A Rare Polymorphic Variant of NBS1 Reduces DNA Repair Activity and Elevates Chromosomal Instability

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Abstract
Failure to expeditiously repair DNA at sites of double-strand breaks (DSB) ultimately is an important etiologic factor in cancer development. NBS1 plays an important role in the cellular response to DSB damage. A rare polymorphic variant of NBS1 that resulted in an isoleucine to valine substitution at amino acid position 171 (I171V) was first identified in childhood acute lymphoblastic leukemia. This polymorphic variant is located in the N-terminal region that interacts with other DNA repair factors. In earlier work, we had identified a remarkable number of structural chromosomal aberrations in a patient with pediatric aplastic anemia with a homozygous polymorphic variant of NBS1-I171V; however, it was unclear whether this variant affected DSB repair activity or chromosomal instability. In this report, we demonstrate that NBS1-I171V reduces DSB repair activity through a loss of association with the DNA repair factor MDC1. Furthermore, we found that heterozygosity in this polymorphic variant was associated with breast cancer risk. Finally, we showed that this variant exerted a dominant-negative effect on wild-type NBS1, attenuating DSB repair efficiency and elevating chromosomal instability. Our findings offer evidence that the failure of DNA repair leading to chromosomal instability has a causal impact on the risk of breast cancer development. Cancer Res; 74(14); 3707–15. ©2014 AACR.

Introduction
Nijmegen breakage syndrome, an autosomal recessive human disease, is because of a mutation in the NBS1 gene (1). The clinical features of this syndrome include growth retardation, immunodeficiency, and increased susceptibility to malignancies (1). A gene product of the nibrin gene [NBN, also known as the Nijmegen breakage syndrome 1 gene (NBS1)] is a member of the MRE11/RAD50/NBS1 (MRN) protein complex, which is involved in the repair of double-strand break (DSB) in DNA (2). NBS1 consists of 2 functional regions (Fig. 1A). Its C-terminal region contains binding motifs of MRE11 meiotic recombination 11 homolog A (Saccharomyces cerevisiae; MRE11A, also known as MRE11) and ataxia telangiectasia–mutated (ATM) kinase (3–5), whereas its N-terminal region contains forhead-associated (FHA) and breast cancer C-terminal (BRCT) domain that mediates phospho-dependent protein–protein interactions (6–8). A rare polymorphic variant of NBS1 that resulted in an isoleucine to valine substitution at amino acid position 171 (I171V) was first identified in childhood acute lymphoblastic leukemia (ALL; ref. 9). The NBS1-I171V polymorphic variant is located in the BRCT domain, which is highly conserved in human, mouse, rat, chicken, and African clawed frog (Fig. 1B). We have previously described a patient with aplastic anemia (AA) in a Japanese child with a homozygous polymorphic variant of NBS1-I171V (10). We also determined that the chromosomes of lymphoblastic cell lines derived from this patient contained a remarkable number of structural chromosomal aberrations (10). However, it is unclear whether the NBS1-I171V polymorphic variant affects DSB repair activity and genomic instability.

In this study, we showed that NBS1-I171V decreased the localization of the MRN complex to sites of plural DSBs through its loss of association with MDC1. This aberrant localization resulted in decreased production of repairable single-stranded DNA and reduced DSB repair activity. We also showed that the heterozygous NBS1-I171V variant increased the risk of breast cancer in Japanese women. The NBS1-I171V reduced the DSB repair activity of endogenous NBS1 in a dominant-negative manner, and increased chromosomal instability.

