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Patients With Hereditary Gastric Cancer Linked to a Family History of Hereditary Breast and Ovarian Cancer (HBOC)

Takuma Hayashi[1], Kaoru Abiko[2], Nobuo Yaegashi[3], Shoko Nishikawa[2], Ikuo Konishi[3]

1 Kyoto Medical Center
2 National Hospital Organization Kyoto Medical Centre
3 Japan Agency for Medical Research and Development (AMED)

Funding: This clinical research was performed with research funding from the following: Japan Society for Promoting Science for TH (Grant No. 19K09840, 23K08881), Tokyo, Japan, and START-program Japan Science and Technology Agency for TH (Grant No. STSC20001), Tokyo, Japan and the National Hospital Organization Multicenter clinical study for TH (Grant No. 2019-Cancer in general-02), Tokyo, Japan, and The Japan Agency for Medical Research and Development (AMED) (Grant No. 22ym0126802j0001), Tokyo, Japan.

Potential competing interests: No potential competing interests to declare.

Abstract

Helicobacter pylori (*H. pylori*), a bacteria which chronically infects the stomach of approximately half people in the world, is a risk factor for the development of gastric cancer (GC). However, the detailed mechanism by which *H. pylori* infection induces GC development remains unclear. Intermittent injection of the *H. pylori* CagA protein in to its host cell, inhibits the nuclear translocation of BRCA1/BRCA2-the DNA repair proteins involved prominently in breast cancer development. Interestingly, hereditary breast and ovarian cancer syndrome (HBOC) is associated with GC development. Here, we aimed to understand the molecular link between *H. pylori* infection, *BRCA1/2* pathogenic variants (PVs), GC and higher incidence of GC in HBOC families. To do so, in this retrospective clinical observation study, we checked the database of Japanese patients undergoing precision treatment using cancer genomic medicine. Indeed, we found a higher incidence of GC in HBOC families having germline pathogenic variants (GPVs) of *BRCA1/2* (2.95 % vs. 0.78% (in non-HBOC families). Next we, found that 96.1 % *H. pylori* infected patients received cancer genomic medicine examination for advanced GC, and > 16% advanced GC patients had *gBRCA2* PVs. Moreover, histological examination of GC tumor tissue showed that gastric mucosa epithelial cells infected with CagA-positive *H. pylori* strains were lacking in nuclear BRCA1. Further, we found that expressing wildtype BRCA1/2 in the *Gan* mice (a mouse model of human GC), inhibited GC development. Thus, *gBRAC1/2* PVs and *H. pylori* infection synergistically increase the risk of GC development. Our study thus highlights the need for early eradication of *H. pylori* infection in HBOC family members to prevent GC development. Further, it indicates that that poly(ADP-ribose) polymerases (PARPs) inhibitors will be effective in halting development and progression of GCs in HBOC families with *gBRCA1/2* PVs.

Takuma Hayashi[1], **Kenji Sano**[2], **Mako Okada**[1], **Takashi Ura**[3], and **Ikuo Konishi**[1,4]

[1] *Cancer Medicine, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Kyoto*

[2] *Pathological Division, Shinshu University Hospital, Matsumoto, 390-0877, Nagano*

[3] *Medical Oncology, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Kyoto*

[4] *Kyoto University School of Medicine, Kyoto 606-8507, Kyoto*

[*]**Corresponding author:**

Takuma Hayashi

Cancer Medicine, National Hospital Organization Kyoto Medical Center,

Fushimi-ku, Kyoto 612-8555, Kyoto

e-mail: yoyoyo224@hotmail.com

Keywords: Gastric cancer, Helicobacter pylori, BRCA1, BRCA2, HBOC.

Introduction

During GC development, external stimuli turn mucosal epithelial cells (lining the stomach wall) into cancer precursor cells which are further transformed into cancerous cells that proliferate uncontrollably [1]. As the tumor grows (which is formed by cancer cell proliferation), the GC cells gradually infiltrate (deep and outward) into the submucosa, muscularis propria, and serosa [2] and reach the outside of the serosa[3]. Further, these GC cells directly infiltrate the large intestine, pancreas, diaphragm, and liver, which are located near the stomach [4]. Thus, GC cells spread to the surrounding area and adhere to other tissues. Moreover, GC cells crossing to outside of the serosa, are scattered within the abdomen and pelvis, resulting in peritoneal dissemination. Furthermore, GC cells invade lymph vessels and blood vessels, move along with the flow of lymphatic fluid and blood to distant organs, resulting in distant metastasis in multiple organs. Notably, there is a type of stomach cancer— called scirrhous GC—that spreads while making the stomach wall hard and thick [5]. Scirrhous GC progresses rapidly and is characterized by a high tendency for peritoneal dissemination. Furthermore, scirrhous GC (which is also a refractory malignant tumor) is difficult to diagnose by endoscopic examination.

