의 학 석 사 학 위 논 문

위암에서 병원성 BRCA 유전자 돌연변이와 백금기반 항암화학요법에의 반응성 및 분자 아형과의 관련성에 대한 연구

BRCA-mutated gastric adenocarcinomas are associated with chromosomal instability and responsiveness to platinum-based chemotherapy

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이 논문을 의학석사학위 논문으로 제출함

2023년 8월

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Abstract

Background

Homologous recombination defect is an important biomarker of chemotherapy in certain tumor types and the presence of pathogenic or likely pathogenic mutations involving *BRCA1* or *BRCA2* (pBRCA) mutations is the most well-established marker for the homologous recombination defect. Gastric cancer, one of the most prevalent tumor types in Asia, also harbors pBRCA mutations.

Methods

366 cases of gastric cancer from 1820 cases analyzed through next-generation sequencing (NGS) and investigated whether the presence of biallelic or heterozygous pBRCA mutations is associated with certain molecular subtypes, and responsiveness to platinum-based chemotherapy.

Chi-square test and Wilcoxon signed-rank test were performed for pairwise comparisons. For non-parametric analysis between groups and variants, Kruskal-Wallis test was used. The Kaplan-Meier method was performed for survival analyses and the Mantel-Cox log-rank (MC) test was used to evaluate whether certain factors affect survivals.

Results

The presence of pBRCA mutations was enriched in the chromosomal instability subtype, which was detected by the proportion of copy-number altered genomic segments, and microsatellite instability subtype. Although patients with gastric cancer harboring pBRCA mutations demonstrated no better response, gastric cancer harboring biallelic pBRCA mutations showed a better response to platinum-based chemotherapy.

Conclusions

Gastric cancers with biallelic pBRCA mutations are associated with platinum-based chemotherapy. Patients with gastric cancer harboring biallelic pBRCA mutations could be considered candidates for poly(adenosine diphosphate ribose) polymerase (PARP) inhibitor therapy, and screening for pBRCA mutations is advised in gastric cancers of chromosomal instability subtype.

Keywords: Homologous Recombination; Gastric Cancer; Poly(ADP-ribose) Polymerase Inhibitors; Chromosomal Instability; Genes, BRCA2

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Introduction

Gastric cancer is the fifth most common cancer, worldwide and the most common tumor in Korea (1, 2). Many patients with gastric cancer require chemotherapy and platinum-based chemotherapy is one of the widely used chemotherapy (3-7). Although many combinations of cytotoxic chemotherapy have been developed, none of them could achieve satisfactory clinical outcomes. Thus, many molecularly targeted therapies have been tested; however the efficacy of those drugs was limited in gastric cancer, except for anti-ERBB2 therapies (1, 8).

The *BReast CAncer* (*BRCA*) gene performs a homology-directed repair, which is one of the repair systems for double-strand DNA breaks (9). Thus, pathogenic mutations involving *BRCA1* or *BRCA2* (pBRCA) mutations lead to homologous recombination deficiency (HRD). Patients with ovarian cancers showing HRD are known to respond to platinum-based chemotherapy or poly (adenosine-diphosphate ribose) polymerase (PARP) inhibitors through synthetic lethality action (10). The association between HRD and responsiveness to platinum-based chemotherapy or PARP inhibitors has been reported in several tumor types, namely, ovary, prostate, pancreas, and breast cancers (11, 12).

For other tumor types, the clinical significance of the HRD is poorly understood. Even if gastric cancer is the most common cancer among germline pBRCA mutation carriers (13), the significance of germline pBRCA mutations in this context is also poorly investigated. Furthermore, the clinical significance of somatic pBRCA mutations in gastric cancer is also poorly understood, which is probably because the zygosity of pBRCA mutations was not considered in most previous studies and gastric cancer is a heterogeneous disease in which four distinct molecular subtypes have been reported (12, 14-15). To solve this issue, we analyzed next-generation sequencing (NGS) data focusing on the presence of pBRCA mutations and their zygosity in the conjunction with the degree of chromosomal instability (CIN) and microsatellite instability (MSI) status. Then, we investigated whether the presence of pBRCA mutations is associated with responsiveness to platinum-based chemotherapy in the context of molecular subtypes.

