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Aberrant recombination and repair during immunoglobulin class switching in BRCA1-deficient human B cells

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Breast cancer type 1 susceptibility protein (BRCA1) has a multitude of functions that contribute to genome integrity and tumor suppression. Its participation in the repair of DNA double-strand breaks (DSBs) during homologous recombination (HR) is well recognized, whereas its involvement in the second major DSB repair pathway, nonhomologous end-joining (NHEJ), remains controversial. Here we have studied the role of BRCA1 in the repair of DSBs in switch (S) regions during immunoglobulin class switch recombination, a physiological, deletion/recombination process that relies on the classical NHEJ machinery. A shift to the use of micro-homology-based, alternative end-joining (A-EJ) and increased frequencies of intra-S region deletions as well as insertions of inverted S sequences were observed at the recombination junctions amplified from BRCA1-deficient human B cells. Furthermore, increased use of long microhomologies was found at recombination junctions derived from E3 ubiquitin-protein ligase RNF168-deficient, Fanconi anemia group J protein (FACJ), BRIP1-deficient, or DNA endonuclease RBBP8 (CTIP)-compromised cells, whereas an increased frequency of S-region inversions was observed in breast cancer type 2 susceptibility protein (BRCA2)-deficient cells. Thus, BRCA1, together with its interaction partners, seems to play an important role in repairing DSBs generated during class switch recombination by promoting the classical NHEJ pathway. This may not only provide a general mechanism underlying BRCA1’s function in maintaining genome stability and tumor suppression but may also point to a previously unrecognized role of BRCA1 in B-cell lymphomagenesis.

Significance

DNA double-strand breaks (DSBs) are one of the most deleterious types of DNA lesions and may pose a severe threat to genome integrity. Breast cancer type 1 susceptibility protein (BRCA1) is a multifunctional DNA damage response factor that is known to protect the chromosome/genome stability by participating in one of the major DSB repair pathways, homologous recombination (HR). Here we show that in human B cells BRCA1 is also required for another major DSB repair pathway, nonhomologous end-joining (NHEJ) during immunoglobulin class switch recombination (CSR), probably by inhibition of resection and microhomology-mediated end-joining (MEEJ), as well as promotion of long-range recombination. Our study provides previously unrecognized insights into BRCA1’s function in maintaining genome stability and tumor suppression.


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Serine-protein kinase ATM, E3 ubiquitin ligase proteins RING finger (RNF) 8 and RNF168, tumor suppressor p53-binding protein 1 (53BP1), telomere-associated protein RIF1, and the MRN (Mre11, Rad50, Nbs1) complex (2, 6).

The gene encoding the DDR factor BRCA1 was first mapped to chromosome 17 in 1994 as a breast and ovarian cancer susceptibility gene (7). Since then, BRCA1 has been implicated in a vast number of processes, ranging from checkpoint control and chromatin remodeling to transcription and HR (8–10). Its numerous functions could be attributed to its ability to interact with various proteins through its different domains. The N-terminal RNF domain binds to BRCA1-associated RING domain protein 1 (BARD1) and promotes its E3 ubiquitin ligase activity (9). Furthermore, regions within the same domain have been reported to bind, independently of BARD1, to the c-NHEJ–factor Ku80 (11, 12). The BRCA1 C terminus contains two BRCT repeats, which are involved in forming the A-, B-, and C-complexes by interaction with Abraxas, Fanconi anemia group J protein (FACJ, BRIP1), and DNA endonuclease RBBP8 (ChIP), respectively (8, 9). Furthermore, BRCA1 can, through its coiled-coil domain, form a complex with partner and localizer of BRCA2 (PALB2) and breast cancer type 2 susceptibility protein (BRCA2). All of the above-mentioned complexes have, in addition to other functions, been linked to HR. Although the involvement of BRCA1 in HR and cell-cycle checkpoint control seems to be most important for its tumor suppression activities, other functional properties of BRCA1 might also contribute (8–10).

Whether BRCA1 is involved in NHEJ remains controversial (9, 11–18). Notably, BRCA1 forms a large complex, termed the BRCA1-associated genome surveillance complex, with a number of DNA damage repair proteins that have been shown to be involved in CSR, including ATM, the MRN complex, and the MMR proteins MSH2, MSH6, and MLH1 (19). It is thus possible that BRCA1 directly or indirectly regulates NHEJ during CSR, and we therefore tested this hypothesis by analyzing the recombination junctions generated from in vivo switched B cells from individuals carrying mutations within the BRCA1 gene. To further elucidate the mechanisms of its actions, CSR mutants from individuals with defects in the BRCA1 interaction partners BRIP1, ChIP, and BRCA2, as well as RNF168, an ubiquitin ligase that recruits both BRCA1 and 53BP1 to DSBs, were studied.

