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ARTICLE

Increased chemosensitivity via BRCA2-independent DNA damage in DSS1- and PCID2-depleted breast carcinomas

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Abstract

Breast cancer, the most common malignancy among women, is closely associated with mutations in the tumor suppressor gene *BRCA*. DSS1, a component of the TRanscription–EXport-2 (TREX-2) complex involved in transcription and mRNA nuclear export, stabilizes BRCA2 expression. DSS1 is also related to poor prognosis in patients with breast cancer owing to the induction of chemoresistance. Recently, BRCA2 was shown to be associated with the TREX-2 component PCID2, which prevents DNA:RNA hybrid R-loop formation and transcription-coupled DNA damage. This study aimed to elucidate the involvement of these TREX-2 components and BRCA2 in the chemosensitivity of breast carcinomas. Our results showed that compared with that in normal breast tissues, DSS1 expression was upregulated in human breast carcinoma, whereas PCID2 expression was comparable between normal and malignant tissues. We then compared patient survival time among groups divided by high or low expressions of *DSS1*, *BRCA2*, and *PCID2*. Increased *DSS1* expression was significantly correlated with poor prognosis in recurrence-free survival time, whereas no differences were detected in the high and low *BRCA2* and *PCID2* expression groups. We performed in vitro analyses, including propidium iodide nuclear staining, single-cell gel electrophoresis, and clonogenic survival assays, using breast carcinoma cell lines. The results confirmed that *DSS1* depletion significantly increased chemosensitivity, whereas overexpression conferred chemoresistance to breast cancer cell lines; however, *BRCA2* expression did not affect chemosensitivity. Similar to *DSS1*, *PCID2* expression was also inversely correlated with chemosensitivity. These results strongly suggest that DSS1 and PCID2 depletion is closely associated with increased chemosensitivity via BRCA2-independent DNA damage. Together with the finding that DSS1 is not highly expressed in normal breast tissues, these results demonstrate that DSS1 depletion confers a druggable trait and may contribute to the development of novel chemotherapeutic strategies to treat DSS1-depleted breast carcinomas independent of *BRCA2* mutations.

Introduction

Breast cancer is the most common and deadly malignancy affecting women worldwide. Approximately two million women are newly diagnosed, and more than 600,000 die of breast cancer each year. One-quarter to one-third of breast cancers develop owing to the inheritance of one or more susceptibility genes, such as *BRCA1* and *BRCA2*. Mutations in the tumor suppressor gene *BRCA* are responsible for 80–90% of single-gene familial breast cancers and 3–6% of all breast cancers. BRCA plays an important role in maintaining genomic integrity by repairing double-strand DNA breaks via homologous recombination [1]. Poly(adenosine diphosphate-ribose) polymerase (PARP) is involved in the detection and initiation of single-strand DNA break repair;

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thus, PARP inhibitors such as olaparib cause BRCA-mutated breast cancer cell death due to synthetic lethality [2].

DSS1, originally identified as a candidate gene for split hand/split foot malformation type 1, is located on human chromosome 7q21.3–q22.1 and is involved in multifunctional biological and cellular processes, including messenger RNA (mRNA) nuclear export, protein degradation, and DNA repair [3, 4]. DSS1 is homologous with yeast SEM1, which comprises the TRanscription–EXport 2 (TREX-2) complex with THP1, SEM1, and CDC31 and contributes to mRNA nuclear export in *Saccharomyces cerevisiae* [3]. Nascent RNA binds with nuclear RNA export factor to form messenger ribonucleoproteins (mRNPs); mRNPs are subsequently exported from the nucleus to the cytoplasm by TREX-2 complex [5]. Thus, yeast SEM1 implicates the intervening roles of mRNA transcription and nuclear export processes [3]. Similarly, in mammals, the TREX-2 component counterparts GANP (SAC3), PCID2 (THP1), DSS1 (SEM1), and Centrin3/4 (CDC31) are associated with mRNA nuclear export [6–8].

TREX-2 complex colocalizes with nuclear pore complexes at the nuclear periphery, contributing to mRNA nuclear export via the nuclear pore [9, 10]. The downregulation of TREX-2 expression and mRNA nuclear export disruption results in the hybridization of mRNA retained in the nucleus with one of two DNA strands. The hybrid DNA:RNA structure, called an "R-loop," frequently causes DNA breaks, genomic instability, and hyperrecombination, leading to carcinogenesis [11]. The DNA breaks induced by R-loop formation are called "transcription-coupled DNA damage." Furthermore, several reports have indicated that aberrant GANP expression causes transcription-coupled DNA damage that is closely associated with the tumorigenesis of several cancers, such as breast carcinomas, malignant lymphomas, malignant melanomas, liver fluke-associated cholangiocarcinomas, and glioblastomas [12–17]. Similarly, aberrant DSS1 and PCID2 expression may alter the structure of TREX-2, contributing to transcription-coupled DNA damage and tumorigenesis.

A recent report showed that DSS1 serves as a critical cofactor for BRCA2 protein stabilization in human cell lines [18]. Aberrant DSS1 expression may result in downregulation of BRCA2 expression followed by DNA damage leading to carcinogenesis even if no *BRCA2* germline mutations have occurred. In fact, DSS1 overexpression was observed in uterine cervix squamous cell carcinoma compared with that in normal endocervical squamous cells [19]. BRCA2 is also associated with PCID2 and prevents R-loop formation; however, whether PCID2 directly binds to and stabilizes BRCA2 has yet to be elucidated [20].

This study aimed to clarify how DSS1 and PCID2 are associated with breast carcinoma chemosensitivity. We found that DSS1 protein expression was higher in non-invasive and invasive ductal carcinoma (IDC) than in normal breast tissues, whereas PCID2 protein was equally expressed in both normal and malignant mammary ductal epithelia. In addition, we found a significant difference in survival time between patients with breast carcinoma with high and low DSS1 expression. We investigated the effects of anticancer drugs on cell death and DNA damage in breast carcinoma cell lines and demonstrated that both *DSS1* and *PCID2* depletion increased the chemosensitivity of breast carcinoma cells. These findings indicate that *DSS1* knockdown is a promising strategy for improving anticancer drug efficacy through transcription-coupled DNA damage associated with TREX-2-dependent mechanisms.

Materials and methods

Patients and breast carcinoma tissue samples

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks of breast carcinoma surgical specimens ($n = 20$), most of which simultaneously included normal breast tissues, ductal carcinoma in situ (DCIS), and IDC, were retrieved from Fujita Health University Hospital. The analysis of FFPE breast carcinoma tissues was approved by the Ethics Committee of Fujita Health University.

Frozen invasive breast carcinoma samples were obtained from female patients admitted to Nagoya City University Hospital between 1997 and 2005 ($n = 269$) [21]. Patients with other malignancies or bilateral breast cancer were omitted from this study. Tissue samples were snap-frozen in liquid nitrogen at the time of surgical resection and then stored at −80 °C until total RNA extraction. Postoperative follow-ups were conducted every 3 months until 3 years, then 6 months until 5 years, and then 12 months thereafter. The median follow-up period was 131 months (range: 1–221 months). Neoadjuvant therapy including chemotherapy and hormonal therapy was administered to 12 patients. Forty-two percent of patients underwent breast-conserving surgery, most of which were postoperatively treated with radiotherapy. Axillary lymph node dissection was performed on 91% of patients. Postoperatively, adjuvant hormonal therapy was administered to 200 patients, 131 patients received adjuvant chemotherapy, and no patients were treated with trastuzumab. The analysis of frozen breast carcinoma samples was approved by the Ethics Committee of the Graduate School of Medical Sciences, Nagoya City University.