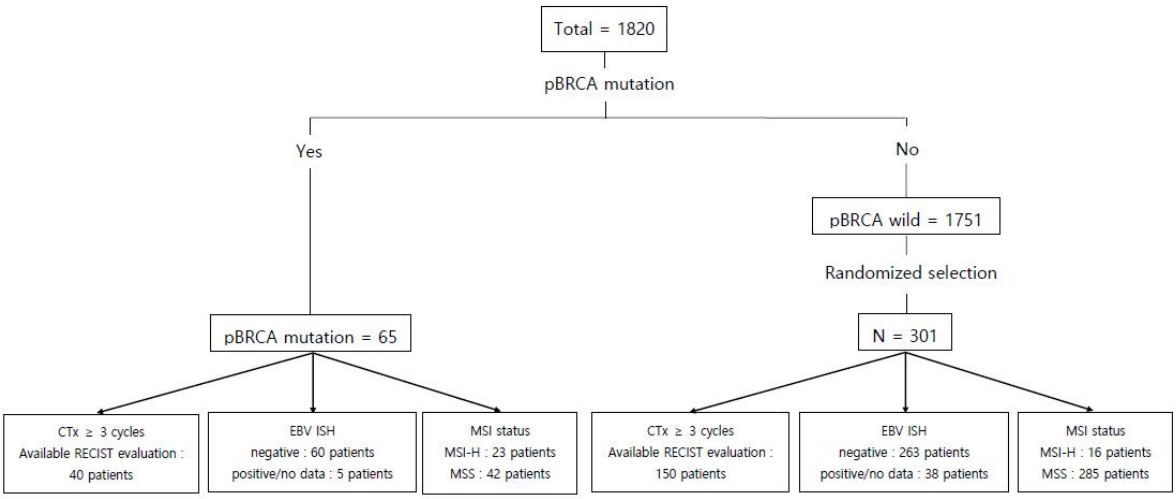

Figure 1. Patient selection process

CTx, platinum-based chemotherapy; EBV ISH, Ebstein-Barr virus in situ hybridization stain; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSS, microsatellite stable; p-BRCA , pathogenic BRCA mutation; RECIST, modified Response Evaluation Criteria in Solid Tumors

Materials and methods

Case selection and mutation analysis

Overall, 1820 patients who were pathologically diagnosed with adenocarcinoma of the stomach and underwent clinical NGS of the tumor tissues between January 2017 and July 2022 were initially included. Among them, 65 patients with gastric adenocarcinoma harboring pBRCA mutations were identified (Figure 1). The functional effects of *BRCA1* or *BRCA2* mutations were predicted using the BRCA mutation database of the Department of Pathology, University of Utah (URL: https://arup.utah.edu/database/BRCA/). In this database, 'likely pathogenic' and 'definitely pathogenic' classified mutation regarded as pathogenic BRCA mutation. These mutations have a probabilty > 0.95, disrupting gene function, and are highly likely to cause clinical consequences by sequence-based genetic tests (16). For BRCA wild-type cases, 301 of 1751 patients with BRCA-wild type tumors were randomly selecte. The MSI status was determined by our validated NGS as described previously (17**)**. Ambiguous cases were confirmed by immunohistochemistry of MLH1, MSH2, MSH6, and PMS2. Ebstein-Barr virus (EBV) in situ hybridization results were reviewed when available. Finally, HER2 amplification status was assumed by IHC of C-ERB B2 immunohistochemistry results. If the IHC results were ambiguous, a SISH test was conducted, and the results were used. This study was approved by the Ethics Committee of Asan Medical Center, Seoul, Korea (IRB no.2022-0956).

NGS analysis

The NGS study was performed using our clinically validated OncoPanel AMC v4.3 panel which is a DNA-based, hybrid capture targeted gene panel using the NextSeq 550Dx Sequencing System (Illumina, San Diego, CA, USA) as described previously (17). Briefly, this panel was approximately 1.2 Mbp with 33,524 probes targeting 382 genes, including entire exons of 199 genes, 184 hot spots, and partial introns for eight genes often rearranged in cancer. For DNA extraction, tumor area was macro-dissected from formalin-fixed, paraffin-embedded tissue blocks to achieve more than 20 % tumor purity. Sequenced reads processing and variant calling were described in detail. (18); briefly sequenced reads were processed by GATK (ver.4.2.6.1) pipeline, variant calling was performed using VarDict (ver. 1.6). Common and germline variants from somatic variant candidates were filtered out with the common dbSNP build 141 (found in >1% of samples), Exome Aggregation Consortium release 0.3.1 (http://exac.broadinstitute.org), and Korean Reference Genome database (http://152.99.75.168/KRGDB) and an in-house panel of normal variants except BRCA1 and BRCA2.

Two pathologists manually reviewed NGS data to remove false positives or false negatives and curated functional impacts of annotated *BRCA1* or *BRCA2* gene mutations. All detected SNV and InDel types, including synonymous and non-synonymous mutations in all exonic regions and splice sites, were used to calculate tumor mutation burden (TMB) (19)

Copy number analysis

Copy number burden was calculated by the fraction of copy number altered regions across the human genome. The copy number altered regions were detected using CNVkit [*PLoS computational biology* 12, e1004873, https://doi.org/10.1371/journal.pcbi.1004873 (2016)] with default parameters without normal tissue (19). Then, we classified tumors into CN-high (chromosomal instability) and CN-low (genomically stable) based on the relative copy number burden within EBV-negative/MSS tumors. Briefly, we first sorted EBV-negative/MSS tumors in ascending order according to copy number burden, and then tumors with more than 25 percentile values were classified as CN-high tumors. This method was based on the previously published TCGA data where the frequency of the CN-high tumors was 72% while that of the CN-low (genomically stable) tumors was 28%, respectively, among the EBV-negative/MSS tumors (15) .

Inference of the zygosity of pBRCA mutations

Because *BRCA1* and *BRCA2* genes are known to be tumor suppressors, complete loss of protein function might be biologically important. Thus, the zygosity of pBRCA mutations, or the loss of heterozygosity (LOH) of the pBRCA-mutant allele in tumor tissues was necessary. To achieve this, tumor purity information was re-estimated through an analysis of variant allelic fraction (VAF) patterns of the detected variants rather than through pathologists' estimation as described previously (20) with slight modification. Briefly, treuncal mutations were carefully chosen. Truncal mutations are usually well-known oncogenes that are usually heterozygous. However, such oncogenes undergo LOH or mutant allele amplification on some occasions because of tumor-specific biology or selective pressure. Thus, we also considered local copy-number profiles and re-reviewed tumor slides from which DNA had been extracted.