East Asian countries (Japan, China, and South Korea) have the highest incidence of GC worldwide; and > 50,000 GC deaths are recorded in Japan, annually [6]. Importantly, *Helicobacter pylori* (*H. pylori*) is highly prevalent in these East Asian countries [6]. *H. pylori* causes inflammation and ulcers in the stomach and small intestine.*H. pylori* infection is associated with the development of GC and some malignant lymphomas [7]. Almost 90% of the GC patients are positive for *H. pylori* infection; and *H. pylori* infection is a risk factor for the development of GC[8][9]. Thus, possibly GC in East Asia is caused by *H. pylori* infection.

H. pylori is broadly classified—depending on the presence of Cytotoxin-associated gene A antigen (CagA)—into CagA-positive and CagA-negative strains. *H. pylori* isolated in Western countries has a higher predominance of CagA-negative strains; CagA-positive/CagA-negative strains ratio in the West is approximately 6:4 [10]. However, *H. pylori* strains isolated in East Asia, including Japan, are predominantly CagA-positive. CagA-positive strains have a much stronger ability to induce gastric mucosal lesions than CagA-negative strains [11][12][13][14]. Thus, global variations in growth, spread and

prevalence of *H. pylori* strains and GC incidence rates seems to be associated. For instance, the incidence rate of GCs in Japan (male 6.07%, female 2.11%) is about 5x -10x times higher than that in Western countries (USA: male 0.65%, female 0.33%, UK: male 0.66%, female 0.28%), [15].

Most likely *H. pylori* infects gastric mucosal epithelial cells and causes genetic mutations within the infected cells. Accumulation of multiple cancer-related gene mutations within a single cell leads to cancer development. However, the molecular mechanism of *H. pylori* infection induced GC remains unclear. Breast cancer susceptibility genes I and II (BRCA1, and BRCA2 **note 1**) which are strongly linked to the onset of hereditary breast and ovarian cancer (HBOC), may also be involved in the onset of GC [16][17][18][19]. Therefore, here, based on multi-center retrospective observational clinical study, we compared the incidence of gastric cancer in 78 HBOC families, (i.e., a family having one or more patients with breast cancer or ovarian cancer and with a germline pathogenic variant of *BRCA1/2 genes* (g*BRCA1/2* PVs)), with that in 86 non-HBOC families. Further, using a transgenic gastric cancer mouse model (*Gan*) modified to express wild type *BRCA1/BRCA2*, we test whether wild type *BRCA1/BRCA2* rescues tumorigenesis in the *Gan* mice. This will help establish a link between BRCA1/2 PVs, gastric cancer and higher incidence of gastric cancer in patients with *BRCA1/2* GPVs. Next, we verify if *H. pylori* infections occur in a majority of gastric cancer patients belonging to HBOC. Thus, here, we try to establish that *H. pylori* infection and *gBRCA1/2* PVs synergistically increase the risk of acquiring gastric cancer. Our results lead us to conjecture that that oral administration of PARP inhibitors (which are effective against platinum-sensitive HBOC) will be effective against gastric cancer with *gBRCA1/2* PVs.

Materials and Methods

1. Producing $Gan^{tgBrca1}$ and $Gan^{tgBrca2}$ mice

$Gan^{tgBrca1}$ and $Gan^{tgBrca2}$ mice were created by crossing C57BL/6J^{tgBrca1} or C57BL/6J^{tgBrca2} mice with the Gan mice (kindly provided by Dr. Oshima M (Kanazawa University School of Medicine, Kanazawa, Ishikawa, Japan)). The *Gan* mice are compound transgenic mice created by crossing C2mE mouse and Wnt mouse. In the Gan mice, both the COX-2/PGE2 pathway and Wnt signaling are activated in the gastric mucosa. The Gan mice spontaneously develop ductal GC accompanied by an inflammatory response, with 100% efficiency. The Gan mice are considered as models of human GC, wherein tumors develop through the interaction of Wnt signal activation and inflammatory responses.

C57BL/6J^{tgBrca1} and C57BL/6J^{tgBrca2} mice used to obtain $Gan^{tgBrca1}$ and $Gan^{tgBrca2}$ mice were produced as follows-

MMTV-*BRCA1* transgenic constructs: Diagram of the breast cancer susceptibility gene I (BRCA1) cDNAs used for the generation of transgenic animals is shown in fig (Supplementary). In MMTV-BRCA1 transgenic constructs, the expression of wild type *BRCA1*, is controlled by the mouse mammary tumor virus-long terminal repeat (MMTV-LTR) promoter. The *BRCA1* cDNAs were each inserted into the third exon of the rabbit *β-globin* gene (*β-g*). The bar indicates the Really Interesting New Gene finger motif, the hatched region corresponds to the nuclear localization signals and the negative symbols {m/-/} indicate the negatively charged C-terminal domain with transactivation function.

Generation and Maintenance of BRCA1 Transgenic (C57BL/6J^{tgBrca1}**) Mice:** The Xho I *BRCA1* fragments were microinjected into the male pronucleus of C57BL/6x DBA/2 F1 fertilized mice embryos (obtained from CLEA Japan, Inc. Meguro, Tokyo, Japan) and implanted into pseudo pregnant ICR surrogate mice at the Transgenic/ Embryonic Stem Cell Shared Resource Facility (Japan) to obtain founder mice. Founder mice were bred with C57BL/6J mice (obtained from CLEA Japan, Inc. Meguro, Tokyo, Japan) to establish the C57BL/6J^{tgBrca1} transgenic mice line.