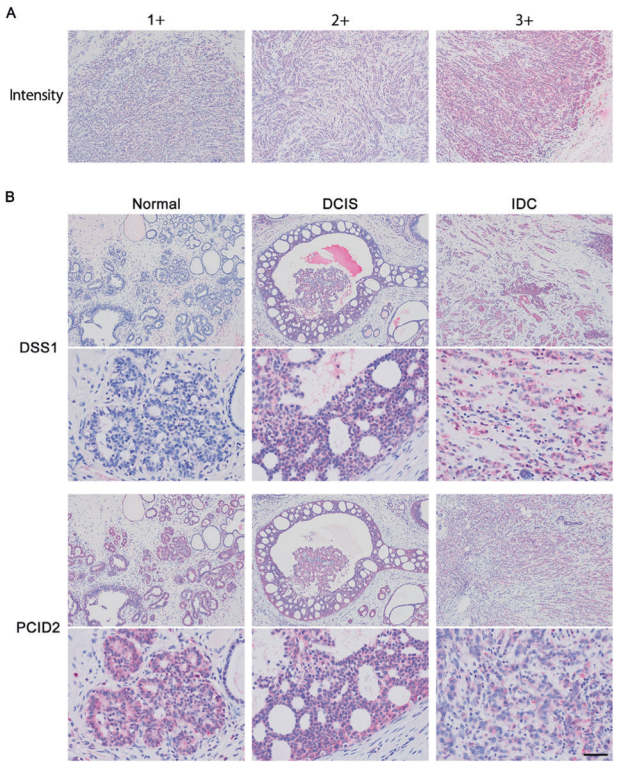

Fig. 1 DSS1 and PCID2 expression levels among normal breast tissues, ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC). A Representative immunohistochemistry of 1+, 2+, and 3+ intensity expression scores. **B** Representative DSS1 and PCID2 immunohistochemistry results in normal breast tissues, DCIS, and IDC. DSS1 expression tended to be higher with increased malignancy, whereas PCID2 was always expressed. Bar = 200 μm for lower magnification fields, 50 μm for higher magnification fields.

These studies involving human data and tissues were performed in accordance with the Declaration of Helsinki.

Immunohistochemical analysis

Immunohistochemical analysis for DSS1 and PCID2 was performed as described previously with minor modifications [22]. In brief, FFPE tissue blocks were cut into 3-μm sections. After deparaffinization, rehydration, antigen retrieval, and blocking, the sections were incubated for 60 min with either anti-DSS1 antibody (rabbit polyclonal, 1:50; Proteintech, Rosemont, IL, USA) or anti-PCID2 antibody (rabbit polyclonal, 1:20; Thermo Fisher Scientific, Rockford, IL, USA), then incubated with Histofine® Simple Stain™ AP (MULTI) (Nichirei Biosciences, Tokyo, Japan). The color reaction was developed using the Vulcan Fast Red Chromogen Kit 2 (Biocare Medical, Concord, CA, USA).

The immunohistochemistry results were scored by expert anatomic pathologists. As described previously [23], an expression score from 0 to 300 was calculated by multiplying the proportion and intensity scores. The proportion was calculated as the percentage of cells that were immunohistochemically positive, and intensity was scored as 0 (none), 1 (weak), 2 (intermediate), or 3 (strong) (Fig. 1A).

Kaplan–Meier plotter database

The Kaplan–Meier plotter database (https://kmplot.com/analysis/) is capable of evaluating the effects of more than 20,000 genes in 4,000 breast cancer samples. We used this database to calculate the overall survival (OS) and recurrence-free survival (RFS) of patients with *DSS1*^{high}, *DSS1*^{low}, *BRCA2*^{high}, and *BRCA2*^{low} breast carcinomas.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Single-strand cDNA was synthesized from total RNA extracted from breast carcinoma tissues as described previously [21]. A TaqMan™ gene expression assay (qRT-PCR; *PCID2*; Hs00218107, *gapdh*; Hs99999905) was conducted to determine the amount of *PCID2* transcripts using the ABI7500 and Sequence Detection System software (Thermo Fisher Scientific). We classified breast carcinomas into *PCID2*^{high} and *PCID2*^{low} groups using *PCID2*/*gapdh* ≥70 as the cutoff point.

Cell lines and cell culture

The human breast carcinoma cell lines MCF7, T47D, MDA-MB-231, and MDA-MB-468 were purchased from the American Type Culture Collection (Manassas, VA, USA). DSS1-overexpressed and GFP-overexpressed MCF7 (MCF7/DSS1 and MCF7/GFP) were previously established [24]. To establish PCID2-overexpressed MCF7 (MCF7/PCID2), a retroviral vector designated pFB-PCID2-IRES-GFP was transfected into PLAT-GP cells (Cellbiolabs, San Diego, CA, USA) using polybrene transfection reagent (Sigma-Aldrich, St. Louis, MO, USA) as described previously; GFP-positive cells were sorted [24]. All breast carcinoma cells were cultured in Roswell Park Memorial Institute 1640 medium (Sigma-Aldrich) supplemented with 10% fetal calf serum (BioFluids, Rockville, MD, USA), 2-mM L-glutamine, and 50-μM 2-mercaptoethanol. The packaging cells, EcoPack293, were maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10% fetal calf serum. In some experiments, doxorubicin (DXR) and paclitaxel (PTX) were added to the culture medium at final concentrations of 5 μM. Overexpression of DSS1 and PCID2 was quantitatively confirmed using qRT-PCR (Supplementary Fig. 1).

siRNA treatment

DSS1 siRNA (si*DSS1*) was obtained as previously described [24]. si*BRCA2* and si*PCID2* were purchased from Life Technologies (Carlsbad, CA, USA). All siRNAs were transfected into breast carcinoma cells using Lipofectamine™ RNAiMAX Transfection Reagent (Life Technologies) as described previously [7]. The knockdown efficiency of *BRCA2*, *DSS1*, and *PCID2* was verified using qRT-PCR and immunoblot analysis (Supplementary Fig. 1).

Propidium iodide (PI) nuclear staining

Cells were lysed with 2× PI solution (0.1% sodium citrate, 0.1% Triton X-100, and 50-μg/mL PI) on ice for 2 h. Sub-G1 fractions were analyzed using FACSCalibur (BD Biosciences, San Jose, CA, USA) and FlowJo software (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) [8].

Single-cell gel electrophoresis

Single-cell gel electrophoresis (comet assay) was performed as previously described [25]. DNA damage, such as single- and double-strand DNA breaks in si*DSS1*- or si*BRCA2*-treated cells, was analyzed using a CometAssay® Kit (Trevigen, Gaithersburg, MD, USA). The damage was quantified by evaluating the tail moments from at least 100 cells using CometScore 1.5 software (Tritek Corp., Sumer Duck, VA, USA).

Clonogenic survival assay

A clonogenic survival assay was performed as described previously [26, 27]. The cell concentration was adjusted to 500 cells/mL, and 1 mL of cell suspension was plated in six-well plates. After 5 days, anticancer drugs were added to each well, and cells were cultured for an additional 1, 2, or 3 days. The cells were then fixed and stained with 1% crystal violet for 10 min. ImageJ software (National Cancer Institutes, Bethesda, MD, USA) was used to count the number of colonies (≥50 cells).