As a simplified model, if the truncal mutation is heterozygous (or LOH-), tumor purity (T) would be two times of VAF (V): T = 2V. If the truncal mutation is biallelic (or LOH+), tumor purity (T) would be equal to VAF (V): T = V. Based on the calculated tumor purity, the zygosity of pBRCA mutations could be determined.

If the reported VAF of the pBRCA mutation was T/2, such a mutation was thought to be heterozygous (or LOH-). If it was the same as T, the pBRCA mutation was thought to be biallelic (or LOH+). In the concept that germline non-neoplastic cells have one mutant allele and neoplastic cells lose wild allele during cancerogenesis (20), If the reported VAF of the pBRCA mutation was similar to T + (1-T)/2, the pBRCA mutation was suggested to be a germline plus somatic LOH. The distinction between biallelic somatic and germline with or without LOH was not always possible. Thus, we grouped biallelic somatic and germline with or without LOH.

Evaluation of response to platinum-based chemotherapy

To evaluate responsiveness to platinum-based chemotherapy, we further selected 40 patients with *BRCA*-mutated gastric cancer and 150 patients with *BRCA*-wild type gastric cancer who underwent more than 3 cycles of platinum-based palliative chemotherapy and had valid response evaluation data (Table 2). Response to platinum-based chemotherapy was evaluated according to the modified Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) (21). When multiple combinations of chemotherapy were given, the initial response to the first platinum-based chemotherapy was used.

Statistics

Chi-square test and Wilcoxon signed-rank test were performed for pairwise comparisons. For non-parametric analysis between groups and variants, the Kruskal-Wallis test was used. The Kaplan-Meier method was performed for survival analyses and the Mantel-Cox log-rank (MC) test was used to evaluate whether certain factors affect survivals. P-values of < 0.05 were considered statistically significant. All statistical analyses and data visualizations were performed using IBM SPSS Statistics version 28.0.1 (IBM Corp., Armonk, NY, USA) and R-Studio using R Version 4.2.2 (RStudio, Boston, MA, USA)

Results

The presence of pBRCA mutation is associated with platinum chemotherapy response in gastric carcinoma.

Table 1. Clinicopathological features of the 366 gastric cancer patients with complete NGS.

	pBRCA mutation	
Variables	**Yes (N=65)**	**No (N=301)**
Age (mean)	61.3	58.5
Sex		
Male	51 (78.3%)	198 (65.7%)
Female	14 (21.7%)	103 (34.3%)
Initial stage		
Ⅰ	8 (11.6%)	13 (4.4%)
Ⅱ	5 (8.7%)	33 (10.8%)
Ⅲ	14 (23.2%)	82 (26.9%)
Ⅳ	38 (56.5%)	173 (57.9%)
C-ERB B2 IHC positivity	2 (3.1%)	40 (13.3%)
Resectable at the time of diagnosis	27 (43.5%)	129 (42.4%)
Recurrence/Metastasis after surgery	16 (30.3%)	97 (32.7%)
Histologic diagnosis		
Adenocarcinoma WD, MD	21 (31.9%)	99 (33.0%)
Adenocarcinoma PD	20 (30.4%)	50 (16.5%)

Others	24 (37.7%)	152 (50.5%)

Most clinicopathological variables except for chemotherapy response were comparable between pBRCA mutant cases and BRCA wild type cases (Table 1). Owing to the low prevalence of pBRCA mutations, the finally selected patients were heterogeneous in terms of initial staging, treatment, and follow up method. When RECIST was employed in the evaluation of patients with evaluable diseases before the initiation of platinum-based chemotherapy, patients with gastric cancer harboring pBRCA mutation responded to the chemotherapy got no differences than those with BRCA-wild type gastric cancer (p = 0.833).

Additionally, a survival analysis was performed. There is also no difference between the BRCA mutant and wild-type groups in overall survival and progression-free survival length (Log Rank test, p = 0.341, 0.596) (Fig.2).

Loss of heterozygosity (LOH) in pBRCA mutant allele is an important predictor of responsiveness to platinum-based chemotherapy in gastric cancer.

We hypothesized that the presence of LOH in pBRCA mutant allele may result in complete loss of BRCA protein function, and hence may show a stronger association with responsiveness to platinum-based chemotherapy. Indeed, patients with gastric cancer harboring pBRCA mutations accompanied by LOH showed a stronger correlation with responsiveness to platinum-based chemotherapy (P = 0.001) LOH of pBRCA mutated tumor groups had better chemotherapy response to platinum-based chemotherapy (odds ratio 0.575 [95% confidence interval 0.506 - 0.653]). However, the presence of the LOH did not affect overall survival within the pBRCA mutated group (Figure 2A). Although pBRCA mutation was associated with the initial response to platinum-based chemotherapy, it did not affect progression-free survival regardless of the LOH of the mutant allele (Figure 2B).