CMV-*BRCA2* transgenic constructs: To construct *p236BRCA2*, the *pcDNA3* vector was first modified by inserting a 236-bp fragment of the 5' untranslated region of *BRCA2* between the KpnI and NotI sites. The assembled full-length *BRCA2* cDNA was then inserted at the XhoI site of this plasmid. The 5' UTR of *BRCA2* was obtained by RT-PCR using primers 5'-GGTACCGGTG GCGCGAGCTT CTGA-3' and 5'-GCGGCCGCAACTACGATATTCCTCCAAT-3'. The *pcDNA3* 236HSC WT (*BRCA2*) was a gift from Mien-Chie Hung (Addgene plasmid # 16246 ; http://n2t.net/addgene:16246 ; RRID:Addgene_16246).

Generation and Maintenance of *BRCA2* Transgenic (C57BL/6J^{tgBrca2}**) Mice:** The *BRCA2* gene was generated using recombineering, and transgenic mice were generated as described previously in the section describing BRCA1 transgenic mice.

All animals used in these studies were handled in strict compliance with Shinshu University School of Medicine, Animal Care Committee regulations (Approved number: Shinshu University 567-5).

2. Immunostaining for detection of BrdU positive cells in tumors

Estimating BrdU-labeling index: Mice were injected i.v. with 200 µL of BrdU solution (Roche Diagnostics, IN, USA) 1 h before euthanasia. Tissue samples were fixed in 70-96 % ethanol, embedded and sectioned at 5-µm thickness. These sections were stained with anti-BrdU antibody (Roche Diagnostics). The labeling index was calculated by dividing the number of BrdU- positive cells with the total number of nucleated cells.

3. Clinical Research

We carried out a multi-center retrospective observational clinical study of subjects who underwent cancer genomic medicine at cancer medical facilities in Kyoto, Japan. This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) on November 18, 2020, and Kyoto University School of Medicine (Kyoto, Japan) on August 24, 2022, with approval codes NHO R4-04 and M237. All participants agreed to take part in the present study. We have obtained Informed Consent Statements from people participating in clinical studies and our clinical research complies with the Helsinki Statement.

Cancer genomic medicine is being carried out using cancer gene panel testing, which was approved by the Japanese Ministry of Health, Labor and Welfare in June 2019. The following panels are tested for– OncoGuide^{TM} NCC oncopanel*; Gene mutation analysis set for cancer genome profiling test (Sysmex Corporation Kobe, Hyogo, Japan), Foundation One CDx**; Foundation One CDx liquid; Foundation One CDx’s cancer genome test (Foundation Medicine, Inc., Cambridge

MA, USA); OncoGuide^{TM} NCC oncopanel*; Foundation One CDx**.

Informed consent

All participants agreed to take part in the present study. We have obtained Informed Consent Statements from people participating in clinical studies.

4. Institutional Review Board Approval

Institutional Review Board Statement and Consent to Participate: These experiments with human cancer genome information derived from results by cancer genome gene panels are conducted at Kyoto University, affiliated hospitals and National Hospital Organization Kyoto Medical Center in accordance with institutional guidelines (i.e., IRB approval no. M192, H31-cancer-2). Subjects signed an informed consent form when they were briefed on the clinical study and agreed with content of the research.

Ethic Committee Name: Institutional Review Board (IRB) of Kyoto University Approval Code: M192 Approval Date: April 05, 2014, and June 16, 2016.

Ethic Committee Name: Institutional Review Board (IRB) of National Hospital Organization Headquarter Approval Code: H31-cancer-2 Approval Date: November 09, 2019, and June 17, 2022.

5. Ethical Compliance with Human/Animal Study

This manuscript contains personal and/or medical information about an identifiable individual. This manuscript also contains a case report/case history about identifiable individual. All authors confirmed this manuscript is sufficiently anonymized in line with our anonymization policy. Authors obtained directly Consent from patient. This study involves human participants and was approved by an Ethics Committee(s) and Institutional Board(s). This study involves the research studies with animals.

The authors attended research ethics education through the Education for Research Ethics and Integrity (APRIN e-learning program (eAPRIN)). The completion numbers for the authors are AP0000151756, AP0000151757, AP0000151769, and AP000351128. Consent to participate was required as this research was considered clinical research.

6. Statistical Analysis

All data are expressed as the mean and standard error of the mean. Normality was verified using the Shapiro–Wilk test. For comparing two groups, the unpaired two-tailed *t* test or Mann–Whitney *U* test was used. Multiple comparisons were performed using a one-way analysis of variance with a Tukey post hoc test or a Kruskal–Wallis analysis with a post hoc Steel–Dwass or Steel test. A *p*-value of < 0.05 was considered statistically significant. All statistical analyses were

conducted using the JMP software (SAS Institute, Cary, NC, USA).

7. Data Availability

The following was selected - The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Details of Materials and Methods are indicated in Supplementary files, which are available online.

