancer cell. Among the suspension cell lines, the IC_{50} values for both platinum agents were higher in the SNU1 and SNU620 cells compared with the normal HFE-145 gastric cell line. The IC_{50} value of cisplatin was lower in SNU5 and SNU16 cells compared with HFE-145 cells; however, the IC_{50} value of oxaliplatin was higher in the SNU5 and SNU16 cells compared with HFE-145 cells. Among the adherent cell lines, the IC_{50} values for both platinum agents were lower in AGS, SNU216, SNU484, SNU601 and NCI-N87 cells compared with the normal HFE-145 cells. The IC_{50} value of cisplatin was lower in Hs746T cells compared with HFE-145 cells; however, the IC_{50} value of oxaliplatin was higher in Hs746T cells compared with HFE-145 cells. The mixed type cell line KATO III was identified to exhibit a higher resistance to both platinum agents compared with HFE-145 cells.

Correlation between BRCA1 expression and chemosensitivity to platinum agents. To investigate the effects of BRCA1 expression on the chemosensitivity of gastric cancer cells to platinum agents, correlation coefficients between BRCA1 protein expression level and the IC_{50} of cisplatin or oxaliplatin were calculated

Table I. IC_{50} values of cisplatin and oxaliplatin in suspended cells and control HFE-145 cells.

Cell line	IC_{50} of cisplatin, μM[a]	P-value[b]	IC_{50} of oxaliplatin, μM[a]	P-value[b]
SNU1	19.78±1.20	0.01770	23.13±4.52	0.01140
SNU5	11.28±2.07	0.05880	49.2±3.44	$1.8x10^{-5}$
SNU16	9.66±0.47	0.00160	29.62±0.27	0.00024
SNU620	25.15±1.28	0.00001	32.11±2.50	0.00001
HFE-145	15.68±1.57	-	14.64±2.00	-

[a]Data are presented as the mean ± standard deviation of three or four experiments. [b]IC_{50} of each drug in HFE-145 cells vs. each other cell line, as assessed by one-way analysis of variance followed by Dunnett's multiple comparison test using R.

Table II. IC_{50} values of cisplatin and oxaliplatin in adherent cells, mixed type KATO III cells, and control HFE-145 cells.

Cell line	IC_{50} of cisplatin, μM[a]	P-value[b]	IC_{50} of oxaliplatin, μM[a]	P-value[b]
Hs746T	10.37±2.61	0.0329	15.75±0.79	0.90159
AGS	14.60±2.20	0.9794	9.87±0.81	0.00741
SNU216	14.77±1.38	0.9918	13.43±2.02	0.85964
SNU484	9.06±0.75	0.0071	13.45±0.85	0.87080
SNU601	14.44±1.62	0.9249	9.11±1.72	0.00250
NCI-N87	13.07±1.67	0.5008	8.07±0.76	0.00037
KATO III	35.68±3.37	$7.5x10^{-11}$	37.02±2.39	$<2.0x10^{-16}$
HFE-145	15.68±1.57	-	14.64±2.00	-

[a]Data are presented as the mean ± standard deviation of three or four experiments. [b]IC_{50} of each drug in HFE-145 cells vs. each other cell line, as assessed by one-way analysis of variance followed by Dunnett's multiple comparison test using R.

(Fig. 2). The suspension cell lines demonstrated a significant positive correlation between BRCA1 protein expression level and the IC_{50} value of cisplatin ($P<0.05$). In addition, significant positive correlations were identified between the BRCA1 protein expression level and the IC_{50} values of cisplatin ($P<0.01$) and oxaliplatin ($P<0.001$) in the adherent cell lines.

Discussion

Gastric cancer was the third leading cause of cancer-associated mortality worldwide in 2018 (27). Due to late detection and diagnosis at an advanced stage, patients with gastric cancer often have a poor prognosis (28). In Korea, gastric cancer was estimated to be the fourth leading cause of cancer-associated mortality both in males and females in 2018 (29). As one of the major types of cancer, gastric cancer remains a global health burden; therefore, there is a requirement to identify markers that may improve prognosis and treatment.