Statistical analysis

Differences between two groups were analyzed using *t*-tests; differences among three groups were analyzed using one-way ANOVA and Games–Howell multiple comparison test. A χ^2 test along with Haberman's residual analysis was performed to determine associations between categorical variables in contingency tables when all the expected values were >1.0 and at least 20% of the expected values were >5; otherwise, Yate's correction for continuity was used to adjust the χ^2 test results. A trend analysis was performed using a χ^2 test for trends (Cochran–Armitage test). Effect sizes were estimated by r in the *t*-test and Games–Howell multiple comparison test, η^2 in one-way ANOVA, and Cramer's V in χ^2 test. The magnitude of the effect sizes was interpreted as follows: r and Cramer's $V = 0.10$ as small, 0.30 as medium, and 0.50 and above as large; $\eta^2 = 0.01$ as small, 0.06 as medium, and 0.14 and above as large. The *t*-test and Kaplan–Meier analysis were conducted using JMP software, version 8.0.2 (SAS Institute Japan, Tokyo, Japan); all other tests were performed using R version 4.0.3 (The R Foundation for Statistical Computing, Vienna, Austria). For all tests, P values of <0.05 were considered statistically significant.

Results

DSS1 protein is highly expressed and PCID2 protein is normally expressed in carcinomas

As described in "Materials and methods," we calculated the expression score (0–300) by multiplying the proportion and intensity scores. The representative immunohistochemistry of 1+, 2+, and 3+ intensity scores is shown in Fig. 1A. Immunohistochemical analyses revealed that DSS1 was expressed in breast carcinoma cells regardless of invasiveness but was absent or weakly identified in normal ductal cells (Fig. 1B). As shown in Fig. 2, DSS1 protein expression scores were statistically higher in DCIS and IDC than in normal breast tissues with a large effect size (DCIS vs. normal: $P < 0.001$, $r = 0.86$; IDC vs. normal: $P < 0.001$, $r = 0.93$); the DSS1 expression score did not significantly differ between DCIS and IDC ($P = 0.269$, $r = 0.26$). However, no significant differences in PCID2 protein expression were noted among normal breast tissues, DCIS, and IDC ($P = 0.203$, $\eta^2 = 0.07$) (Figs. 1B and 2).

Low DSS1 expression is associated with longer survival time

The Kaplan–Meier plotter database analysis revealed that OS and RFS were shorter in patients with $DSS1^{high}$ breast carcinoma than in patients with $DSS1^{low}$ breast carcinoma (OS; $P = 0.017$, RFS; $P < 0.001$) (Fig. 3A, upper panel). However, no significant difference in RFS was noted among the $BRCA2^{high}$ and $BRCA2^{low}$ groups ($P = 0.948$), although OS was shorter in the $BRCA2^{high}$ group ($P = 0.005$) (Fig. 3A, lower panel). These differences in survival time indicate that DSS1 may be a critical factor influencing chemosensitivity in breast carcinoma, independent of BRCA2.

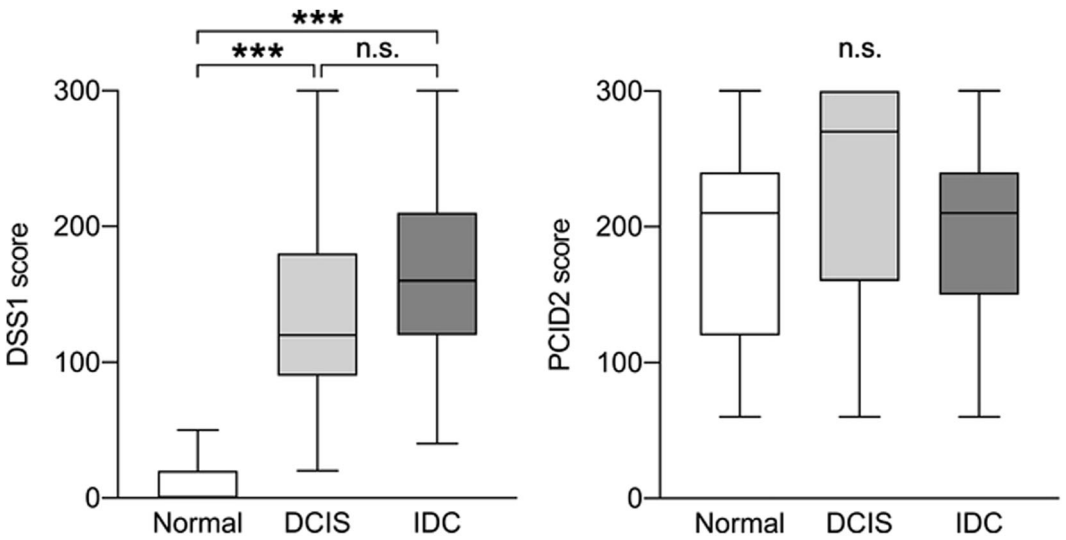

Fig. 2 DSS1 (left panel) and PCID2 (right panel) expression scores among normal breast tissues, ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC). The expression score (0–300) was calculated by multiplying the intensity (0, 1, 2, and 3) and proportion (0–100%) of immunohistochemical staining. Box plots illustrate the medians and 25th and 75th percentiles. $^{***}P < 0.001$; n.s. not significant.

Low PCID2 expression is associated with lower nuclear grade carcinoma

We performed a comprehensive analysis of *PCID2* mRNA expression levels by qRT-PCR using 276 invasive breast carcinoma tissue samples and arbitrarily classified them into *PCID2*^{high} and *PCID2*^{low} groups. The survival times were not significantly different between the *PCID2*^{high} and *PCID2*^{low} groups (OS; $P = 0.122$, RFS; $P = 0.156$); however, the *PCID2*^{high} group showed a weak trend toward shorter OS and RFS (Fig. 3B). Moreover, as shown in Supplementary Table 1, the χ^2 test for trends revealed a statistically significant ($P = 0.013$, Cramer's $V = 0.16$) positive correlation between nuclear grade and *PCID2* expression in breast carcinoma tissues with a small effect size. Haberman's residual analysis revealed that *PCID2* expression tended to be lower in grade 1 breast carcinomas ($P = 0.024$), although no correlations were detected between *PCID2* expression and age, tumor size, lymph node metastases, histology, and ERα, PR, and HER2 status. High *PCID2* expression is associated with nuclear grading in breast carcinomas, which may lead to a weak trend of poor prognoses in patients with *PCID2*^{high} breast carcinomas.

DSS1 depletion contributes to chemosensitivity independent of BRCA2

To determine the effect of DSS1 expression on breast carcinoma cells, we performed PI nuclear staining and single-cell gel electrophoresis (comet assay) using DSS1-overexpressed and DSS1-depleted breast carcinoma cells. As shown in Fig. 4A, B, DSS1 overexpression renders MCF7 cells more resistant to DXR (an intercalating agent) compared with GFP overexpression in control cells. The chemoresistance in DSS1-overexpressing cells was not affected by BRCA2 RNA interference. These results indicate that expression of DSS1 but not BRCA2 is associated with genomic stability and resistance against DNA-damaging agents. As shown in Fig. 5A, PI nuclear staining revealed that DSS1-depleted MCF7 cells were more chemosensitive to DXR and PTX (microtubule depolymerization-inhibiting agent), both of which are usually administered to patients with breast cancer in standard regimens [17]. Similarly, si*DSS1* treatment influenced DXR chemosensitivity in other breast carcinoma cells, including T47D, MDA-MB-231, and MDA-MB-468 cells (Fig. 5B). Similar results were obtained in the single-cell gel electrophoresis assay (Fig. 5C). These findings demonstrate that DSS1 depletion contributes to chemosensitivity in breast carcinomas.