**Results**

**Longer Microhomologies at Sγ–Sα Junctions in BRCA1-Deficient B Cells.** Complete absence of BRCA1 is most likely not compatible with life, as biallelic, deleterious mutations result in embryonic lethality in mice (20). Sγ–Sα switch fragments were thus amplified from in vivo switched B cells from 15 individuals with heterozygous mutations in BRCA1 (Fig. 1 and SI Appendix, Table S1), using our previously described nested PCR assay (21, 22). Altogether, 227 switch fragments representing unique CSR events, were characterized from BRCA1-deficient individuals. Of these, 219 contained Sγ–Sα junctions, representing a direct switch from IgM to IgG3, to IgG1, and then to IgG2; and (C) ISD.

The CSR junctions from the BRCA1-deficient cells were further grouped based on the effect of the mutations carried by the patients (SI Appendix, Table S1). Notably, in the BRCT domain–affected group, the direct end-joining was totally absent. The repair by long MHS (≥10 bp), on the other hand, was strongly increased in both the BRCT and RNF domain–affected groups (SI Appendix, Table S2). The junctions from the BRCA1-deficient individuals with truncating mutations in the middle of the gene, which are likely to cause haploinsufficiency (24), and the patient with a splice site mutation also showed an increased frequency of repair by long MHS (≥10 bp) (SI Appendix, Table S2).

**Fig. 1.** Schematic figure of the human BRCA1 gene. The boxes represent the exons (ex), which are numbered from 1 to 24. The positions of the RNF domain, the coiled-coil domain, and the BRCT repeats are underlined. Proteins interacting with these domains are written underneath the domains. The approximate positions of mutations, carried by the individuals included in the CSR junctional analysis, are indicated by arrows (blue, nonlymphoma patients; red, diffused large B-cell lymphoma patients). All mutations are heterozygous.
Increased Sequential Switching in Sμ−Sγ Junctions from BRCA1-Deficient B Cells. Altogether 137 Sμ−Sγ recombination junctions from seven BRCA1-deficient individuals were compared and described with our previously published 59 Sμ−Sγ junctions from adult controls (25, 26). The Sμ−Sγ junctions derived from BRCA1-deficient individuals showed a significant reduction in use of 1–3 bp MH and a borderine increased frequency of repair by ≥4 bp MH (SI Appendix, Table S3). Notably, among the Sμ−Sγ junctions derived from P1, who carries a mutation in the RNF domain, two junctions had an unusually long MH of 9 bp. In controls, no Sμ−Sγ junction with ≥6 bp MH was ever observed.

A proportion of Sμ−Sγ junctions (11%) from the BRCA1-deficient individuals also exhibit “footprints” of sequential switching (illustrated in Fig. 2B; see also Table 2). This has previously been detected in Artemis-deficient patients but never in controls and could suggest an impaired repair through c-NHEJ during CSR (23). In conclusion, the c-NHEJ pathway seems to be affected in BRCA1-deficient B cells, during the processes of both IgA and IgG switching.

Increased Frequency of Intraswitch Region Recombination in BRCA1-Deficient B Cells. Aberrant CSR, but normal or enhanced intraswitch recombination (resulting in intraswitch deletions, ISDs), has been observed in mouse cells deficient in several DDR or NHEJ factors (27–30). In contrast to the joining of two heterologous S regions, which could be located several hundreds of kilobases apart, ISDs occur when DSBs are introduced and repaired within the same S region. It may thus be an indication of failed synopsis of distant S regions and long-range recombination (27). Another, nonexclusive, explanation is that ISDs are caused by increased resection and/or use of A-EJ pathway(s), as the probability of finding a homologous template for MH-dependent repair is increased within the same S region, which consists of highly repetitive sequences (31, 32).

As illustrated in Figs. 2C and 4, by analyzing CSR junctions derived from our PCR assay, it is possible to detect ISDs. The proportion of Sμ−Sγ and Sμ−Sγ junctions containing ISDs was significantly increased in the BRCA1-deficient group (Table 2). A similar increase was found at CSR junctions from individuals with mutations in the RNF and BRCT BRCA1 domains (SI Appendix, Tables S2 and S3). Taken together, BRCA1 might thus be involved in the synopsis and long-range recombination of S regions and/or in preventing resection and A-EJ during CSR.