BRCA1 expression level is widely used to predict the prognosis of breast cancer and ovarian cancer. BRCA1 expression is lower in sporadic and inherited breast cancer (30), and >50% of epithelial ovarian cancer cases exhibit a BRCA1-deficient status (8,31). In addition to the identified association between BRCA1 expression and breast and ovarian cancer, previous studies have reported that BRCA1 expression is associated with the prognosis of gastric cancer (14-16). A high TNM stage or poorly differentiated tumor is associated with a BRCA1-negative status (14-17). SNU484 and SNU601 cells, and all suspension gastric cancer cell lines used in the present study are poorly differentiated (32,33).

Treatment regimes involving cisplatin and oxaliplatin have been widely used to treat gastric cancer (26). In general, the current study revealed that the IC_{50} values of both platinum agents were higher in the suspension cell lines (16.76±6.4 for cisplatin; 34.01±10.89 for oxaliplatin) compared with the adherent cell lines (12.68±2.75 for cisplatin; 11.63±3.00 for oxaliplatin). Previous clinical studies have reported that poorly differentiated or advanced-stage cancer cases are more likely to have a BRCA1-negative status (14-17). The suspension cell lines used in the present study were poorly differentiated and exhibited similar properties to advanced-stage cancer, which suggests they would be sensitive to platinum agents. However, the suspension cell lines were identified to possess a higher resistance to cisplatin and oxaliplatin compared with the adherent cell line. Considering a secondary mutation that may restore BRCA1 function (34), further studies are required to assess if the suspension cell lines could gain another mutation. When correlation coefficients between BRCA1 expression and IC_{50} values were determined, lower correlation coefficients were revealed between BRCA1 expression levels and IC_{50} values for the suspension cell lines compared with the adherent cell lines, which indicates that BRCA1 influences advanced-stage cancer cells to a lesser extent compared with cancer cells at an earlier stage.