DSS1 is closely associated with the stabilization of BRCA2, a pivotal molecule involved in DNA repair. Therefore, to determine whether increasing chemosensitivity in DSS1-depleted cells is caused by downregulation of BRCA2 expression, we performed PI nuclear staining on BRCA2-silenced breast carcinoma cells. As shown in Fig. 6A, B, chemosensitivity was not significantly increased in several breast carcinoma cell lines after siBRCA2 treatment. Differences in chemosensitivity were detected among BRCA2-depleted and siDSS1-treated cells (also see Fig. 5). Moreover, the single-cell gel electrophoresis assay showed that siBRCA2 treatment alone did not induce DNA damage in MCF7 and MDA-MB-231 cells (Fig. 6C). These findings suggest that enhanced chemosensitivity in DSS1-depleted breast carcinoma cells is independent of BRCA2 expression.

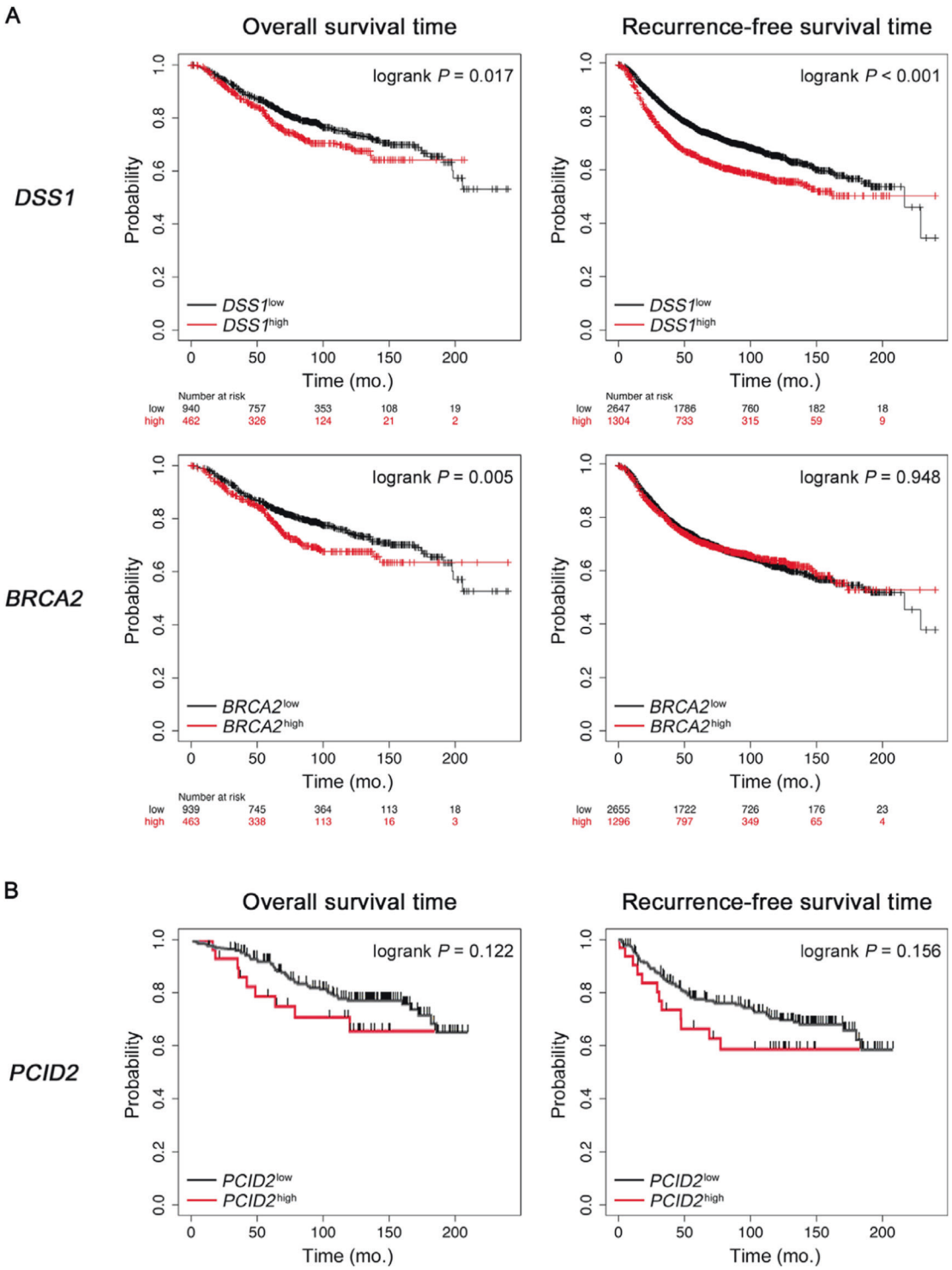

Fig. 3 Survival time analysis for patients with breast carcinoma with high or low DSS1, BRCA2, or PCID2 expression using the Kaplan–Meier method. Survival time analysis for patients with breast carcinoma with high or low *DSS1*, *BRCA2*, or *PCID2* expression using the Kaplan–Meier method for a public database designated KM plotter (**A**) or for 269 patients treated at Nagoya City University Hospital (**B**). Patients with $DSS1^{high}$ breast carcinomas had poorer prognoses than those with $DSS1^{low}$ breast carcinomas, whereas recurrence-free survival time in the $BRCA2^{high}$ and $BRCA2^{low}$ groups were comparable, suggesting that chemosensitivity induced by DSS1 depletion was independent of BRCA2 dysregulation. No differences in overall and recurrence-free survival times between the $PCID2^{high}$ and $PCID2^{low}$ groups were noted.