Table 2. Response to platinum-based chemotherapy according to pBRCA mutation in tumor tissue

Response	**LOH of pBRCA**			**p-value**
	LOH+	**LOH-**	**Wild**	
	n (%)	n (%)	n (%)	
Complete response	1 (6.3%)	1 (4.2%)	5 (3.3%)	
Partial response	15 (93.8%)	8 (33.3%)	86 (57.3%)	
Stable disease	0 (0.0%)	12 (50.0%)	52 (34.7%)	

Progressive disease	0 (0.0%)	3 (12.5%)	7 (4.7%)	
Total	16	24	150	0.001*

*Kruskal-Wallis test

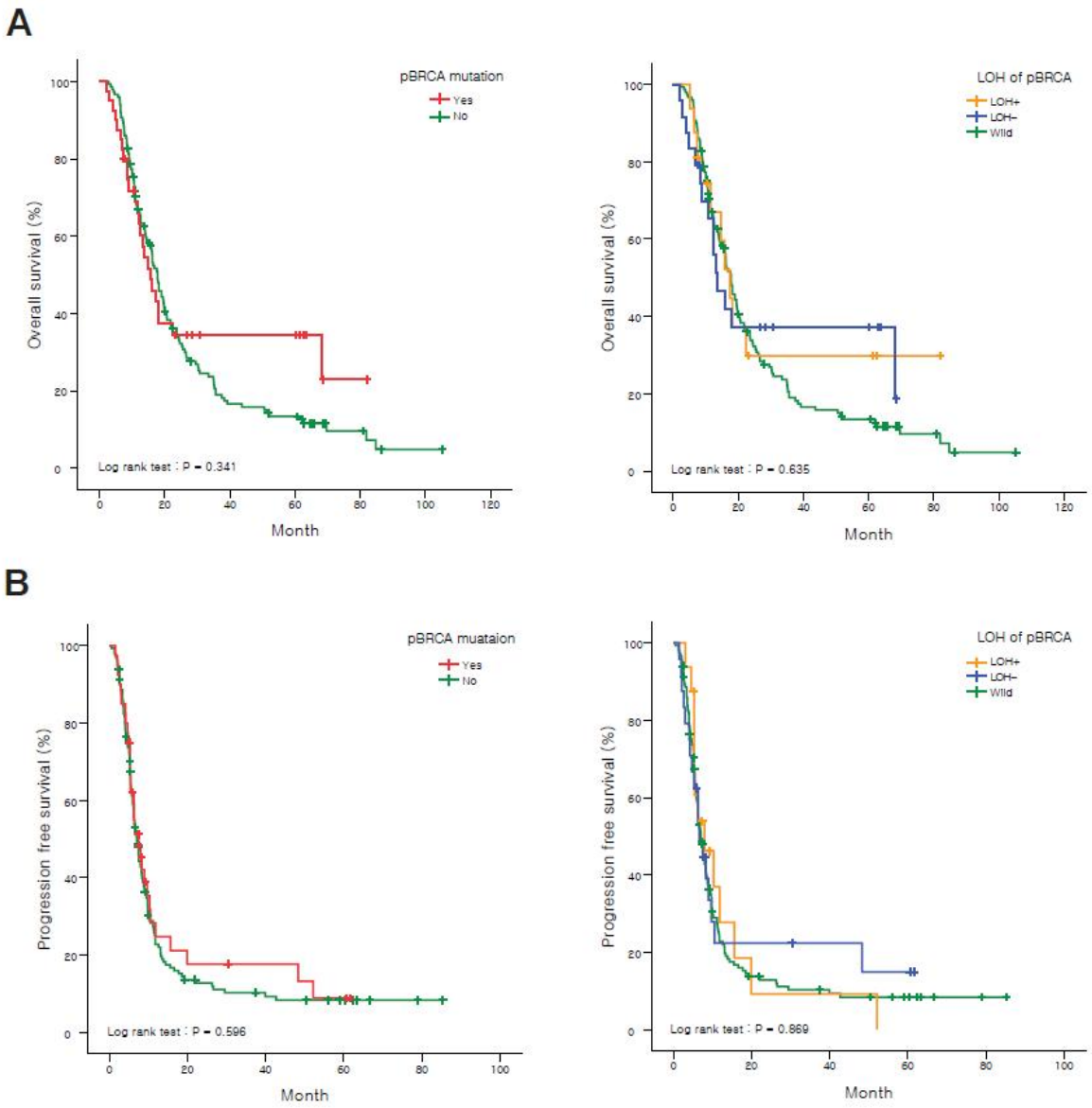

Figure 2. Survival rate according to pBRCA mutation status

A For overall survivals, patients with gastric cancer harboring pBRCA mutations versus wild type BRCA show no differences. **B** For progression survival, p-BRCA mutation and LOH do not affect the prognosis.