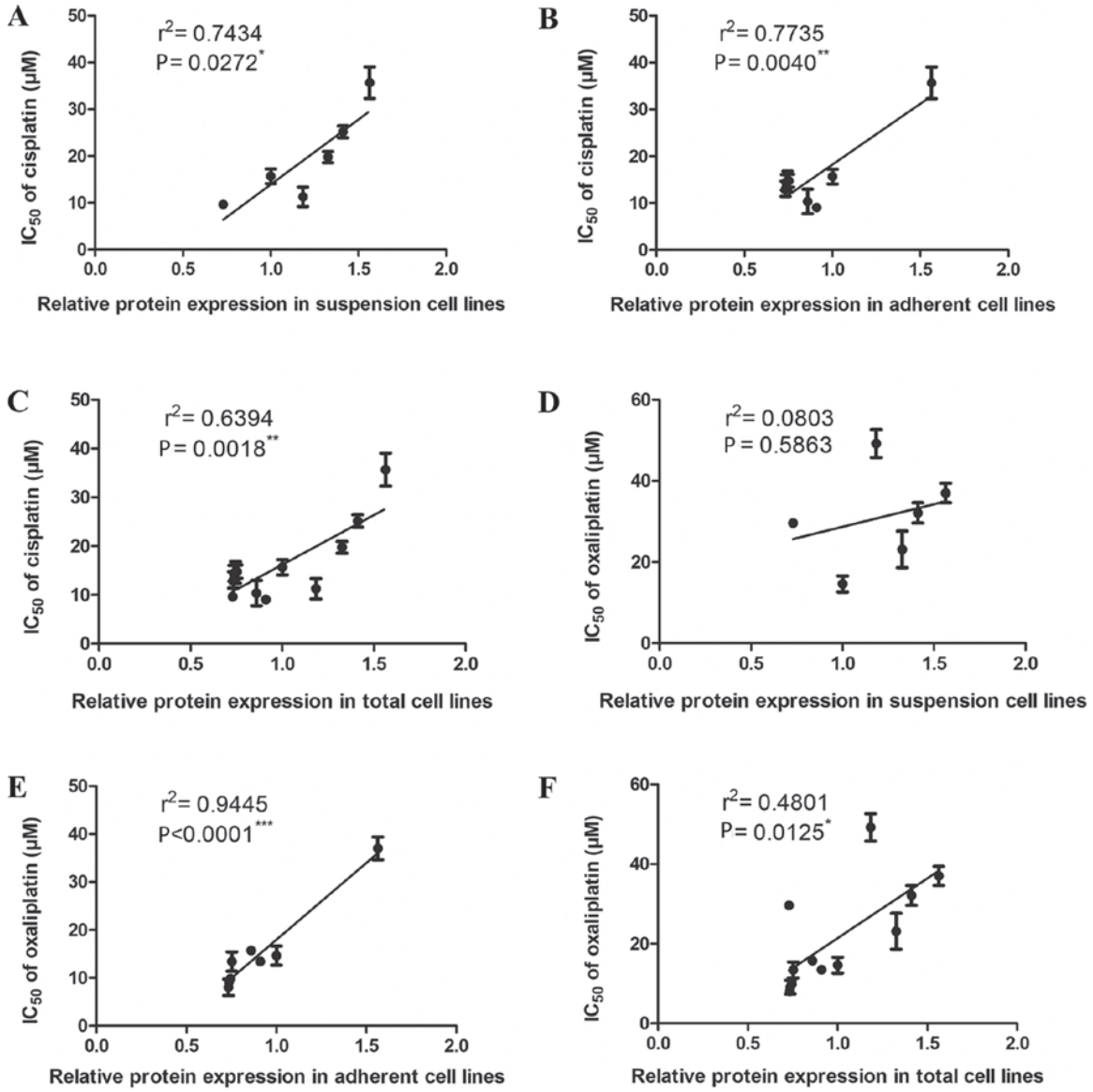

Figure 2. Dot plots representing the correlation between BRCA1 protein expression and IC_{50} values of platinum agents. (A) The IC_{50} value of cisplatin in suspension cell lines plotted against relative BRCA1 expression level. (B) The IC_{50} value of cisplatin in adherent cell lines plotted against relative BRCA1 expression level. (C) The IC_{50} value of cisplatin in all cell lines plotted against relative BRCA1 expression level. (D) The IC_{50} value of oxaliplatin in suspension cell lines plotted against relative BRCA1 expression level. (E) The IC_{50} value of oxaliplatin in adherent cell lines plotted against relative BRCA1 expression level. (F) The IC_{50} value of oxaliplatin in all cell lines plotted against relative BRCA1 expression level. Correlation coefficients and P-values are presented in the corresponding dot plot. The IC_{50} values are presented as the mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001. BRCA1, breast cancer type 1 susceptibility protein.

Cisplatin and oxaliplatin are understood to respond to cancer cells via a similar mechanism, including the formation of adducts to double strands of DNA (26). However, cisplatin and oxaliplatin exhibit different effects on the DNA mismatch repair pathway. Cisplatin-DNA adducts demonstrate a stronger affinity for the mismatch repair proteins MSH2 and MutS compared with oxaliplatin-DNA adducts; therefore, mismatch repair proteins are more susceptible to cisplatin cytotoxicity (35,36). When mismatch repair proteins bind to cisplatin-DNA adducts, the cytotoxicity increases due to an enhancement of the apoptosis pathway (37,38) and DNA translesion synthesis (39). If mismatch repair pathways are deficient or mutated, cisplatin resistance typically occurs (40). In gastric cancer, hypermethylation of the promoter region of the mismatch repair protein MLH1 has been reported (41), which leads to silencing of the MLH1 gene. Hypermethylation of the MLH1 promoter has been identified in >50% of gastric cancer cases, which demonstrate a high level of microsatellite instability (MSI-H) (42-47). Furthermore, patients with MSI-H have been reported to have no MLH1 and MSH2 protein expression (43,48). Downregulation of the mismatch repair gene alone does not promote carcinogenesis (49); therefore, additional alterations in the expression of other genes would be required.