Results

H. pylori infection is a well-known risk factor for GC (GC). However, the contribution of germline pathogenic variants (GPVs) and/or somatic pathogenic variants (SPVs) in cancer-predisposing genes and their effect, when combined with *H. pylori* infection, on the risk of GC has not been widely evaluated.*H. pylori* Cytotoxin-associated gene A antigen (CagA) **(Note 1)** injected into gastric mucosal epithelial cells induces the accumulation of genetic mutations that lead to the development of GC [20]. Furthermore, the CagA genotype majorly influences the development of GC[20][21]. The CagA inhibits the important genetic mutations repair function of BRCA1/2 **(Note 2)**; BRCA1/2 is a tumor suppressor protein in hereditary breast and ovarian cancer (HBOC) [16][17][18][19]. Thus, CagA may cause the accumulation of genetic mutations that trigger the onset of GC. Especially, mutations (i.e., pathogenic variants) in *BRCA1/2* appear to indirectly induce transformation of gastric mucosal epithelial cells. Therefore, HBOC families with germline pathogenic variants (GPVs) of the *BRCA1/2* may have a high number of patients suffering from GC[16][17][18][19]. We investigated the number of patients affected by GC in 78 HBOC and 86 non-HBOC families (study observed up to six generations). We found that, the non-HBOC families had lower proportion of patients suffering from GC than the HBOC families (0.78% vs. 2.95%) (Table 1, Supplementary Figure 1, Supplementary Figure 2).

Table 1. Patients with Hereditary Gastric cancer in HBOC families reflecting the extent of genetic testing [1]

	HBOC family (78 families)		No-HBOC family (86 families)	
Generation	Patients with GC[2] (other tumor[3])	Incidence% of GC[2] (Total people)	Patients with GC[2] (other tumor[3])	Incidence% of GC[2] (Total people)
I	12 (24) cases	4.17% (288 cases)	1 (6) case	0.31% (324 cases)
II	36 (174) cases	5.56% (648 cases)	5 (43) cases	0.74% (674 cases)
III	12 (120) cases	2.38% (504 cases)	9 (36) cases	1.73% (521 cases)
IV	0 (6) cases	0.00% (348 cases)	2 (5) cases	0.55% (364 cases)
V	1 (1) case	0.39% (258 cases)	0 (2) cases	0.00% (263 cases)
VI	0 (0) case	0.00% (24 cases)	0 (0) cases	0.00% (41 cases)
Total cases	61 (325) cases	2.95% (2070 cases)	17 (92) cases	0.78% (2187 cases)

Genetic testing[1]; BRACAnalysis® Diagnostic System (Myriad Genetics G.K., Zurich, Switzerland), GC[2]; gastric cancer, other tumor[3]; HBOC related cancer (hereditary cancer: i.e., breast cancer, ovarian cancer, prostate cancer, pancreatic cancer).

In murine gastric stem and progenitor cells, inactivation of the tumor suppressor genes *BRCA1* and/or *BRCA2* synergizes with *H. pylori* infection to induce DNA damage [22][23]. Furthermore, in GC cells, infection-dependent DNA damage is aggravated by mutational inactivation of *BRCA2* gene, but not by *TRP53*/*Smad4* loss, or *Erbb2* overexpression [22][23].

The *Gan* mice closely mimics human GC, wherein Wnt signal activation and inflammatory responses interact to form tumors. The *Gan* mice are composite transgenic mice created by crossing C2mE-expressing mice and Wnt-expressing mice. Both the COX-2/PGE2 pathway and Wnt signaling are activated in gastric mucosal epithelial cells of the *Gan* mice [24]. Activation of these two signals in the *Gan* mice causes spontaneous development of ductal-type GC accompanied by an inflammatory response, with 100% efficiency [24] (Figure 1A and B, Supplementary Figure 2). To verify if *BRCA1* or *BRCA2* are involved in the development of the ductal-type GC (that is accompanied by an inflammatory response), we created Gan^{tgBrca1} and Gan^{tgBrca2} mice, which constitutively express wildtype BRCA1 and BRCA2, respectively (Figure 1C and D). We found that the proliferation ratio of epithelial cells in the gastric mucosa of wild-type C57BL/6J mice is 8.54% (as represented by the percentage of bromodeoxyuridine (BrdU) positive cells) (Figures 1A and E, Supplementary Figure 2). Further, the proliferation ratio of ductal-type GC cells in the *Gan* mice (34.41%) is higher than that seen in Gan^{tgBrca1} (14.84%) and Gan^{tgBrca2} (13.69%) mice (Figure 1B,C,D,E). These results suggest that expressing wildtype (wt) *BRCA1* or *BRCA2* in the *Gan* mice, suppresses the transformation of mucosal epithelial cells into ductal type GC cells. In particular, surgical pathological findings show that the nuclear atypia of the epithelial cells in the gastric mucus tissue of *Gan*^{gtBrca2} mice is weaker compared to that in the gastric mucus tissue of *Gan*^{gtBrca1} mice (Figure 1Cc, Figure 1Dd). Specifically, the nuclear shape of the epithelial cells in the gastric mucus tissue of *Gan*$^{\mathrm{gtBrca2}}$ mice and C57BL/6J wt mice are similar (Figure 1Aa, Figure 1Dd). These results indicate that *BRCA2* GPVs induce greater genetic changes and transformation into ductal type GC cells, than *BRCA1* GPVs.