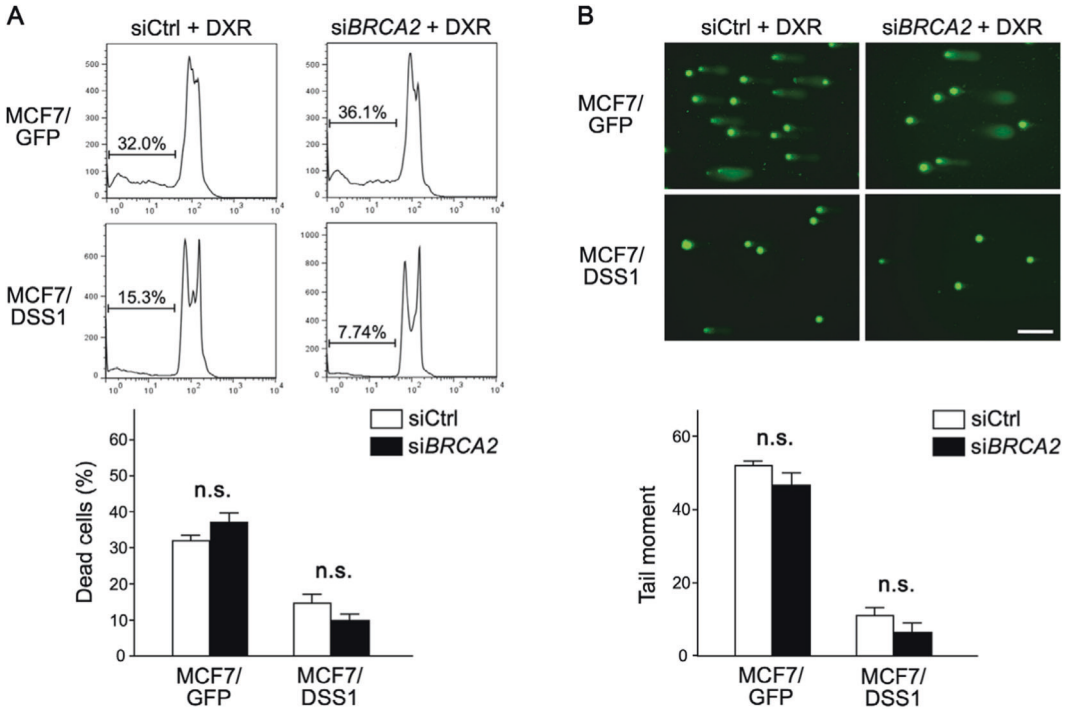

Fig. 4 Effect of *BRCA2* knockdown in DSS1-overexpressing cells. *BRCA2* knockdown in MCF7/GFP and MCF7/DSS1 cells was achieved using RNAi techniques. **A** Cell-cycle profiles of the RNAi transfectants on day 3 of culture with doxorubicin (DXR) (upper panel); proportions of dead cells measured by counting the sub-G1 fraction (lower panel). **B** DNA content pattern observed under fluorescence microscopy on day 3 of culture with DXR (upper panel); DNA damage rate measured as tail moments in fluorescent images using CometScore software (lower panel). Both results indicate that DSS1 but not BRCA2 is closely related to DXR chemoresistance in breast carcinoma cells. n.s. not significant. Bar = 100 μm in fluorescent images.

PCID2 exhibits similar resistance against chemotherapeutic drugs as DSS1

In addition to DSS1, the TREX-2 complex includes PCID2, which binds to BRCA2; thus, we compared the functional roles of these TREX-2 subunits in chemotherapy. Compared with the control cells, a significant increase in the death of *PCID2*-depleted MCF7 and MDA-MB-231 cells was observed after DXR treatment (Fig. 7A). Conversely, *PCID2* overexpression in MCF7 cells led to resistance to DXR (Fig. 7B). Moreover, the clonogenic survival assay revealed that PCID2-overexpressing MCF7 cells and DSS1-overexpressing MCF7 cells exhibited higher chemoresistance to DXR and PTX than control cells (Fig. 8). These results indicate that PCID2 increases resistance to chemotherapeutic drugs and that PCID2 depletion enhances the chemosensitivity of breast carcinoma cells.

Discussion

The findings of this study demonstrate that DSS1 depletion enhances chemosensitivity in breast carcinoma cells. Previously, we identified DSS1 as a candidate molecular target to enhance chemosensitivity in breast cancers, independent of p53 gene status [24]. Although a previous study reported that decreased *BRCA2* mRNA expression predicts a favorable response to docetaxel in breast cancers [28], we found that downregulation of BRCA2 expression by si*BRCA2* does not influence DXR and PTX chemosensitivity. DSS1 has been shown to stabilize the carboxyl-terminal region of BRCA2; thus, we speculated that increased chemosensitivity with DSS1 depletion results from the destabilization and downregulation of BRCA2 expression. However, these results indicate that the mechanism of enhanced chemosensitivity by DSS1 depletion is independent of BRCA2.

Our results also demonstrate that altered expression of another TREX-2 subunit, PCID2, improves chemosensitivity to levels similar to those observed with DSS1 depletion. It has been widely accepted that the TREX-2 complex localizes at the nuclear periphery, playing an important role in the export of nuclear mRNA through the nuclear pore. DSS1 or PCID2 depletion may lead to downregulation of TREX-2 expression, disrupting mRNA export. Our previous study revealed that mRNA metabolic disturbances in the nucleus result in R-loop formation, causing transcription-coupled DNA damage such as DNA breaks, genomic instability, and hyper-recombination [29, 30]. Collectively, these findings suggest that the pharmacological actions of anticancer drugs such as DXR