Increased Proportion of CSR Junctions with Inversions in BRCA1-Deficient B Cells. A small proportion of Sμ−Sγ junctions amplified from BRCA1-deficient cells contained inversions of inverted pieces of S regions, which are rarely observed in controls (Table 2 and SI Appendix, Table S2). Most of these junctions comprised only one inverted S region, whereas some harbored several inverted pieces or in combination with sequential switching, as exemplified by the P6–9 and P4–8 junctions in Fig. 4 and SI Appendix, Fig. S1. The generation of these junctions might have occurred through multiple steps of break/inversion/deletion/recombination processes. Few Sμ−Sγ junctions from BRCA1-deficient B cells also comprised inverted pieces of S regions, whereas this has not been observed in those from control cells. Thus, BRCA1 appears to inhibit inversions during recombination of the S regions.

Repair Pattern at CSR Junctions from Patients with Defects in the BRCA1 BRC Repeat Interaction Partners CTP or BRIP1. Sμ−Sγ junctions from patients with mutations in either BRIP1 or RBBP8 (SI Appendix, Table S1) were subsequently analyzed. These genes encode the BRIP1 and CTP proteins that interact with the BRCA1 BRC repeat through the B and C complexes, respectively (8, 9). As the BRIP1-deficient Fanconi anemia patient was 4.6 y of age at the sampling and we have previously shown that the CSR pattern differs between children and adults [with more MH use in children (23)], the Sμ−Sγ junctions from the patient were compared with those derived from healthy children (1–13 y old) (23, 33). There was a slightly increased use of MH at the CSR junctions derived from the BRIP1-deficient patient. More specifically, the changes were significant only when combining the 4–6 bp and 7–9 bp groups (χ² test, P = 0.022). Surprisingly, the Sμ−Sγ junctions from a heterozygous parent showed a more significant change, with increase in the use of long MHs as well as a reduction of small insertions and direct joining (Table 1 and Fig. 3). The frequency of ISDs and inversions, however, were

Table 1. Characterization of Sμ−Sγ junctions

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>Direct end-joining (%)</th>
<th>Small insertions (%)</th>
<th>1-3 bp (%)</th>
<th>4-6 bp (%)</th>
<th>7-9 bp (%)</th>
<th>≥10 bp (%)</th>
<th>Total no. of S fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1+/−</td>
<td>21 (11)*</td>
<td>40 (18)</td>
<td>45 (21)**</td>
<td>32 (15)</td>
<td>38 (18)†</td>
<td>38 (18)†</td>
<td>214</td>
</tr>
<tr>
<td>BRIP1+/−</td>
<td>1 (3)</td>
<td>2 (6)*†</td>
<td>6 (19)</td>
<td>11 (39)**</td>
<td>10 (32)**†</td>
<td>1 (3)</td>
<td>31</td>
</tr>
<tr>
<td>Ctrl+/−</td>
<td>2 (14)</td>
<td>1 (7)</td>
<td>3 (21)</td>
<td>5 (36)**†</td>
<td>1 (7)</td>
<td>2 (14)</td>
<td>14</td>
</tr>
<tr>
<td>BRCA2+/−</td>
<td>4 (14)</td>
<td>5 (17)</td>
<td>10 (34)</td>
<td>4 (14)</td>
<td>2 (7)</td>
<td>4 (14)</td>
<td>29</td>
</tr>
<tr>
<td>RNF168+/−</td>
<td>2 (6)</td>
<td>2 (8)</td>
<td>3 (12)*†</td>
<td>7 (27)**†</td>
<td>8 (31)**†</td>
<td>4 (19)**†</td>
<td>26</td>
</tr>
<tr>
<td>Controls, adults</td>
<td>41 (16)</td>
<td>56 (22)</td>
<td>91 (36)</td>
<td>29 (11)</td>
<td>25 (10)</td>
<td>14 (5)</td>
<td>256</td>
</tr>
<tr>
<td>BRIP1+</td>
<td>8 (17)</td>
<td>7 (15)</td>
<td>11 (24)</td>
<td>9 (20)</td>
<td>4 (9)</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>BRCA2+/−</td>
<td>2 (6)</td>
<td>9 (29)</td>
<td>4 (13)</td>
<td>5 (16)</td>
<td>6 (19)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Controls, 1−13 y</td>
<td>31 (17)</td>
<td>42 (23)</td>
<td>36 (20)</td>
<td>29 (16)</td>
<td>19 (10%)</td>
<td>26 (14)</td>
<td>183</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using χ² test, and significant differences are indicated in bold. *P < 0.05, **P < 0.01, ***P < 0.001. All individuals were compared with adult controls (n = 31) for statistic calculations, except the BRCA2+/− and BRIP1+/− patients, who were compared with controls with younger ages (1–13 y, n = 20).
largely normal in both the BRIP1-deficient patient and the heterozygous parent (Table 2).