LOH−, no loss of heterozygosity in pathogenic BRCA mutation; LOH+, loss of heterozygosity in pathogenic BRCA mutation; p-BRCA , pathogenic BRCA mutation

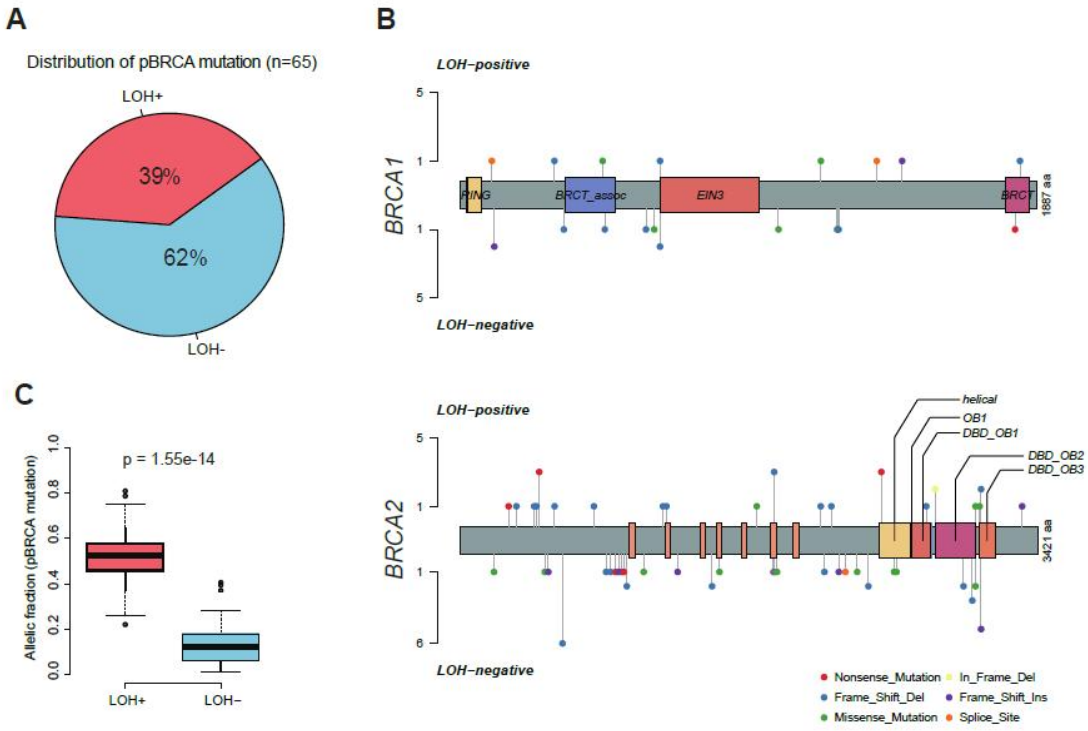

Figure 3. Details of pBRCA mutations.

A Distribution of zygosity status of the pBRCA mutations in 65 patients. 39% considered as loss of heterozyity in pBRCA-mutant allele (LOH+) **B** Distribution of the detected pBRCA mutations. Major domains were presented as box **C** Variant allelic fractions of pBRCA mutations with loss of heterozygosity are significantly higher than those of pBRCA mutations without LOH ($p < 0.001$)

The simultaneous pBRCA mutation and LOH of the mutant allele is associated with increased copy number burden and chromosomal instability (CIN) molecular subtype.

Based on the association with responsiveness to platinum-based chemotherapy, we hypothesized that gastric cancers haboring pBRCA mutation and LOH of the mutant allele might be associated with homologous recombination defect and might be a good candidate for PARP inhibitor therapy. Approximately 40% of the pBRCA mutations were accompanied by the LOH, and the location was scattered throughout the exon as usual tumor suppressors (Fig. 3A & 3B), and variant allelic fractions were significantly higher in the LOH+ group as expected (Fig 3C). The genomic landscape of the included patients was generally consistent with the previously published large-scale sequencing studies,

with *TP53* mutation being most frequent, floowed by *ARID1A* and *CDH1* mutations (Fig.4A) (15, 22). The reatively frequent *BRCA2* mutations were due to selection bias related to study design, and *BRCA2* mutations were far more frequent than *BRCA1* mutations. Interestingly, all pBRCA mutations LOH accompanies were found in microsatellite stable (MSS) tumors, while the vast majority of the pBRCA mutations without LOH were in MSI-high tumors (p = <0.001) (Fig.4B).

Next, we focused on the copy number burden, namely, relative genomic regions showing copy number alterations of both loss and gain because it may serve as a surrogate for homologous recombination defect (HRD) score. Indeed, gastric cancers harboring pBRCA mutations showed a higher copy number burden than those without pBRCA mutations (p = 0.0287, Fig. 5A). Furthermore, within pBRCA mutated tumors, tumors with concomitant LOH of the mutant allele were associated with a higher copy number burden than those without LOH (p = 0.0413, Fig. 5A). Then, we classified cases with all required data, such as MSI status, EBV in situ hybridization result, and relative copy number burden, into the previously published four molecular subtypes, all gastric cancers harboring pBRCA mutations with LOH belonged to CIN subtype while those harboring pBRCA mutations without LOH were highly enriched in MSI-H subtype (Table 3) (15). For tumor mutation burden, MSI-H subtype showed higher tumor mutation burden as expected, and pBRCA mutated tumors showed a trend toward higher tumor mutation burden than non-mutated tumors within MSS tumors (Fig. 5B). The representative copy number plots were depicted in Figure 5C.