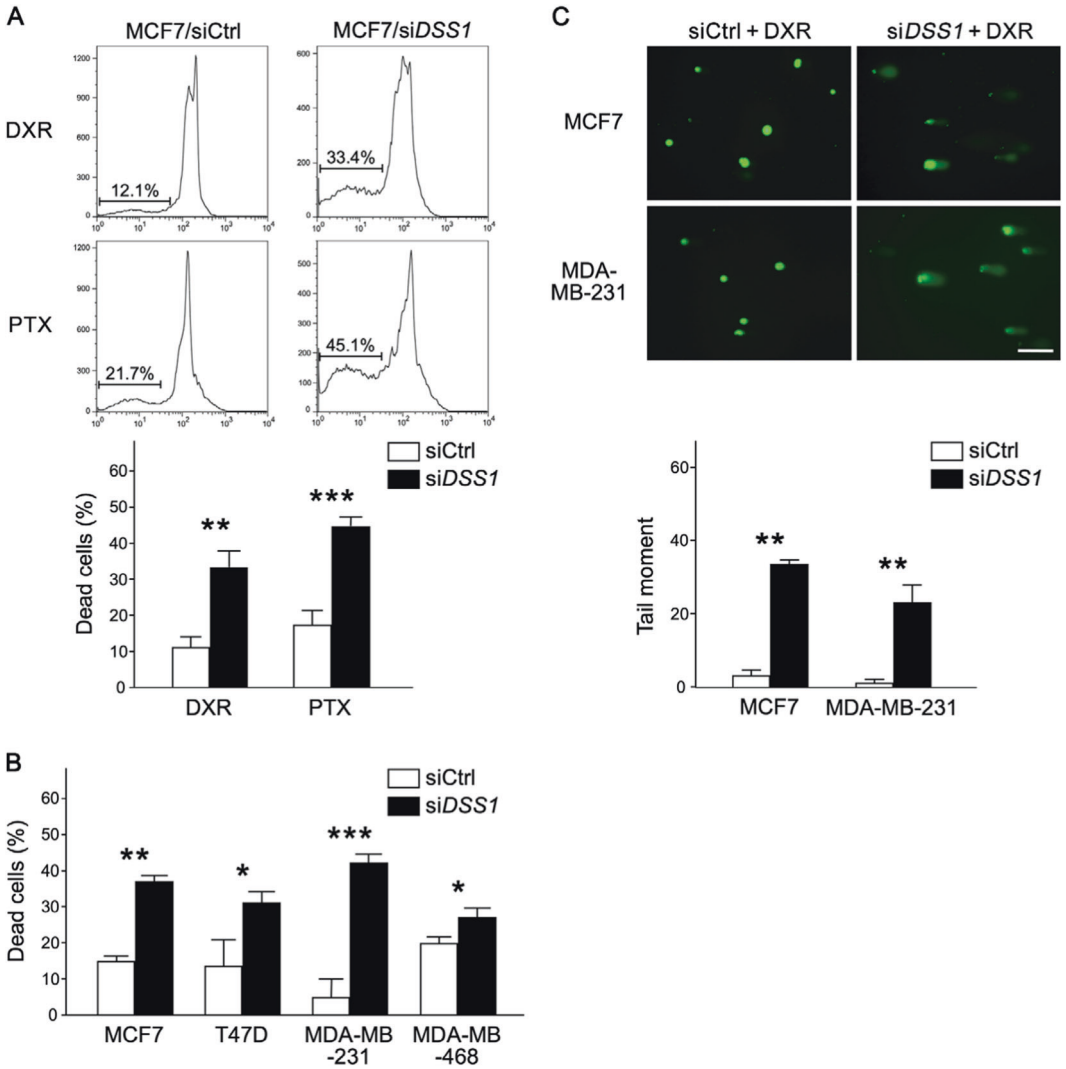

Fig. 5 Chemosensitive effect of *DSS1* knockdown. *DSS1* knockdown was achieved in MCF7 cells and other breast carcinoma cell lines using RNAi techniques. **A, B** Cell-cycle profiles of the RNAi transfectants on day 2 of culture with doxorubicin (DXR) or paclitaxel (PTX) (upper panel); proportions of dead cells measured by counting the sub-G1 fraction (lower panel). **C** DNA content pattern observed under fluorescence microscopy on day 2 of culture with DXR (upper panel); DNA damage rate measured as tail moments in fluorescent images using CometScore software (lower panel). These results indicate that DSS1 depletion is associated with DXR and PTX chemosensitivity in breast carcinomas. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Bar = 100 μm in fluorescent images.

and PTX may be enhanced by the transcription-coupled DNA damage that results from DSS1- and PCID2-depletion-induced TREX-2 downregulation. These results provide a basis for developing a novel approach to expand the application of existing chemotherapeutic agents with reduced side effects.

A previous report showed that DSS1 expression is upregulated in premalignant and malignant endocervical epithelial cells compared with that in nonmalignant cells [19]. In an independent single-hospital-based study, we demonstrated that *DSS1*^{high} breast carcinomas exhibit resistance to chemotherapy, leading to poor progression-free survival in sporadic breast cancers [24]. In this study, we determined the expression level of DSS1 protein in DCIS as well as IDC and found that DSS1 protein was expressed in breast carcinoma with or without stromal invasion. Moreover, our Kaplan–Meier plotter database analysis revealed poorer OS and RFS in *DSS1*^{high} breast carcinomas, although cross-over casts influence the adequacy of the logrank test for comparing survival curves due to a putative violation of the assumption of proportional hazards. These data are consistent with our previous analysis [24], and *DSS1*^{low} breast carcinoma may be more sensitive to breast cancer treatment, especially chemotherapy. Furthermore, these results indicate that DSS1 expression in breast carcinomas is similar to that observed in endocervical cancer cells.

In this study, we clarified that two TREX-2 subunits, DSS1 and PCID2, contribute to changes in the sensitivity threshold for anticancer drugs. Although DSS1 and PCID2

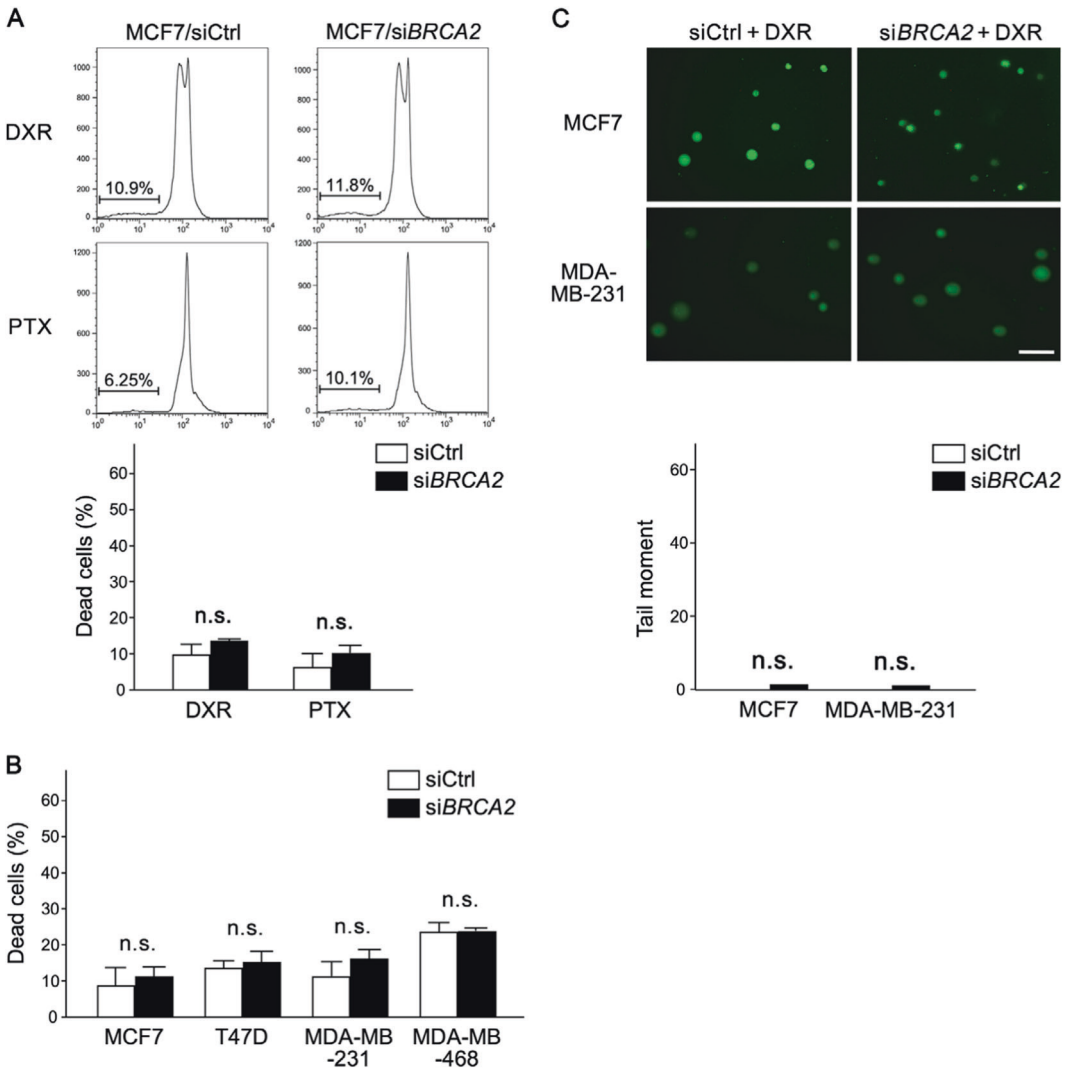

Fig. 6 Effect of *BRCA2* knockdown. *BRCA2* knockdown was achieved in MCF7 cells and other breast carcinoma cell lines using RNAi techniques. **A**, **B** Cell-cycle profiles of the RNAi transfectants on day 2 of culture with either doxorubicin (DXR) or paclitaxel (PTX) (upper panel); proportions of dead cells measured by counting the sub-G1 fraction (lower panel). **C** DNA content pattern observed under fluorescence microscopy on day 2 of culture with DXR (upper panel); DNA damage rate measured as tail moments in fluorescent images using CometScore software (lower panel). These results suggest that enhanced chemosensitivity by DSS1 depletion in breast carcinoma cells is independent of BRCA2 expression, even though BRCA2 is stabilized by DSS1. n.s. not significant. Bar = 100 μm in fluorescent images.

associate as components of the TREX-2 complex, only DSS1 expression is specifically upregulated in breast carcinoma. No significant differences in OS and RFS were detected among the *PCID2*^{high} and *PCID2*^{low} breast carcinoma groups; however, in vitro studies indicated that PCID2 overexpression in breast cancer cells also suppresses chemotherapeutic effects. There may be functional differences between DSS1 and PCID2; interactions between DSS1 with BRCA2 and 26S proteasome components may facilitate DNA machinery and protein degradation via the proteasome [3, 31]. However, no interactions between PCID2 and proteasomes have been detected. Proteasome activity may be partly involved in DNA repair through DSS1 [32]; DSS1 depletion may cause transcription-coupled and proteasome-related DNA damage, leading to apoptotic cell death [33], whereas PCID2 depletion alone may not drastically affect cell viability.