Materials and Methods
Cell lines and antibodies
HeLa, HCC1937, GM07166VA7, GM07166VA7 transfected with DR-GFP reporter, and HS-SY-I cells were obtained from the American Type Culture Collection (ATCC), Dr. K. Komatsu (Kyoto University, Japan), and Dr. S. Sonobe (Kouchi Medical...
the presence of 800 or 600 DR-GFP reporter, and HeLa cells. The cells were cultured in
infected into GM07166VA7 cells, GM07166VA7 cells containing
malian expression vector (Wako). These vectors were trans-
by using PCR and then ligated into the pEB-Multi-Neo mam-
(WT) with a 3xFlag-His6-HA-tag at C-terminus was generated
2 weeks, after which clones were isolated and selected on the

B, modified sequence alignment of the NBS1-I171V polymorphic
variant region of various NBS1 from human, mouse, rat, chicken, and
ACF (African clawed frog, Xenopus laevis). C, expression vectors
containing 3xFlag-His-HA -NBS1-WT cDNA, -NBS1-I171V cDNA, or no
cDNA (vector) were stably transfected into GM07166VA7 cells.
Extracts from the cell lines were immunoprecipitated (IP) with anti-Flag
antibody and then incubated with antibodies directed against MRE11,
RAD50, or NBS1.

Figure 1. The polymorphic variant of NBS1-I171V is located in the BRCT
domain. The structure of human NBS1 and the sequence alignment of the
NBS1-I171V polymorphic variant region of various NBS1. A, NBS1
consists of functional regions: FHA and BRCT (BRA1 C-terminus)
domains at the N-terminus, MRE11, and ATM interacting motifs at the
C-terminus, two ATM/ATR-phosphorylated serine residues (S278, S343).
B, modified sequence alignment of the NBS1-I171V polymorphic
variant region of various NBS1 from human, mouse, rat, chicken, and
ACF (African clawed frog, Xenopus laevis). C, expression vectors
containing 3xFlag-His-HA -NBS1-WT cDNA, -NBS1-I171V cDNA, or no
cDNA (vector) were stably transfected into GM07166VA7 cells.
Extracts from the cell lines were immunoprecipitated (IP) with anti-Flag
antibody and then incubated with antibodies directed against MRE11,
RAD50, or NBS1.

School, Japan), respectively. The following commercially avail-
able antibodies were used: mouse anti-Flag M2 monoclonal
antibody (Sigma-Aldrich), rabbit anti-MDC1 antibody, mouse
anti-MDC1 antibody, rabbit anti-p95 NBS1 antibody, rabbit
anti-Mre11 antibody (Abcam), rabbit anti-phospho RPA32 (S4/
S8) antibody (Bethyl Laboratories, Inc.), mouse anti-phospho
histone H2AX (Ser139) antibody (Upstate), rabbit anti-phospho
RAD50, or NBS1.

Plasmids and stable cell lines
The plasmids of pDRGFP and pCBASceI were obtained
from addgene. The full-length human NBS1 cDNA was a gift
from Dr. Komatsu. The cDNA of NBS1-I171V or NBS1-wild-type
(WT) with a 3xFlag-His6-HA-tag at C-terminus was generated by
using PCR and then ligated into the pEB-Multi-Neo mammal-
lian expression vector (Wako). These vectors were trans-
ferred into GM07166VA7 cells, GM07166VA7 cells containing
DR-GFP reporter, and HeLa cells. The cells were cultured in
the presence of 800 or 600 µg/mL G418 (Calbiochem) for
2 weeks, after which clones were isolated and selected on the
basis of their NBS1 expression, with the selected clones express-
ing equivalent levels of protein.

Immunoprecipitation and immunofluorescence analyses
For immunoprecipitation analysis, the cells were washed
with phosphate-buffered saline (PBS) and sonicated in lysis
buffer [150 mmol/L NaCl, 20 mmol/L Tris-HCl (pH 7.9), 20%
glycerol, and 1 mmol/L Pefabloc (a protease inhibitor; Roche)].
The lysate (1 mg) was mixed with anti-Flag M2 Affinity Gel
(Sigma-Aldrich) and incubated for 4 hours at 4°C. The gel was
washed three times with lysis buffer. The immunoprecipitated
proteins were separated by using sodium dodecyl sulfate-
polyacrylamide gel electrophoresis (SDS-PAGE) and then
incubated with the indicated antibodies. For immunofluorescence
analysis, the cells were cultured on glass coverslips, exposed to
10 Gy of ionizing radiation (IR). After 4 hours, the cells were
fixed in ice-cold 4% paraformaldehyde for 1 hour, permeabi-
lied with 0.1% TritonX-100 at room temperature, immersed in
blocking reagent [2% normal swine serum (Funakoshi Inc.),
0.05% TritonX-100] for 30 minutes at room temperature, and
then incubated with the indicated primary antibodies over-
night at 4°C. The cells were incubated with secondary anti-
odies conjugated to Alexa Fluor 488 or Alexa Fluor 555
Invitrogen) for 1 hour at room temperature and stained with
2 µg/mL of 4’,6-diamidino-2-phenylindole dihydrochloride
(DAPI) for 10 minutes at room temperature. Images were
captured with a confocal laser microscope (Carl Zeiss) with
a ×40 water immersion objective.