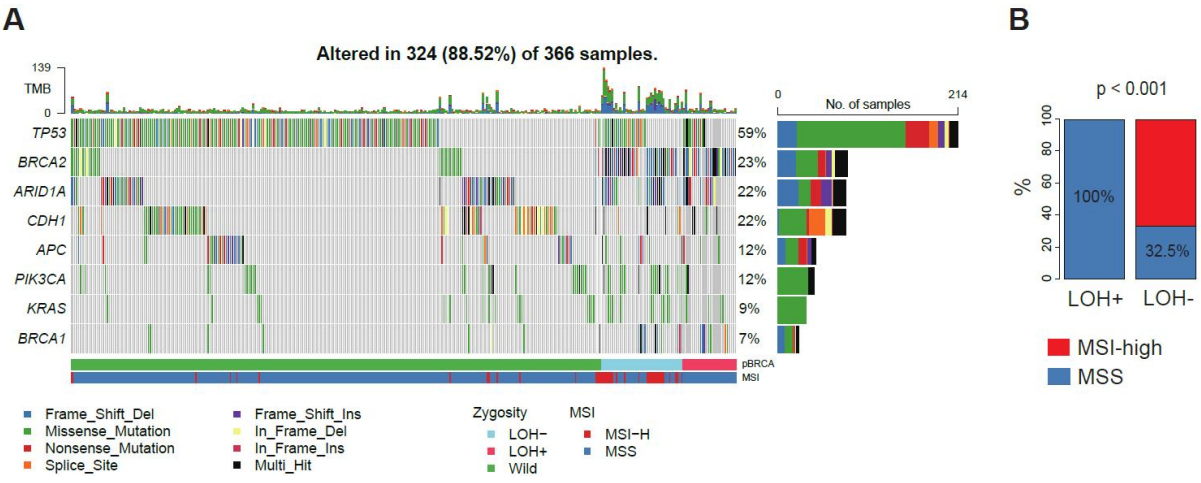

Figure 4. Genomic landscape of selected gastric cancer samples.

A Frequently mutated genes include *TP53, ARID1A,* and *CDH1* and *BRCA2* mutations are far more frequent than BRCA1 mutations. **B** All gastric cancers harboring the p-BRCA mutation and

LOH are MSS while most cases harboring heterozygous p-BRCA mutation are MSI-H.

Table 3. Molecular classification of enrolled gastric cancer.

	EBV	MSI-H	CN-low	CN-high	Total	
LOH+	0	0	0	13 (100%)	13 (100%)	
LOH-	0	24 (61.5%)	4 (10.3%)	11 (28.2%)	39 (100%)	
Wild	13 (6.0%)	12 (5.5%)	48 (22.0%)	145 (66.5%)	218 (100%)	
p-value						< 0.001*

***Kruskal-Wallis test

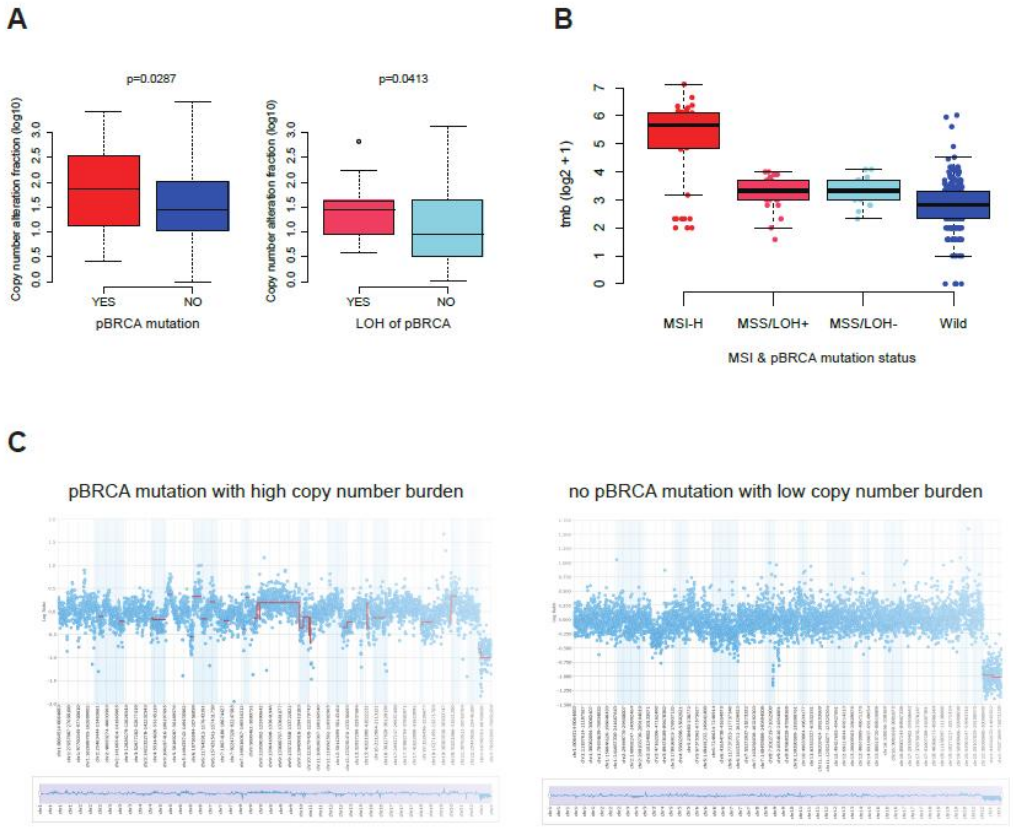

Figure 5. Comparison of copy number and tumor mutation burden.