Synthetic lethality induced by PARP inhibition may be a promising therapy for homologous recombination-deficient cancer cells. Indeed, the PARP inhibitor olaparib is approved for use for BRCA1/2 mutation carriers with ovarian and breast cancers worldwide [34]. However, DSS1 depletion may cause BRCAness (i.e., BRCA2 destabilization and downregulation) even without a BRCA1/2 germline mutation, leading to homologous recombination impairment. Therefore, PARP inhibitors may be effective in

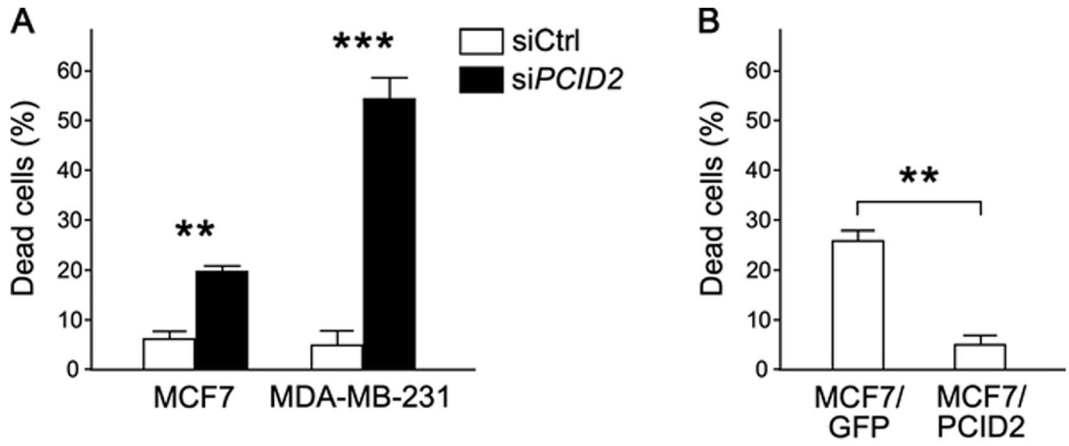

Fig. 7 Effect of *PCID2* under- and overexpression on cell death induced by doxorubicin (DXR). A The sub-G1 fraction proportions of MCF7 and MDA-MB-231 with or without si*PCID2* treatment treated with DXR for 2 days. **B** The sub-G1 fraction proportions of MCF7 with or without *PCID2* overexpression treated with DXR for 3 days. These results indicate that PCID2 contributes to chemoresistance and that PCID2 depletion causes increased chemosensitivity in breast carcinoma cells. $^{**}P < 0.01$; $^{***}P < 0.001$.

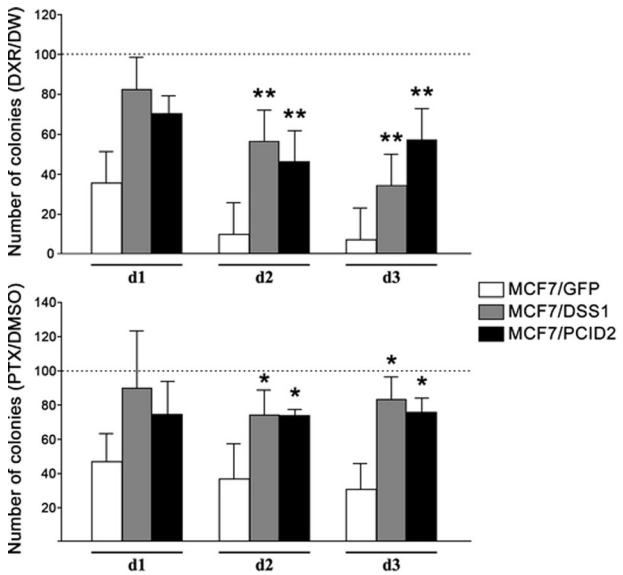

Fig. 8 Clonogenic survival assay to evaluate the inhibition of MCF7/DSS1 and MCF7/PCID2 cell death induced by doxorubicin (DXR) or paclitaxel (PTX). This result indicates that DSS1 and PCID2 overexpression confers chemoresistance against DXR and PTX. $^{*}P < 0.05$; $^{**}P < 0.01$.

patients with BRCA wild-type, DSS1-depleted breast cancer. Anticancer drug sensitivity may improve, and PARP inhibitors may also be effective against DSS1-depleted breast cancers; therefore, DSS1 is a suitable molecular target for developing breast cancer chemotherapy regimens.

Several chemotherapeutic drugs are commonly used to treat breast cancer before or after surgery or as a treatment for cancer recurrence. In particular, triple-negative breast cancers, which account for 10–20% of all invasive breast cancers, lack druggable receptors such as hormonal receptors and HER2; thus, chemotherapy is the sole treatment available to improve patient prognosis. In this study, we demonstrated the chemotherapeutic effects of DSS1 depletion. Similar experimental results obtained with DSS1 and PCID2 suggest that the effect on chemotherapy is associated with a unique function of the TREX-2 complex. The chemotherapeutic effect of PCID2 expression was less effective than that of DSS1 in the cohort study; therefore, DSS1 depletion may provide better clinical anticancer outcomes. Further research to elucidate the molecular mechanism of enhanced chemosensitivity via DSS1 depletion may provide pertinent information regarding druggable molecules for clinical application.

Data availability

All data collected and analyzed in this study are available from the corresponding author, KK, upon reasonable request. Some data are publicly available in the Kaplan–Meier plotter database at https://kmplot.com/analysis/.

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Author contributions Conceptualization: AR and KK; formal analysis: NG, YS, ZZ, YH, SP, YK, MS, SO, HI, TT, AR, and KK; funding acquisition: NG, YS, and KK; investigation: NG, YS, ZZ, YH, YK, MS, SO, AR, and KK; project administration: KK; resources: YH, YK, MK, MS, HI, TT, and KK; supervision: KK; validation: YS, TT, AR, and KK; visualization: NG, YS, and KK; writing—original draft: NG, YS, and KK; writing—review & editing: YS, AR, and KK.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval and consent to participate The collection of human tissue samples was approved by the Ethics Committee of Nagoya City University Graduate School of Medical Sciences and Fujita Health University. Sample collection was performed in strict accordance with the approved guidelines. All participants signed informed consent documents.