DR-GFP assay
Homologous recombination (HR) repair frequency in the
cell lines using DR-GFP system was performed as described
previously (11, 12).

Cell-cycle assay
An appropriate number of cells was plated and then exposed
to 10 Gy of IR. After 6 hours, the cell-cycle phase in the cells
was analyzed by the Cell-Clock Mammalian Cell Cycle Assay Kit
(biocolor life science assays).

Cell survival assay
An appropriate number of cells was plated and then exposed
to IR, mitomycin C (MMC), or a poly-(ADP-ribose) polymerase
1 (PARP1) inhibitor (AZD2281). After 10 days of incubation,
the surviving fractions were calculated by counting the number
of colonies.

Small interfering RNA analysis
For the small interfering RNA (siRNA) experiments, the
siRNA for control (4390844; Life Technologies), MDC1
(s18578; Life Technologies), and NBS1 (s9291 and s9292; Life
Technologies) were used. Transfection was performed as
described previously (13).

Cytogenetic analysis
After exposure to IR, the cells were cultured for 3 days and
then treated with colcemid (0.02 µg/mL) for 2 hours before
being harvested. Chromosome slides were prepared by using standard protocols and then stained with a 5% Giemsa solution (Wako) for 30 minutes. For each cell line, about 1,600 well-spread metaphase chromosomes were screened for structural chromosomal aberrations.

Sample collection, genotyping, and statistical analysis
We obtained DNA samples of 1,524 breast cancer cases and 1,462 controls from the BioBank Japan Project as described previously (PMID: 22951594). As part of this project, patients’ DNA samples were collected through a collaborative network of 66 hospitals throughout Japan. A list of participating hospitals can be found at the following website: http://biobankjp.org/plan/member_hospital.html. Genotyping of the NBS1 polymorphic variants of the NBS1 gene. First, we constructed the NBS1 cDNA to construct expression vectors that encoded a protein with either isoleucine (NBS1-WT) or valine (NBS1-I171V) at amino acid position 171. The vectors were stably transfected into human GM07166VA7 cells, which contain MRE11 and RAD50 homolog (S. cerevisiae; RAD50; Fig. 1C, lanes 4 and 5), suggesting that NBS1-I171V can incorporate into the MRN protein complex. We also used these NBS1-expressing cell lines to assess cell survival in response to DNA damage caused by IR or the cross-linking agent MMC. An analysis of cell survival revealed that the cell line expressing NBS1-I171V was more sensitive to IR and MMC than was the cell line expressing NBS1-WT (Fig. 2A).