The BRCA1-associated genome surveillance complex (BASC) is composed of numerous proteins, including BRCA1, MSH2, MSH6, MutL homolg 1 (MLH1), ATM, bloom syndrome RecQ like helicase and replication factor C, and the RAD50-MRE11-nibrin protein complex. BRCA1 and MLH1 or BRCA1 and the MSH2-MSH6 heterodimer interact with each other within the complex (50). In addition, a study

investigating hereditary nonpolyposis colon cancer, which increases the risk of GC (51), revealed an interaction between BRCA1 and the MSH2-MSH6 complex (52), which suggests BASC serves a role in the pathogenicity of gastric cancer. Therefore, further studies with a focus on mismatch repair proteins, including MSH2, MSH6 and MLH1, are required to improve understanding regarding the association between BRCA1 and the cytotoxicity of platinum agents.

In conclusion, the present study revealed that the expression level of BRCA1 is variable in different types of gastric cancer. In addition, BRCA1 expression level in adherent gastric cancer cells was identified to be correlated with the treatment response to cisplatin and oxaliplatin. Furthermore, a correlation was observed in the suspension cell lines for cisplatin. Therefore, the current study suggests that BRCA1 may be used as a therapeutic marker to predict the sensitivity for platinum based anticancer agents in gastric cancer.

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Availability of data and materials

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

Authors' contributions

HM conceived and designed the experiments. GK, JK and SYH performed the experiments and collected the data. GK, IGH, HSK and HM analyzed the data and prepared the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Rosen EM, Fan S, Pestell RG and Goldberg ID: BRCA1 gene in breast cancer. J Cell Physiol 196: 19-41, 2003.
2. Roy R, Chun J and Powell SN: BRCA1 and BRCA2: Different roles in a common pathway of genome protection. Nat Rev Cancer 12: 68-78, 2011.
3. Joo WS, Jeffrey PD, Cantor SB, Finnin MS, Livingston DM and Pavletich NP: Structure of the 53BP1 BRCT region bound to p53 and its comparison to the Brca1 BRCT structure. Genes Dev 16: 583-593, 2002.
4. Mullan PB, Quinn JE and Harkin DP: The role of BRCA1 in transcriptional regulation and cell cycle control. Oncogene 25: 5854-5863, 2006.
5. Streff H, Profato J, Ye Y, Nebgen D, Peterson SK, Singletary C, Arun BK and Litton JK: Cancer incidence in first- and second-degree relatives of BRCA1 and BRCA2 mutation carriers. Oncologist 21: 869-874, 2016.
6. Linger RJ and Kruk PA: BRCA1 16 years later: Risk-associated BRCA1 mutations and their functional implications. FEBS J 277: 3086-3096, 2010.
7. Wilson CA, Ramos L, Villaseñor MR, Anders KH, Press MF, Clarke K, Karlan B, Chen JJ, Scully R, Livingston D, *et al*: Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. Nat Genet 21: 236-240, 1999.
8. Sun C, Li N, Yang Z, Zhou B, He Y, Weng D, Fang Y, Wu P, Chen P, Yang X, *et al*: miR-9 regulation of BRCA1 and ovarian cancer sensitivity to cisplatin and PARP inhibition. J Natl Cancer Inst 105: 1750-1758, 2013.
9. Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, Thornton A, Norquist BM, Casadei S, Nord AS, *et al*: Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res 20: 764-775, 2014.
10. Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, Harkes IC, Repasky EA, *et al*: Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92: 564-569, 2000.
11. Moskwa P, Buffa FM, Pan Y, Panchakshari R, Gottipati P, Muschel RJ, Beech J, Kulshrestha R, Abdelmohsen K, Weinstock DM, *et al*: miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. Mol Cell 41: 210-220, 2011.
12. Liu Z, Liu J, Segura MF, Shao C, Lee P, Gong Y, Hernando E and Wei JJ: MiR-182 overexpression in tumourigenesis of high-grade serous ovarian carcinoma. J Pathol 228: 204-215, 2012.
13. Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY and Stratton MR: A mutational signature in gastric cancer suggests therapeutic strategies. Nat Commun 6: 8683, 2015.
14. Zhang ZZ, Liu YJ, Yin XL, Zhan P, Gu Y and Ni XZ: Loss of BRCA1 expression leads to worse survival in patients with gastric carcinoma. World J Gastroenterol 19: 1968-1974, 2013.
15. Chen W, Wang J, Li X, Li J, Zhou L, Qiu T, Zhang M and Liu P: Prognostic significance of BRCA1 expression in gastric cancer. Med Oncol 30: 423, 2013.
16. Kim JW, Cho HJ, Kim M, Lee KH, Kim MA, Han SW, Oh DY, Lee HJ, Im SA, Kim TY, *et al*: Differing effects of adjuvant chemotherapy according to BRCA1 nuclear expression in gastric cancer. Cancer Chemother Pharmacol 71: 1435-1443, 2013.
17. Chen XR, Zhang WZ, Lin XQ and Wang JW: Genetic instability of BRCA1 gene at locus D17S855 is related to clinicopathological behaviors of gastric cancer from Chinese population. World J Gastroenterol 12: 4246-4249, 2006.
18. Wang K, Xu L, Pan L, Xu K and Li G: The functional BRCA1 rs799917 genetic polymorphism is associated with gastric cancer risk in a Chinese Han population. Tumour Biol 36: 393-397, 2015.
19. Shim HJ, Yun JY, Hwang JE, Bae WK, Cho SH, Lee JH, Kim HN, Shin MH, Kweon SS, Lee JH, *et al*: BRCA1 and XRCC1 polymorphisms associated with survival in advanced gastric cancer treated with taxane and cisplatin. Cancer Sci 101: 1247-1254, 2010.
20. Wang D and Lippard SJ: Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 4: 307-320, 2005.
21. Borst P, Rottenberg S and Jonkers J: How do real tumors become resistant to cisplatin? Cell Cycle 7: 1353-1359, 2008.
22. Galluzzi L, Vitale I, Michels J, Brenner C, Szabadkai G, Harel-Bellan A, Castedo M and Kroemer G: Systems biology of cisplatin resistance: Past, present and future. Cell Death Dis 5: e1257, 2014.
23. Zimmermann T, Zeizinger M and Burda JV: Cisplatin interaction with cysteine and methionine, a theoretical DFT study. J Inorg Biochem 99: 2184-2196, 2005.
24. Circu ML and Aw TY: Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 48: 749-762, 2010.
25. Martinez-Balibrea E, Martínez-Cardús A, Ginés A, Ruiz de Porras V, Moutinho C, Layos L, Manzano JL, Bugés C, Bystrup S, Esteller M and Abad A: Tumor-related molecular mechanisms of oxaliplatin resistance. Mol Cancer Ther 14: 1767-1776, 2015.
26. Ajani JA: Evolving chemotherapy for advanced gastric cancer. Oncologist 10 (Suppl 3): S49-S58, 2005.

27. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
28. Carcas LP: Gastric cancer review. J Carcinog 13: 14, 2014.
29. Jung KW, Won YJ, Oh CM, Kong HJ, Cho H, Lee JK, Lee DH and Lee KH: Prediction of cancer incidence and mortality in Korea, 2016. Cancer Res Treat 48: 451-457, 2016.
30. Mueller CR and Roskelley CD: Regulation of BRCA1 expression and its relationship to sporadic breast cancer. Breast Cancer Res 5: 45-52, 2003.
31. McMillen BD, Aponte MM, Liu Z, Helenowski IB, Scholtens DM, Buttin BM and Wei JJ: Expression analysis of MIR182 and its associated target genes in advanced ovarian carcinoma. Mod Pathol 25: 1644-1653, 2012.
32. Park JG, Frucht H, LaRocca RV, Bliss DP Jr, Kurita Y, Chen TR, Henslee JG, Trepel JB, Jensen RT, Johnson BE, *et al*: Characteristics of cell lines established from human gastric carcinoma. Cancer Res 50: 2773-2780, 1990.
33. Park JG, Yang HK, Kim WH, Chung JK, Kang MS, Lee JH, Oh JH, Park HS, Yeo KS, Kang SH, *et al*: Establishment and characterization of human gastric carcinoma cell lines. Int J Cancer 70: 443-449, 1997.
34. Swisher EM, Sakai W, Karlan BY, Wurz K, Urban N and Taniguchi T: Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Res 68: 2581-2586, 2008.
35. Fink D, Nebel S, Aebi S, Zheng H, Cenni B, Nehmé A, Christen RD and Howell SB: The role of DNA mismatch repair in platinum drug resistance. Cancer Res 56: 4881-4886, 1996.
36. Zdraveski ZZ, Mello JA, Farinelli CK, Essigmann JM and Marinus MG: MutS preferentially recognizes cisplatin-over oxaliplatin-modified DNA. J Biol Chem 277: 1255-1260, 2002.
37. Nehmé A, Baskaran R, Aebi S, Fink D, Nebel S, Cenni B, Wang JY, Howell SB and Christen RD: Differential induction of c-Jun NH2-terminal kinase and c-Abl kinase in DNA mismatch repair-proficient and -deficient cells exposed to cisplatin. Cancer Res 57: 3253-3257, 1997.
38. Nehmé A, Baskaran R, Nebel S, Fink D, Howell SB, Wang JY and Christen RD: Induction of JNK and c-Abl signalling by cisplatin and oxaliplatin in mismatch repair-proficient and -deficient cells. Br J Cancer 79: 1104-1110, 1999.
39. Vaisman A, Varchenko M, Umar A, Kunkel TA, Risinger JI, Barrett JC, Hamilton TC and Chaney SG: The role of hMLH1, hMSH3, and hMSH6 defects in cisplatin and oxaliplatin resistance: Correlation with replicative bypass of platinum-DNA adducts. Cancer Res 58: 3579-3585, 1998.
40. Martin LP, Hamilton TC and Schilder RJ: Platinum resistance: The role of DNA repair pathways. Clin Cancer Res 14: 1291-1295, 2008.
41. Strathdee G, MacKean MJ, Illand M and Brown R: A role for methylation of the hMLH1 promoter in loss of hMLH1 expression and drug resistance in ovarian cancer. Oncogene 18: 2335-2341, 1999.
42. Yamamoto H, Perez-Piteira J, Yoshida T, Terada M, Itoh F, Imai K and Perucho M: Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. Gastroenterology 116: 1348-1357, 1999.
43. Wu MS, Lee CW, Shun CT, Wang HP, Lee WJ, Chang MC, Sheu JC and Lin JT: Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes. Genes Chromosomes Cancer 27: 403-411, 2000.
44. Bacani J, Zwingerman R, Di Nicola N, Spencer S, Wegrynowski T, Mitchell K, Hay K, Redston M, Holowaty E, Huntsman D, *et al*: Tumor microsatellite instability in early onset gastric cancer. J Mol Diagn 7: 465-477, 2005.
45. Leung SY, Yuen ST, Chung LP, Chu KM, Chan AS and Ho JC: hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. Cancer Res 59: 159-164, 1999.
46. Pinto M, Oliveira C, Machado JC, Cirnes L, Tavares J, Carneiro F, Hamelin R, Hofstra R, Seruca R and Sobrinho-Simões M: MSI-L gastric carcinomas share the hMLH1 methylation status of MSI-H carcinomas but not their clinicopathological profile. Lab Invest 80: 1915-1923, 2000.
47. Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, Zou TT, Abraham JM, Kong D, Smolinski KN, *et al*: Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. Cancer Res 59: 1090-1095, 1999.
48. Scartozzi M, Galizia E, Freddari F, Berardi R, Cellerino R and Cascinu S: Molecular biology of sporadic gastric cancer: Prognostic indicators and novel therapeutic approaches. Cancer Treat Rev 30: 451-459, 2004.
49. Eshleman JR and Markowitz SD: Mismatch repair defects in human carcinogenesis. Hum Mol Genet 5: 1489-1494, 1996.
50. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ and Qin J: BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14: 927-939, 2000.
51. Caldas C, Carneiro F, Lynch HT, Yokota J, Wiesner GL, Powell SM, Lewis FR, Huntsman DG, Pharoah PD, Jankowski JA, *et al*: Familial gastric cancer: Overview and guidelines for management. J Med Genet 36: 873-880, 1999.
52. Wang Q, Zhang H, Guerrette S, Chen J, Mazurek A, Wilson T, Slupianek A, Skorski T, Fishel R and Greene MI: Adenosine nucleotide modulates the physical interaction between hMSH2 and BRCA1. Oncogene 20: 4640-4649, 2001.