In total, 14 Sμ–Sγ junctions were isolated from the two CtIP-compromised Seckel syndrome patients. A significant increase in the use of 4–6 bp MH was observed (Table 1 and Fig. 3). The proportions of ISDs and inversions were, on the other hand, normal in these patients (Table 2). Taken together, the repair pattern at the Sμ–Sγ junctions from the CtIP-compromised and BRIP1-deficient patients and the parent with heterozygous mutations in BRIP1 showed some similarity with the BRCA1-deficient individuals—that is, a shift in using the MH-mediated A-EJ. However, the increased frequency of ISDs and inversions seems to be more specific for BRCA1-deficient individuals.

Repair Pattern at CSR Junctions from BRCA2-Deficient Individuals. BRCA2 is another breast and ovarian cancer susceptibility gene, which encodes a protein that is known to be important for HR, but has not been implicated in NHEJ (34). It forms a complex with BRCA1 and PALB2 through the BRCA1 coiled-coil domain. Sμ–Sγ junctions from a Fanconi anemia patient with compound heterozygous mutations in BRCA2 were analyzed, and these junctions had a slightly elevated MH use and reduced frequency of direct joining compared with the children controls, albeit not to a significant degree (Table 1 and Fig. 3). The Sμ–Sγ junctions from the heterozygous mother, who developed breast cancer at 38 y of age, showed a similar repair pattern as adult controls. Thus, it seems that the role of BRCA1 in NHEJ is independent from its interaction with BRCA2. Nevertheless, the frequency of inversions at CSR junctions was significantly elevated in the BRCA2 heterozygous mother (Table 2).

Altered Pattern of CSR Junctions from RNF168-Deficient Cells. Several studies have shown an involvement of BRCA1 and 53BP1 in DSB repair pathway choice, where 53BP1 promotes NHEJ and BRCA1 facilities end resection and HR (35–37). As 53BP1 has also been implicated in NHEJ during CSR (2, 38), it is some-

Table 2. Frequencies of ISDs, inversions, and sequential switching at CSR junctions

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>ISDs (%)</th>
<th>Inversions (%)</th>
<th>Sequential switching (%)</th>
<th>Total no. of S junctions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sμ–Sγ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA1−/−</td>
<td>85 (37)*</td>
<td>16 (11)**</td>
<td>8 (4)*</td>
<td>227</td>
</tr>
<tr>
<td>BRIP1−/−</td>
<td>11 (34)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>32</td>
</tr>
<tr>
<td>CtIP−/−</td>
<td>2 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>14</td>
</tr>
<tr>
<td>BRCA2−/−</td>
<td>14 (44)</td>
<td>2 (6)*†</td>
<td>1 (3)</td>
<td>32</td>
</tr>
<tr>
<td>RNF168−/−</td>
<td>11 (42)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>26</td>
</tr>
<tr>
<td>Controls, adults</td>
<td>78 (29)</td>
<td>2 (1)</td>
<td>7 (3)</td>
<td>268</td>
</tr>
<tr>
<td>BRIP1−/−</td>
<td>15 (31)</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>48</td>
</tr>
<tr>
<td>BRCA2−/−</td>
<td>8 (25)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>32</td>
</tr>
<tr>
<td>Controls, 1–13 y</td>
<td>59 (32)</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>187</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using χ² test, and significant differences are indicated in bold. *P < 0.05, **P < 0.01, ***P < 0.001. All individuals were compared with adult controls (n = 31 for Sμ–Sγ junctions and n = 33 for Sμ–Sy junctions) for statistical analysis, except the BRIP1−/− and BRCA2−/− patients, who were compared with controls with younger ages (1–13 y, n = 20).