Results and Discussions
NBS1-I171V polymorphic variant reduced DSB repair activity
To explore the biological consequences of the NBS1-I171V substitution, we generate cell lines that express the polymorphic variants of the NBS1 gene. First, we constructed the NBS1 cdNA to construct expression vectors that encoded a protein with either isoleucine (NBS1-WT) or valine (NBS1-I171V) at amino acid position 171. The vectors were stably transfected into human GM07166VA7 cells, which contain homozygous for the 657del5 mutation in exon 6. The mutation of NBS1 determines the synthesis of two truncated proteins of 26 kDa (p26) and 70 kDa (p70) (14). We isolated and selected clones with equivalent levels of NBS1 expression for further analysis (Fig. 1C, lanes 1 and 2, and Supplementary Fig. S1A). Immunoprecipitation analysis in these cloned cell lines indicated that both NBS1-I171V and NBS1-WT bound to MRE11 and RAD50 homolog (S. cerevisiae; RAD50; Fig. 1C, lanes 4 and 5), suggesting that NBS1-I171V can incorporate into the MRN protein complex. We also used these NBS1-expressing cell lines to assess cell survival in response to DNA damage caused by IR or the cross-linking agent MMC. An analysis of cell survival revealed that the cell line expressing NBS1-I171V was more sensitive to IR and MMC than was the cell line expressing NBS1-WT (Fig. 2A). We also found that other cell line expressing NBS1-I171V was more sensitive to IR and MMC than was other cell line expressing NBS1-WT (Supplementary Fig. S1B). Next, we analyzed HR repair frequency in the NBS1-expressing cell lines with DR-GFP system (11, 12). When I-Scel expression was induced, the cell line expressing NBS1-I171V showed a 3-fold lower frequency in HR repair compared with the cell line expressing NBS1-WT (Fig. 2B). We also found that other cell line expressing NBS1-I171V showed lower frequency in HR repair compared with other cell line expressing NBS1-WT (Supplementary Fig. S1C). It was reported that a PARP1 inhibitor (AZD2281) reduced growth of HR repair–deficient cells such as BRCA1 and BRCA2 mutated cells (15, 16). Therefore, we examined cell survival in response to AZD2281. We found that the cell line expressing NBS1-I171V was more sensitive to AZD2281 than was the cell line expressing NBS1-WT, but was more resistant to AZD2281 than was the cell line expressing a vector (Fig. 2C and Supplementary Fig. S2A). This result indicated that damages

![Figure 2](image-url)
by the PARP inhibitor were different from those by IR or MMC in Fig. 2A. We showed that HR repair activity of GM07166VA7 cells expressing NBS1-I171V was almost similar to that of GM07166VA7 cells expressing a vector in Fig. 2B. These results suggested that damages by the PARP inhibitor were repaired by HR repair–dependent and –independent manners of NBS1. We speculate that NBS1-I171V has the HR repair–independent activity to repair damages by the PARP inhibitor. However, the activity of NBS1-I171V is still unclear. Thus, further research of NBS1-I171V function could be necessary.

It was reported that NBS cells exposed to IR showed an abnormal cell cycle (17). Therefore, we examined the cell-cycle status of the cells that had been exposed to IR. We found that the ratio of S phase in the cell expressing the NBS1-WT was reduced by IR treatment, but not in the cell expressing the NBS1-I171V or the vector (Supplementary Fig. S2B). This result suggests that G1–S phase arrest is failed in cells expressing NBS1-I171V. These results suggest that the DSB repair activity of cells expressing NBS1-I171V is reduced compared with that of cells expressing NBS1-WT and prompted a more in-depth analysis of these cell lines.

**NBS1-I171V polymorphic variant decreased the localization of the MRN complex to sites of DSB**

NBS1 is known to be involved in an early step of DSB repair (2). Thus, we used immunofluorescence to examine whether NBS1-I171V localizes to sites of DSB in cells exposed to IR. We observed NBS1 foci in cells expressing NBS1-WT, but not in those expressing NBS1-I171V (Fig. 3A and B). MRE11 foci were also identified in cells expressing NBS1-WT, but not in those expressing NBS1-I171V (Fig. 3C and D). Phosphorylated H2A histone family member X (γH2AX) foci were identified in both the cells expressing...
NBS1-I171V and those expressing NBS1-WT, where they colocalized with the NBS1-WT foci or MRE11 foci (Fig. 3). These results suggest that after exposure to IR, the localization of NBS1-I171V to sites of DSB is less prominent than that of NBS1-WT.