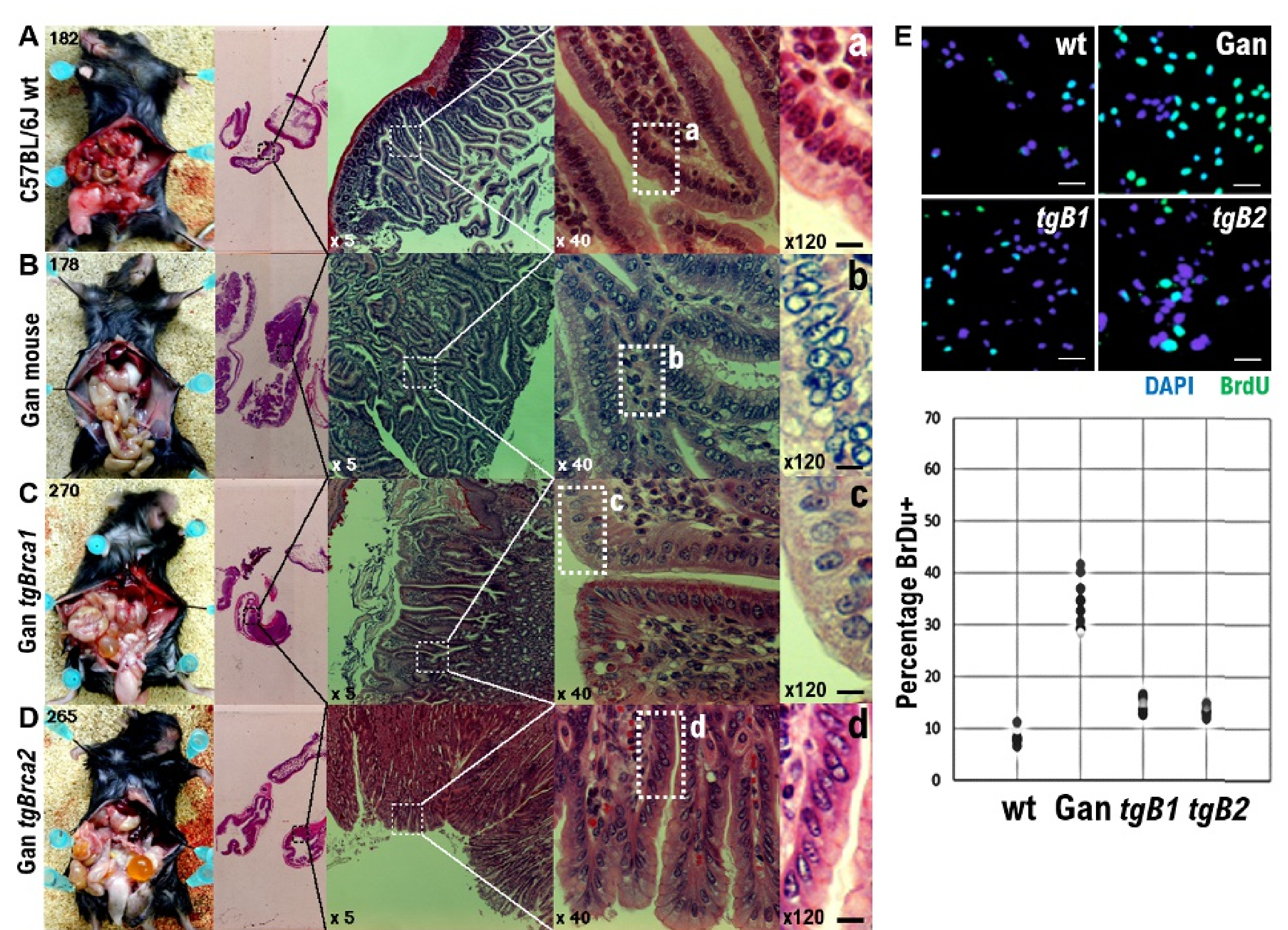

Figure 1. Effects of wildtype *BRCA1* and *BRCA2* expression on gastric cancer (GC) development in the *Gan* mice, a mouse model of GC, **A.** Photograph of surgical pathological findings of normal epithelial cells in gastric mucosal tissue of wild type C57BL6J mice; **B.** Photo of surgical pathological findings of highly atypical epithelial cells in gastric mucosal tissue developed in the *Gan* mice; **C.** Photograph of surgical pathological findings of mildly atypical epithelial cells in the gastric mucosal tissue developed in $Gan^{tgBrca1}$ mice that constitutively express BRCA1. Atypia is reduced; **D.** Photograph of surgical pathological findings of epithelial cells in the gastric mucosal tissue developed in $Gan^{tgBrca2}$ mice that constitutively express BRCA2. has been significantly reduced. The gastric mucosal tissue of $Gan^{tgBrca2}$ mice resembles normal tissue surgically pathologically; **E.** Each genetically modified mouse was inoculated with a BrdU solution, and the proliferation of epithelial cells in the gastric mucosal tissue of each genetically modified mouse was examined using the number of BrdU-positive cells as an indicator. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of wild-type C57BL6J mice is extremely small. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of the *Gan* mice was extremely large. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of $Gan^{tgBrca1}$ mice was reduced. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of $Gan^{tgBrca2}$ mice was significantly reduced; In the graph, the number of BrdU-positive cells observed in the epithelial cells of the gastric mucosal tissue of each genetically modified mouse is plotted.