A Copy number alteration fraction showed meaningful differences by not only *BRCA* mutation status but also by zygosity status. **B.** Tumor mutation burden comparison. Four groups showed statistically significant differences (Kruskal–Wallis test, p = 3.6e-12). Microsatellite-unstable tumors (MSI-H) had a high tumor mutation burden. The *BRCA* mutation groups (MSS/LOH+ and MSS/LOH− groups) also had a higher tumor mutation burden than the no *BRCA* gene mutation and MSS groups (wild type) (p= 0.0025 each). The tumor mutation burden between the two *BRCA* genes that mutated in the microsatellite-stable groups was not statistically different (p = 0.91). **C**

NGS data plots of the copy number burden. Cases of *BRCA* mutation with a high copy number burden had a waving copy number ratio.

Discussion

In this study, we showed that gastric cancer harboring pBRCA mutation and LOH of the mutant allele is highly enriched in CIN molecular subtype and is associated with platinum-based chemotherapy. Although we could not directly evaluate HRD score and responsiveness to PARP inhibition, our findings suggest that patients with gastric cancer harboring biallelic pBRCA mutations constitute a distinct, genetically defined subgroup that shows HRD phenotype and, hence might be a potential candidate for PARP inhibitor therapy. Considering the fact that molecularly targeted therapies are pretty limited in gastric cancer, our findings shed light on potential PARP inhibitor therapy for patients with gastric cancer harboring biallelic pBRCA mutations. In addition, the absence of improvement in progression-free survival by platinum-based chemotherapy implies that the PARP inhibitor therapy would be tried in an adjuvant setting rather than initially metastatic disease like high-grade serous carcinoma.

In terms of selecting patients who would benefit from PARP inhibitor therapy, the most validated criteria is the presence of pBRCA mutations. However, it has not been clearly defined whether the pBRCA mutations should be accompanied by the LOH of the mutant allele. We found that the biological significance of pBRCA mutation differs depending on the general genomic context. Patients with gastric cancer harboring pBRCA mutations and LOH of the mutant allele showed the most striking correlation with platinum-based chemotherapy, while those with gastric cancer harboring heterozygous pBRCA mutations were more like those with BRCA-wild type tumors. Furthermore, the vast majority of the heterozygous pBRCA mutations were found in microsatellite unstable tumors, and all pBRCA mutations found in MSI-H gastric cancers were heterozygous. It can be explained by the biological characteristics of the MSI-H tumors that frameshift insertion or deletion mutations rapidly accumulate during tumor evolution, and those "passenger" pBRCA mutations may hit quite a large exonic area of *BRCA1* or *BRCA2* genes (23). As such, most pBRCA mutations in MSI-H gastric cancers are just passengers that are not associated with the HRD phenotype. Finally, concomitant LOH of the pBRCA mutant allele represents selection pressure toward HRD and may lead to complete loss of BRCA protein function and HRD phenotype. Indeed, the strong

association between responsiveness to platinum-based chemotherapy and pBRCA mutation plus LOH remained significant within the MSS subgroup (p = 0.005) (Table 4).

To further define molecular subgroups that can serve as a target population for HR-targeted therapies, we tried to classify our cases into the previously published four molecular subtypes (15, 19) and found that gastric cancers harboring pBRCA mutations plus LOH are exclusively CIN subtypes. Thus, we propose that potential targets for HR-targeted therapies should be screened in the CIN subtype of gastric cancers. During the assignment of molecular subtypes, we assumed that our study population is similar to that of the TCGA project rather than unsupervised clustering. We admit that our assumption may not be true because our sample set has a profound selection bias due to the case-control study design. External validation using independent sample sets with complete multi-omics and chemotherapy response data might be necessary to overcome this limitation. In this study, we could not directly measure HRD score for various reasons, and we used relative copy number burden as a surrogate marker. This may be a fundamental limitation because not all aneuploidies are associated with the HRD phenotype. Furthermore, we chose responsiveness to platinum-based chemotherapy as a clinical outcome because no patient has received PARP inhibitor therapy. To overcome those limitations, further studies involving direct measurement of HRD score or randomized clinical trials of PARP inhibitor therapies in genomically defined patient populations are needed.

Because our study population has a selection bias and potential confounders, the prognostic significance is hard to determine. In a multivariate analysis including tumor stage, pBRCA mutation, MSI status, ERBB2 status, and age as covariates, only tumor stage was independently associated with overall survival (data not shown). Notably, the pBRCA mutation status plus LOH was not associated with better progression-free survival despite its association with better response to platinum-based chemotherapy. The efficacy of platinum-based chemotherapy may be limited in the presence of measurable disease. Thus, the potential use of PARP inhibition should be considered in an adjuvant setting after curative resection like high-grade serous carcinoma (24). Finally, our findings suggest that the potential candidates for PARP inhibitor therapy should be searched in the CIN subtype of gastric cancer because all gastric cancers harboring pBRCA mutation and LOH of the mutant allele were exclusively found in the CIN subtype.

Taken together, patients with gastric cancer harboring pBRCA mutation and LOH of the mutant allele could be considered as potential candidates for PARP inhibitor therapy in an adjuvant setting after curative resection, and such patients can be screened if their

gastric cancers are of CIN molecular subtype. Although direct assessment of HRD scores by validated methods and randomized clinical trials involving PARP inhibitors are required, we think our findings opened a new opportunity for molecularly targeted therapy in a subset of patients harboring CIN subtype gastric cancer.