**NBS1-I171V polymorphic variant reduced DSB repair activity through loss of association with MDC1**

Because mediator of DNA-damage checkpoint 1 (MDC1) recruits NBS1 to sites of DSB after IR treatment (18, 19), we examined MDC1 localization in cells that had been exposed to IR. MDC1 foci were identified at sites of DSB in cells expressing either NBS1-I171V or NBS1-WT (Fig. 4A and B). These results suggest that MDC1’s ability to localize to sites of DSB after IR treatment is unaffected by the NBS1-I171V substitution. We also determined that MDC1 foci localized at sites of DSB with NBS1-WT foci but not with NBS1-I171V foci after IR treatment (Fig. 4C and D). Next, we characterized the interaction between NBS1-I171V and MDC1 by conducting an immunoprecipitation analysis. After exposure to IR, a large amount of MDC1 coprecipitated with NBS1-WT, but not with NBS1-I171V (Fig. 4E, lanes 4 and 5). This result is consistent with the reports that both of FHA and BRCT domains in NBS1 are important for its association with MDC1 (8, 20), and NBS1-K160M mutant that resulted in a lysine to methionine substitution at amino acid position 160 within the BRCT domain reduces its binding activity to MDC1 (8). Therefore, we speculate that NBS1-I171V mutant within the BRCT domain affects a structure of the BRCT domain and abolishes the interaction between NBS1 and MDC1. The results in Fig. 4 suggest that the decreased localization of NBS1-I171V to sites of IR-induced DSB results

**Figure 4.** Reduced association of the NBS1-I171V polymorphic variant with MDC1. A and C, focus formation of γH2AX, MDC1, and NBS1. The GM07166VA7 cells expressing NBS1-WT (WT), NBS1-I171V (I171V), and the vector were irradiated with 10 Gy of IR. After 4 hours, the cells were incubated with antibodies directed against γH2AX, MDC1, or NBS1 and then stained with DAPI. B and D, scatter plots of MDC1 or NBS1 focus counts per cell in Fig. 4A or C are shown (n = 30). Red bars, median. E, the GM07166VA7 cells expressing NBS1-WT, NBS1-I171V, and the vector were irradiated with 10 Gy of IR. After 4 hours, whole cell lysates were prepared. The cell lysates were immunoprecipitated with anti-Flag antibody and then incubated with antibodies directed against MDC1 or NBS1.
from its inability to bind to MDC1. We also found that the
knockdown of MDC1 expression using MDC1-specific siRNA
did not affect the sensitivity to IR in cell line expressing
NBS1-I171V or the vector (Supplementary Fig. S3A). However,
the knockdown of MDC1 expression increased the
sensitivity to IR in cell line expressing NBS1-WT (Supple-
mental Fig. S3A). This result suggests that NBS1-I171V
impaired the MDC1–NBS1 pathway.

The MRN complex initiates resection of DSB ends to
create single-stranded 3′-overhangs that can be repaired by
HR system (21). In addition, the complex recruits replication
protein A (RPA), containing phosphorylated 32 kDa repli-
cation protein A2 subunit (RPA2, also known as RPA32), to
single-stranded DNA at sites of DSB (22). We examined the
production of single-stranded DNA at the ends of DSB by
using immunofluorescence to analyze the localization of
phosphorylated RPA32 in cells exposed to IR. Phosphory-
lated RPA32 foci were identified at sites of DSB in cells
expressing NBS1-WT, but not in those expressing NBS1-
I171V or the vector (Fig. 5A and B). Because RAD51
homolog (S. cerevisiae; RAD51) also was recruited to the
single-stranded 3′-overhangs after IR treatment (23), we
examined RAD51 localization in cells that had been exposed
to IR. RAD51 foci were identified at sites of DSB in cells
expressing NBS1-WT, but not in those expressing NBS1-
I171V or the vector (Fig. 5C and D). It was reported that
BRCA1 (familial breast cancer susceptibility protein) also
promoted induction of the single-stranded DNAs at sites of
DSB (24). Therefore, we examined BRCA1 localization in
cells that had been exposed to IR. BRCA1 foci were identified
at sites of DSB in cells expressing NBS1-WT, but not in
cells expressing NBS1-I171V or the vector (Supplementary
Fig. S3C and S3D). These results are consistent with our
finding that the localization of NBS1-I171V to sites of IR-
induced DSB was less pronounced than that of NBS1-WT
(Fig. 3A and B), and suggest that NBS1-I171V decreases the
localizations of the MRN complex and BRCA1 to sites of
DSB. These aberrant localizations result in decreased pro-
duction of repairable single-stranded DNA and reduced DSB
repair activity.