The treatment history of a total of 35311 cases (enlisted in the cancer genomic medicine precision treatment at Japanese national universities between December 2019 to September 2023) were re-examined using cancer genome panels– 8130 cases were examined using OncoGuide^{TM} NCC oncopanel test (Riken genesis Co., Ltd. Japan); and 27181 cases were examined using FoundationOne® CDx test (MF Inc. USA). New therapies for a total of 2291 cases of advanced GC in Japanese patients were tested using cancer genomic panel examinations. We found that, 96.1% of advanced GC patients

with *H. pylori* infection (2200/2291) underwent cancer genomic panel examinations; recent clinical studies have reported that 5 to 10% of Japan's total population is infected with *H. pylori*. The GPVs and/or SPVs of *BRCA2* was detected in 382 patients with advanced GC (16.67%: 382/2291). These results showing that *BRCA2* GPVs and/or SPVs are associated with the onset and aggravation of advanced GC are consistent with our experimental findings showing that constitutive wt BRCA2 expression reduces the aggravation of epithelial cells in the gastric mucosal tissue of *Gan*^{tgBrca2} mice. *ERBB2* GPVs and/or SPVs was detected in 521 patients with advanced GC (22.74%: 521/2291). Our results, here, from analyzing cancer genomic medicine database are similar to those obtained in clinical research conducted by other research institutions [22].

Discussion

GC is the third largest leading cause of cancer-related mortality, accounting for approximately 10% of all cancer-related deaths [25]. Infection of the stomach with *H. pylori* is the greatest risk factor for GC development. The CagA protein produced by *H.pylori* invades the gastric mucosal epithelial cells, binds to intracellular proteins, causes abnormalities in signal transductions that influence cell proliferation and subsequently promotes canceration [26]. However, the physiological mechanism by which gastric mucosal epithelial cells are transformed in a CagA-dependent manner has not been clarified. The CagA protein of *H. pylori* impairs the function of *BRCA1*—which is s homologous recombination repair related (HR) gene controlling genome stability—leading to the accumulation of genetic mutations required for canceration of gastric mucosal epithelial cells [27][28][29]. *BRCA1/2* are tumor suppressors because inactivating mutations in them cause hereditary breast and ovarian cancers. There exists a common carcinogenic mechanism between the pathogeneses of GC caused by *H. pylori* infection and hereditary breast and ovarian cancers [16]. Here, the results of our clinical study reveals that HBOC families had higher number of GC cases compared to non-HBOC families. The cancer genome panel examination conducted for precision/cancer genome medicine, revealed that 382 patients with advanced GC (16.67%: 382/2291) had *GPVs* and/or SPVs of *BRCA2*. PARP inhibitors are effective against platinum-sensitive HBOC. Therefore, oral administration of PARP inhibitors, may be effective against these GCs.

The CagA injected by *H. pylori* into infected cells (host cells) binds to the oncogenic phosphatase—SRC homology phosphatase 2 (SHP2) **(Note 3)** —and enhances its activity. Furthermore, CagA also binds to the phosphorylating enzyme partitioning-defective 1b (PAR1b) **(Note 4)** and CagA-PAR1b complex is formed near the cell membrane [16][23]. The CagA-PAR1b complex suppresses the activation of PAR1b kinase [16][23]. For nuclear translocation of BRCA1/2, their serine residue (Ser616) located near the nuclear translocation signal **(Note 5)** must be phosphorylated by PAR1b [30]. Specifically, the nuclear translocation of BRCA1/2 is inhibited due to the PAR1b kinase inactivation by CagA. As a result, BRCA1 decreases in the nucleus, leading to BRCAness **(Note 6)**. Subsequently, homologous recombination repair **(Note 7)** of DNA double-strand breaks (DSBs) **(Note 8)** in the DNA replication forks **(Note 9)** by BRCA1/2 fails to occur [16][23]. Specifically, *H. pylori* infected cells exhibit BRCA1-specific repair dysfunction. This may lead to high number of GC cases in HBOC families which also contain GPVs of *BRCA1/2* genes. To ascertain this, we investigated the number of GC cases in 78 HBOC families (one/four/six generations of these families were studied). We found that, HBOC families had a higher

proportion of GC cases (2.95%) compared to non-HBOC families (0.78%) (Table 1, Supplementary Figure 1, Supplementary Figure 2).