Table 4. Response to platinum-based chemotherapy according to pBRCA mutation and MSS in tumor tissue

	LOH of pBRCA in MSS tumor			
Response	**LOH+**	**LOH-**	**Wild**	**p-value**
	n (%)	n (%)	n (%)	
Complete response	1 (6.3%)	0 (0.0%)	5 (3.5%)	
Partial response	15 (93.8%)	6 (50.0%)	82 (57.7%)	
Stable disease	0 (0.0%)	5 (41.7%)	48(33.8%)	
Progressive disease	0 (0.0%)	1 (8.3%)	7 (4.9%)	
Total	16	12	142	0.005*

*****Kruskal-Wallis test

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국문요약

연구배경 및 목적

위암은 전세계적으로 흔하게 발생하는 암이며, 특히 한국에서는 가장 흔하게 발생하는 암이다. 그러나 이러한 위암의 중요도에도 불구하고 항암요법의 다양화나 사용군의 세분화는 그리 발전되지 못하였다. 특히, 많은 위암에서 Next generation sequencing (NGS)가 도입되어 그 유전이상이 충분히 확인될 수 있음에도 불구하고 표적치료에서 유전이상이 이용되는 경우는 HER2-양성을 보이는 위암 환자군에서의 표적치료 정도에 그친다.

BRCA 유전이상은 1990 년대 처음 발견된 이래로 여러 암종과의 연관성 연구가 활발히 진행되었다. BRCA 유전이상이 있는 암들이 DNA 불안정성을 유발하는 platinum 기반 항암요법에 효과적으로 반응하기에 실제로 치료에 적용되어왔다.

위암에서는 BRCA 돌연변이가 확인 된 경우에도 이에 대한 해석과 치료와의 연관성 역시 연구된 바가 드물다. 이에 본 연구에서는, BRCA 유전이상의 접합성 분류에 따른 분자병리적 세분화가 위암에서 platinum 계 항암 치료에 유의미한 상관관계를 보일 수 있는지를 확인해 보고자 한다.

연구재료와 연구방법

2017년도 1월부터 2022년 7월까지 서울아산병원에서 esophagogastric adenocarcinoma와 gastric cancer로 진단 분류된 조직검사 및 수술 검체 중 NGS 검사가 완료되어 진단에 사용된 1820건이 연구에 모집되었다. 이 중에서 BRCA 돌연변이가 확인된 65개의 케이스와 난수표를 사용한 무작위 추출을 통하여 BRCA 야생형으로 분류된 케이스 중 301건의 케이스가 연구에 사용되었다.

BRCA 돌연변이가 확인된 암종들은 접합성에대한 조사가 수행되었으며 3 회 이상의 platinum 기반 항암치료를 받은 환자군의 항암치료 반응효과 평가가 RECIST 에 의해 수행되었다.

BRCA mutation 의 여부와 항암치료의 효과를 비교하기 위하여 Fisher's exact test 및 Wilcoxon signed-rank test 가 진행되었고 생존율을 비교하기 위하여 Kaplan-Meier method 가 사용되고 Mantel-Cox log-rank (MC) test 로 비교되며 생존분석이 진행되었다.

연구결과

BRCA 돌연변이 암과 BRCA 야생형 암의 항암치료 반응을 비교한 결과, 두 군의 항암치료에 대한 반응의 통계학적 차이는 없었다. (p-value 0.833). 그러나, BRCA 유전자의 이형접합성 소실의 경우 다른 군에 비해 항암치료 반응이 좋은 것이 확인되었으며 (p-value 0.001) 오즈비는 0.575 이었다. (95% 신뢰구간 0.506-0.653) 이 외에 조사된 여러 변수 중 항암치료 반응에 유의한 결과를 도출하는 다른 인자는 없었다.

BRCA 유전자 이형접합성 소실 돌연변이의 경우, copy number alteration 의 정도가 다른 종양군에 비해 높았으며, 이는 기존의 TCGA 정보 기반 위암의 분자적 분류에 따르면 chromosomal instability (CIN) 아형에 가장 가까울 것으로 생각되었다.

결론

현재 위암의 전신치료에 있어 유전이상 연구가 임상적으로 효과적으로 사용하는 경우도 드물다. 그러나 본 연구를 통하여, BRCA 의 병리적 유전이상이 존재하는 경우 platinum 기반 항암치료를 받을 때 치료 반응이 유의미하게 좋았음이 확인되었다. 이는 전통적으로 BRCA 돌연변이 및 HRD 라는 표현형을 보일 때 platinum 항암치료가 유의미함이 이미 밝혀진 몇 가지 암종과 유사한 결과였다.

이를 바탕으로 위암 환자에서 치료 이후의 결과를 예측하지 않고 고식적으로 '맹검 치료'를 진행하기 보다 유전 검사를 결과를 통하여 미리 치료 효과에 대하여 추측할 수 있는 시작이 될 수 있을 것을 기대한다. 나아가, 이형접합성 소실 BRCA 돌연변이가 특히 HRD 표현형과의 의미 있는 연관성을 보인다면 이러한 특정 위암 환자군에서 유방암, 난소암 등과 마찬가지로 PARP inhibitor 사용이라는 새로운 기회를 열어줄 수 있을 것이다.