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References

1. Chen CC, Feng W, Lim PX, Kass EM, Jasin M. Homology-directed repair and the role of BRCA1, BRCA2, and related proteins in genome integrity and cancer. Annu Rev Cancer Biol. 2018;2:313–36.
2. Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmaña J, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol. 2015;33:244–50.
3. Kragelund BB, Schenstrøm SM, Rebula CA, Panse VG, Hartmann-Petersen R. DSS1/Sem1, a multifunctional and intrinsically disordered protein. Trends Biochem Sci. 2016;41:446–59.
4. Wei SJ, Williams JG, Dang H, Darden TA, Betz BL, Humble MM, et al. Identification of a specific motif of the DSS1 protein required for proteasome interaction and p53 protein degradation. J Mol Biol. 2008;383:693–712.
5. Luna R, González-Aguilera C, Aguilera A. Transcription at the proximity of the nuclear pore: a role for the THP1-SAC3-SUS1-CDC31 (THSC) complex. RNA Biol. 2009;6:145–8.
6. Rondón AG, Jimeno S, Aguilera A. The interface between transcription and mRNP export: from THO to THSC/TREX-2. Biochim Biophys Acta. 2010;1799:533–8.
7. Okamoto N, Kuwahara K, Ohta K, Kitabatake M, Takagi K, Mizuta H, et al. Germinal center-associated nuclear protein (GANP) is involved in mRNA export of Shugoshin-1 required for centromere cohesion and in sister-chromatid exchange. Genes Cells. 2010;15:471–84.
8. Nakaya T, Kuwahara K, Ohta K, Kitabatake M, Toda T, Takeda N, et al. Critical role of Pcid2 in B cell survival through the regulation of MAD2 expression. J Immunol. 2010;185:5180–7.
9. Jani D, Lutz S, Hurt E, Laskey RA, Stewart M, Wickramasinghe VO. Functional and structural characterization of the mammalian TREX-2 complex that links transcription with nuclear messenger RNA export. Nucleic Acids Res. 2012;40:4562–73.
10. Wickramasinghe VO, Laskey RA. Control of mammalian gene expression by selective mRNA export. Nat Rev Mol Cell Biol. 2015;16:431–42.
11. García-Muse T, Aguilera A. R loops: from physiological to pathological roles. Cell. 2019;179:604–18.
12. Kuwahara K, Yamamoto-Ibusuki M, Zhang Z, Phimsen S, Gondo N, Yamashita H, et al. GANP protein encoded on human chromosome 21/mouse chromosome 10 is associated with resistance to mammary tumor development. Cancer Sci. 2016;107:469–77.
13. Fujimura S, Xing Y, Takeya M, Yamashita Y, Ohshima K, Kuwahara K, et al. Increased expression of germinal center-associated nuclear protein RNA-primase is associated with lymphomagenesis. Cancer Res. 2005;65:5925–34.
14. Sakai Y, Rezano A, Okada S, Ohtsuki T, Kawashima Y, Tsukamoto T, et al. A novel cytological model of B-cell/macrophage biphenotypic cell Hodgkin lymphoma in *ganp*-transgenic mice. Cancers. 2020;12:204.
15. Kageshita T, Kuwahara K, Oka M, Ma D, Ono T, Sakaguchi N. Increased expression of germinal center-associated nuclear protein (GANP) is associated with malignant transformation of melanocytes. J Dermatol Sci. 2006;42:55–63.
16. Chan-On W, Kuwahara K, Kobayashi N, Ohta K, Shimasaki T, Sripa B, et al. Cholangiocarcinomas associated with long-term inflammation express the activation-induced cytidine deaminase and germinal center-associated nuclear protein involved in immunoglobulin V-region diversification. Int J Oncol. 2009;35:287–95.
17. Ohta K, Kuwahara K, Zhang Z, Makino K, Komohara Y, Nakamura H, et al. Decreased expression of germinal center-associated nuclear protein is involved in chromosomal instability in malignant gliomas. Cancer Sci. 2009;100:2069–76.
18. Li J, Zou C, Bai Y, Wazer DE, Band V, Gao Q. DSS1 is required for the stability of BRCA2. Oncogene. 2006;25:1186–94.
19. Ma YY, Lin H, Chang FM, Chang TC, Trieu T, Pridgen HI, et al. Identification of the deleted in split hand/split foot 1 protein as a novel biomarker for human cervical cancer. Carcinogenesis. 2013;34:68–78.
20. Bhatia V, Barroso SI, García-Rubio ML, Tumini E, Herrera-Moyano E, Aguilera A. BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2. Nature. 2014;511:362–5.
21. Asano T, Sato S, Yoshimoto N, Endo Y, Hato Y, Dong Y, et al. High expression of LMTK3 is an independent factor indicating a poor prognosis in estrogen receptor α-positive breast cancer patients. Jpn J Clin Oncol. 2014;44:889–97.
22. Sakai Y, Hoshino H, Kitazawa R, Kobayashi M. High endothelial venule-like vessels and lymphocyte recruitment in testicular seminoma. Andrology. 2014;2:282–9.
23. Badve S, Turbin D, Thorat MA, Morimiya A, Nielsen TO, Perou CM, et al. FOXA1 expression in breast cancer–correlation with luminal subtype A and survival. Clin Cancer Res. 2007;13:4415–21.
24. Rezano A, Kuwahara K, Yamamoto-Ibusuki M, Kitabatake M, Moolthiya P, Phimsen S, et al. Breast cancers with high DSS1 expression that potentially maintains BRCA2 stability have poor prognosis in the relapse-free survival. BMC Cancer. 2013;13:562.
25. Phimsen S, Kuwahara K, Nakaya T, Ohta K, Suda T, Rezano A, et al. Selective cell death of p53-insufficient cancer cells is induced by knockdown of the mRNA export molecule GANP. Apoptosis. 2012;17:679–90.
26. Hong J, Zhang Z, Lv W, Zhang M, Chen C, Yang S, et al. Icaritin synergistically enhances the radiosensitivity of 4T1 breast cancer cells. PLoS ONE. 2013;8:e71347.
27. Trudeau M, Charbonneau F, Gelmon K, Laing K, Latreille J, Mackey J, et al. Selection of adjuvant chemotherapy for treatment of node-positive breast cancer. Lancet Oncol. 2005;6:886–98.
28. Egawa C, Miyoshi Y, Takamura Y, Taguchi T, Tamaki Y, Noguchi S. Decreased expression of BRCA2 mRNA predicts favorable response to docetaxel in breast cancer. Int J Cancer. 2001;95:255–9.
29. Yoshida M, Kuwahara K, Shimasaki T, Nakagata N, Matsuoka M, Sakaguchi N. GANP suppresses DNA recombination, measured by direct-repeat beta-galactosidase gene construct, but does not suppress the type of recombination applying to immunoglobulin genes in mammalian cells. Genes Cells. 2007;12:1205–13.
30. Sakai Y, Phimsen S, Okada S, Kuwahara K. The critical role of germinal center-associated nuclear protein in cell biology,

immunohematology, and hematolymphoid oncogenesis. Exp Hematol. 2020;90:30–8.
31. Paraskevopoulos K, Kriegenburg F, Tatham MH, Rösner HI, Medina B, Larsen IB, et al. Dss1 is a 26S proteasome ubiquitin receptor. Mol Cell. 2014;56:453–61.
32. Gudmundsdottir K, Lord CJ, Ashworth A. The proteasome is involved in determining differential utilization of double-strand break repair pathways. Oncogene. 2007;26:7601–6.
33. Kristensen CN, Bystol KM, Li B, Serrano L, Brenneman MA. Depletion of DSS1 protein disables homologous recombinational repair in human cells. Mutat Res. 2010;694:60–4.
34. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med. 2017;377:523–33.

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