When *H. pylori* inserts CagA into infected cells, the nuclear translocation of BRCA1/2 is inhibited, and the genetic mutations remain unrepaired, resulting in genome instability [16][23]. As a result, the gastric mucosal epithelial cells become cancer progenitor cells. The recent report demonstrated that the expression and intracellular distribution of BRCA1/2 in surgically excised gastric mucosal tumor tissues [16][23]. We found that BRCA1 was absent in the nucleus of epithelial cells, that make up the surface of the gastric mucosa, where CagA-positive *H. pylori* bacteria exist [23]. Furthermore, in epithelial cells infected with *H. pylori*—in the isthmus within the gastric fundic gland**(note 10)** where stem cells and proliferating cells reside—nuclear BRCA1 was significantly reduced and DSBs formation was observed [23]. The lifetime incidence of breast cancer and ovarian cancer—in women with hereditary breast and/or ovarian cancer (HBOC) syndrome due to germline mutation and/or inactivating mutations in homologous recombination related (HR) genes including the *BRCA1*/2 genes—is 70-80% and 40%, respectively [31]. Moreover, approximately 6% men with HBOC syndrome, who also carry a *BRCA1* mutation, develop breast cancer [32]. Additionally, *BRCA1/2* is expressed in all cells including mammary gland cells, ovarian epithelial cells, and gastric epithelial cells. Specifically, *BRCA1/2* work to protect the genomic DNA of all somatic cells. However, the reason for the significant increase in the risk of developing specific cancers (such as breast, ovarian, and GCs) with deletion/inactivation of germline *BRCA1/2* is still unknown.

H. pylori is a gram-negative microaerophilic spiral bacillus that sustains infection in the harsh, highly acidic stomach environment [33]. Normally, the *H. pylori* infection established during childhood persists lifelong, unless aggressively eradicated using drugs [34]. *H. pylori* infects approximately half of the world's population and is a resident bacterium [35]. However, some people infected with *H. pylori* develop atrophic gastritis, peptic ulcers, and even GC, forcing *H. pylori* to be categorized as a pathogenic bacterium [36]. Here, we clearly demonstrate the importance of early eradication of *H. pylori* infections in HBOC family members in preventing GC development. The results of this study indicate that oral administration of PARP inhibitors (**note 11**), which is effective against breast, ovarian, pancreatic, and prostate cancers involving *BRCA1/2* PVs, can be used to treat GCs involving *BRCA1/2* PVs. Therefore, this study will help establish early treatment methods for GC patients with *BRCA1/2* PVs.

Footnote

Note 1. *Breast cancer susceptibility genes I and II* (*BRCA1/BRCA2*) protect DNA replication forks by repairing damage (DNA double-strand breaks (DSBs)) during nuclear genomic DNA replication. In particular, *BRCA1/2* plays an important role in maintaining the stability of cellular genome (genome stability). The *BRCA1/2* genes are typical tumor suppressor genes, and loss-of-function mutations in *BRCA1/2* significantly increase the risk of developing breast and ovarian cancers. Furthermore, loss of *BRCA1/2* functions is involved in the development of some prostate and pancreatic cancers.

Note 2. *H. pylori* CagA: *Helicobacter pylori (H. pylori)* is a pathogenic bacterium that chronically infects the human gastric mucosa, and is estimated to infect 3 billion people, approximately half of the world's population. *H. pylori* infection causes

atrophic gastritis and gastric mucosal lesions (such as gastric ulcers). *H. pylori* is broadly classified as CagA-negative and CagA-positive strains depending on the CagA gene presence. Compared to CagA-negative *H. pylori*, the CagA-positive *H. pylori* causes more severe gastric mucosal lesions. Therefore, possibly only CagA-positive strains are involved in GC development. Using the microscopic needle (type IV secretion mechanism), *H. pylori* directly injects CagA protein into the gastric epithelial cells it comes in contact with.

Note 3. SHP2 (Src homology-2 domain-containing protein tyrosine phosphatase-2) is a tyrosine dephosphorylating enzyme (phosphatase) encoded by the *PTPN11* gene. *H. pylori* CagA targets SHP2, and abnormally activates it. This activates Ras-ERK pathway that promotes cell proliferation through its own phosphatase activity.

Note 4. PAR1b (Polarity-regulating kinase partitioning-defective 1b) is an orthologue of partitioning-defective 1 (PAR1), which was first identified in *C. elegans.* PAR1b is a kinase that phosphorylates serine and threonine residues. PAR1b is also called microtubule affinity-regulating kinase 2 (MARK2). PAR1b regulates the formation of apical-basal polarity of epithelial cells and microtubules (which serve as channels for intracellular substance transport).

Note 5. Nuclear localization signal: Molecules shuttling between the cytoplasm and the nucleus pass through nuclear pore complexes located in the nuclear membrane. A nuclear import signal is an amino acid sequence that serves as a marker for transporting a protein into the nucleus, and generally indicates a region where basic amino acids lysine and arginine are gathered. During nuclear translocation of protein, importin (the transport protein) binds to the GTP-binding protein Ran, allowing the nuclear pore complex to recognize the Ran complex and allow the protein to pass through.

Note 6. BRCAness: The cellular state caused by *BRCA1/2* dysfunction is called BRCAness. *BRCA1/2* play a central role in protecting DNA during replication via homologous recombination repair. Therefore, *BRCA1/2* dysfunction leads to destabilization of replication forks causing DSBs and defective homologous recombination repair function.