**NBS1-I171V polymorphic variant increases the risk of
breast cancer**

To date, the NBS1-I171V polymorphic variant was
detected frequently only in Polish patients with breast
cancer, head and neck cancer, and colorectal cancer
(25–28). However, other groups did not find a similar asso-
ciation in European patients with breast cancer, leukemia,
or lymphoma (29–31). It remains unclear whether this partic-
ular polymorphic variant of the NBS1 gene is associated with
cancer. It was reported that although null mutations in the
mouse NBS1 gene resulted in embryonic lethality at the

![Figure 5. Reduced association of RPA32 and RAD51 at the DSB sites in cells expressing an NBS1-I171V polymorphic variant. A and C, focus formation of γH2AX, phospho-RPA32, and RAD51. The GM07166VA7 cells expressing NBS1-WT (WT), NBS1-I171V (I171V), and the vector were irradiated with 10 Gy of IR. After 4 hours, the cells were incubated with antibodies directed against γH2AX, phospho-RPA32, or RAD51 and then stained with DAPI. B and D, scatter plots of phospho-RPA32 or RAD51 focus counts per cell in Fig. 5A or C are shown (n = 30). Red bars, median.](image-url)
blastocyst stage, heterozygous knockout (NBS1+/−) mice developed a wide array of tumors (32). Moreover, cell-cycle–dependent association of BRCA1 with the MRN protein complex contributes to the activation of HR-mediated DSB repair in S and G2 phases of the cell cycle (33). These reports strongly suggest that the NBS1-I171V polymorphic variant may increase breast cancer risk. Therefore, we analyzed the association of the NBS1-I171V variant with breast cancer in a Japanese population; patient characteristics are presented in Supplementary Table S1. Of the 1,524 Japanese women with cancer, 23 (1.6%) carried the heterozygous polymorphic variant. Only 7 women (0.48%) with the Japanese women with cancer, 23(1.6%) carried the heterozygous polymorphic variant. Only 7 women (0.48%) with the NBS1-I171V substitution, the frequency of patients with the heterozygous NBS1 polymorphic variant in the Japanese breast cancer group [OR, 3.19; 95% confidence interval (CI), 1.36–7.44; P = 0.0048] was significantly higher than that in the control group (Table 1). This result suggests that the NBS1-I171V variant increases the risk of breast cancer in Japanese women.

Table 1. Association of NBS1 variation with breast cancer in Japanese

<table>
<thead>
<tr>
<th>SNP Gene</th>
<th>Allele 1/2</th>
<th>Case Groups</th>
<th>Case</th>
<th>RAF</th>
<th>P †</th>
<th>OR ‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs61754966</td>
<td>G/A</td>
<td>Breast</td>
<td>0</td>
<td>23</td>
<td>1,501</td>
<td>0.0075</td>
</tr>
<tr>
<td>NBS1</td>
<td>0</td>
<td>Control</td>
<td>0</td>
<td>7</td>
<td>1,455</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

†P value and ‡OR were calculated in a dominant model (11 vs. 12 vs. 22).

NBS1-I171V polymorphic variant elevated chromosomal instability

The above finding may suggest that NBS1-I171V exerts a dominant-negative effect on the function of NBS1-WT. To test this hypothesis, we expressed the codon 171 polymorphic variants of NBS1 in HeLa cells, which also express endogenous NBS1. Clones were isolated and selected on the basis of their exogenous and endogenous NBS1 expression, with the selected clones expressing equivalent levels of protein (Fig. 6A, lanes 1 and 2). Using these cell lines, we assessed cell survival in response to DNA damage caused by exposure to IR or MMC. The cell line expressing NBS1-I171V was more sensitive to IR and MMC than was the line expressing NBS1-WT or the vector (Fig. 6B). We also found that other cell line expressing NBS1-I171V was more sensitive to IR and MMC than was other cell line expressing NBS1-WT (Supplementary Figs. S4D, S4E, S5, S6, and S7). These results suggest that the DSB repair activity of endogenous NBS1 was reduced by NBS1-I171V in a dominant-negative manner. Figure 6A showed that total amount of NBS1 protein in HeLa cell line expressing NBS1-I171V or NBS1-WT was almost same as that in HeLa cell line expressing a vector. Because NBS1-I171V incorporates into the MRN protein complex (Fig. 1C), the half of the MRN protein complex contains NBS1-I171V in HeLa cell line expressing NBS1-I171V. Therefore, we think that decreased the amount of MRN protein complex containing wild-type NBS1 in HeLa cell line expressing NBS1-I171V shows the dominant negative effect in response to DNA damage caused by exposure to IR or MMC.