Note 7. Homologous recombination repair: Homologous recombination repair is one of mechanisms of DSB repair. Homologous recombination repair can accurately repair DSBs using the DNA sequence generated by DNA replication as template, without adding or subtracting any base sequence. Contrarily, in cells with reduced homologous recombination repair ability, DNA is repaired by ``non-homologous end ligation'' or ``microhomology-mediated end ligation'' regardless of DNA homology. In these two latter repair mechanisms, the accuracy of repair is not high. Therefore, in cells with reduced homologous recombination repair ability, an incomplete DNA repair mechanism occurs, resulting in genetic mutations.

Note 8. DNA double-strand break (DSB): DNA double-strand breaks (DSBs) are one of the most serious forms of DNA damage to cells. Internal (such as intracellular enzyme activity), and external factors (such as ionizing radiation and drugs such as anticancer drugs) can cause DSB. If DSBs are not repaired and accumulate within cells, loss of genetic information, chromosomal translocation, and cell death occur. Homologous recombination repair is an accurate repair mechanism that does not cause changes in the DNA base sequence.

Note 9. Replication fork: DNA replication proceeds by opening double-stranded DNA in both directions from the origin of replication. The Y-shaped structure where the DNA that has dissociated as replication progresses and the dissociated double-stranded DNA join together is called a replication fork. Replication forks that are stopped by external (such as

ultraviolet light or chemicals) or internal (such as active enzymes) factors become targets of nucleases, which are nucleolytic enzymes. As a result, DSBs are formed and replication fork collapses. BRCA1/2 maintain genome stability by protecting the replication fork from nuclease attack.

Note 10. Fundic gland: The fundus gland is a exocrine gland in the stomach. The gastric fundic gland is composed of parietal cells that secrete gastric acid, mucus cells that secrete mucus (to protect the mucous membrane from gastric acid), and principal cells that secrete pepsinogen (which is the stock solution of the protease enzyme pepsin). The gastric fundic glands are distributed from the fundus to the body of the stomach.

Note 11. PARP inhibitors: These are drugs that inhibit the activity of an enzyme called PARP (poly ADP ribose polymerase), which is involved in DNA repair. Inhibiting PARP prevents the repair of DNA single-strand breaks. Single-strand breaks in DNA lead to double-strand breaks during DNA replication. Normal cells use the homologous recombination repair mechanism to repair DNA double-strand breaks, but cancer cells that are unable to perform homologous recombination are unable to repair double-strand breaks, leading to cell death. PARP inhibitors exhibit antitumor effects by inducing cell death through this mechanism. PARP inhibitors are used as chemotherapy for breast and ovarian cancers having reduced homologous recombination function.

Statements and Declarations

Acknowledgments

We thank all medical staff for clinical research at Kyoto University Hospital, the National Hospital Organization Kyoto Medical Center and Shinshu University School of Medicine. We also thank Dr. Masanori Hatakeyama (The University of Tokyo, School of Medicine, Hongo, Bunkyoku, Tokyo, Japan) for medical scientific discussion regarding gastric cancer and H. pylori. We also thank Dr. Mitsuko Masutani (Nagasaki University School of Medicine, Nagasaki, Nagasaki, Japan) for medical scientific discussion regarding PARP inhibitors and biomedical function of BRCA1 and BRCA2 product.

Funding

This clinical research was performed with research funding from the following: Japan Society for Promoting Science for TH (Grant No. 19K09840, 23K08881), Tokyo, Japan, and START-program Japan Science and Technology Agency for TH (Grant No. STSC20001), Tokyo, Japan and the National Hospital Organization Multicenter clinical study for TH (Grant No. 2019-Cancer in general-02), Tokyo, Japan, and The Japan Agency for Medical Research and Development (AMED) (Grant No. 22ym0126802j0001), Tokyo, Japan.

Competing Interest statement

The authors state No competing interest.

Data Availability

The authors declare that data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) on November 08, 2019, and Kyoto University School of Medicine (Kyoto, Japan) on August 25, 2023, with approval codes NHO H31-02 and M192. The completion numbers for the authors are AP0000151756, AP0000151757, AP0000151769, and AP000351128. As this research was considered clinical research, consent to participate was required. After briefing regarding the clinical study and approval of the research contents, the participants signed an informed consent form.

Clinical Research

A multi-center retrospective observational clinical study of subjects who underwent cancer genomic medicine at a cancer medical facility in Kyoto, Japan. This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) on November 18, 2020, and Kyoto University School of Medicine (Kyoto, Japan) on August 24, 2022, with approval codes NHO R4-04 and M237. All participants agreed to take part in the present study. We have obtained Informed Consent Statements from people participating in clinical studies.

Author Contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. T.H., K.S. and M.O.; Research Conduction, T.H., K.S.; Writing-Original Draft, T.H., T.U. and I.K.; Writing-Review & Editing, I.K.; Visualization, T.H. and I.K.; Supervision, T.H. and I.K.; Funding Acquisition.

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