We also assessed the structural aberrations of the chromosomes in each cell line after exposure to IR and discovered that the number of aberrations in the cell line expressing NBS1-I171V [28 double minutes (DM), 7 chromatid gaps (CTG), and 8 chromatid breaks (CTB)]/~1,600 chromosomes; a representative metaphase spread is shown in Fig. 6C] was significantly higher than that of cell line expressing either NBS1-WT or the vector control (NBS1-WT: 4 DMs and 4 CTGs, vector control: 4 DMs, 1 CTG, and 1 CTB; both/ ~1,600 chromosomes; Fig. 6D). We also found that the number of aberrations in other cell line expressing NBS1-I171V was significantly higher than that of other cell line expressing either NBS1-WT or the vector control (Supplementary Fig. S8). These results suggest that chromosomal instability is elevated in cells expressing NBS1-I171V because its dominant-negative effects on endogenous NBS1 reduce DSB repair. This result is consistent with our previous finding that the chromosomes of lymphoblastic cell lines derived from the patient’s father, who carried the heterozygous polymorphic variant of the NBS1-I171V substitution, contained a remarkable number of structural chromosomal aberrations (10).

Conclusion

We have demonstrated that the NBS1-I171V variant reduces DSB repair activity through loss of association with MDC1. Moreover, the reduced activity of NBS1 in cells expressing the NBS1-I171V variant elevated chromosomal instability in these cells and increased the risk of breast cancer in a
Japanese population. Recently, it was reported that Mre11-mediated DDR restrains mammary hyperplasia by effecting an oncogene-induced G2 arrest (34). Therefore, further research of NBS1-I171V function in the development of breast cancer could be necessary.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: Y. Yamamoto, T. Ohta
Development of methodology: Y. Yamamoto
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Yamamoto, M. Miyamoto, D. Tatsuda, M. Kubo, K. Matsuda, T. Watanabe
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Yamamoto, Y. Nakamura
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Kubo, H. Satoh, T. Watanabe
Study supervision: H. Nakagama, Y. Nakamura

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Figure 6. The DSB repair activity of endogenous NBS1 was repressed by the NBS1-I171V polymorphic variant in a dominant-negative manner. Expression vectors containing 3xFlag-His-HA-NBS1-WT cDNA, -NBS1-I171V cDNA, or no cDNA were stably transfected into HeLa cells. A, the expression levels of NBS1 in the cell extracts were analyzed with antibodies directed against NBS1. The upper arrowhead indicates exogenous NBS1 and the lower arrowhead indicates endogenous NBS1. B, the survival of the cell lines expressing NBS1-WT (WT), NBS1-I171V (I171V) or the vector, or the survival of HeLa cells transfected with NBS1 siRNA (NBS1-siRNA#1 and siRNA#2) or control siRNA (control-siRNA) was analyzed by colony formation assays after exposure to 0, 2, or 4 Gy of IR or 0, 12.5, 25, 37.5, or 50 nmol/L of MMC. Data, mean ± SEM (n = 3). C, a representative metaphase spread of the HeLa cells expressing NBS1-I171V after exposure to 6 Gy of IR. CTBs (white arrowheads), DMs (black arrowheads), and CTGs (arrows) are indicated. Noteworthy, structural chromosomal aberrations are shown at higher magnification in the right panels: CTG (top), CTB (middle), and DM (bottom). D, comparison of the frequencies of aberrations found in the cells used in A. * P < 0.005.
References

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