Discussion

The involvement of BRCA1 during NHEJ has been unclear, with studies showing that BRCA1 promotes, inhibits, or has no effect on the process (11–18). All previous experiments on the participation of BRCA1 in NHEJ have been performed in vitro systems, such as cell lines or cell-free extracts. In this paper, we have analyzed the function of BRCA1 in NHEJ in a more physiological setting, by characterizing the recombination breakpoints derived from in vivo switched B cells in individuals with deleterious mutations in BRCA1. The pattern of these CSR junctions showed a shift from direct end-joining to MH-dependent repair, suggesting that BRCA1 may promote the c-NHEJ pathway and/or inhibit the resection/MH-mediated A-EJ pathway. An indirect role of BRCA1 on CSR through regulation of transcription of genes encoding proteins important for CSR, such as AID, BER, and c-NHEJ factors or DDR proteins including 53BP1, was excluded by mRNA expression analysis in BRCA1-deficient samples (SI Appendix, Figs. S3 and S4). BRCA1 is, however, unlikely

Fig. 4. An example of CSR junction containing sequential switching, ISDs, and inversion. Symbols are explained in the Fig. 2 legend.
to be a component of the core NHEJ machinery, but instead might modulate the NHEJ pathway through a number of mechanisms, as discussed below.

First, BRCA1 may regulate NHEJ through interactions with the NHEJ factors Ku80 and Ku70. Mediated by its N terminus, BRCA1 seems to stabilize the binding of Ku80 to DSBs (11, 12). In the absence of Ku binding, DSBs are preferentially repaired by A-EJ (29).

Second, the functional roles of BRCA1 during NHEJ could be executed through its BRCT repeats that interact with a number of DNA repair factors. The CSR junctions from the heterozygous BRIP1- and CtIP-compromised individuals showed a significant increased use of longer MHs but not as dramatic as those observed from the BRCA1 BRCT-affected individuals. Thus, binding to BRIP1 or CtIP alone does not seem to explain the involvement of the BRCA1 BRCT repeats during NHEJ; instead, there could be a combined effect of impaired formation of several distinct complexes that have varying functions. The BRCT repeats containing A (Abraxas/RAP80/BRCC36) (40) and B (BRIP1) (41) complexes have both been shown to inhibit resection through interaction with MRE11 and/or CtIP, whereas the C (CtIP) complex appears to promote resection and A-EJ (42). Accordingly, one of the functions of BRCA1 during NHEJ could be to modulate the resection process and thus affect the choice between c-NHEJ and A-EJ. This is furthermore supported by the increased use in ISDs, which have been suggested to be caused by extensive resection at the DSBs in the S regions, in BRCA1-deficient B cells (32).

Third, BRCA1 may potentially affect NHEJ during CSR through chromatin remodeling (43). It has been proposed that DDR proteins, including H2AX, ATM, and 53BP1, could induce chromatin conformational changes that facilitate synopsis between distant S regions (44–46). This has been supported by the increased frequency of ISDs in B cells from mice deficient in the above-mentioned proteins (27, 28, 30). BRCA1 might thus collaborate with other DDR factors, such as ATM, which is part of BRCA1-associated genome surveillance complex, to induce changes in chromatin that would promote long-range repair during CSR.

Our results from CtIP-compromised patients differ from previous studies, where a reduction or no change in MH use has been observed at CSR junctions derived from a mouse B-cell line treated with CtIP shRNA (42) or from CtIP-depleted mouse B cells (32). It should be noted that the Seckel syndrome patients in our study did not carry null mutations. Instead, cells from our patients expressed normal levels of wild-type CtIP and, in addition, C-terminal truncated protein with an intact BRCA1 interacting site (44). Hence, the CtIP-BRCA1 complex would be expected to form in patients’ cells, but the resection mechanism would be hampered due to the incorporation of truncated CtIP into the CtIP homodimers. Thus, in contrast to the CtIP-depleted mouse models, CtIP-mediated resection might still occur in the cells from the patients, albeit with lower efficiency, which could possibly favor intermediate lengths of MHs at the CSR junctions.

BRCA2 seems to be less important than BRCA1 for c-NHEJ. The balance between the direct end-joining and MH-mediated A-EJ at CSR junctions from the BRCA2-deficient patients and the individual with a mutation affecting the BRCA2 coiled-coil domain, which is necessary for formation of the BRCA1/PALB2/BRCA2 complex, was not significantly altered. However, CSR junctions from these individuals showed a similar increased frequency of inversions as those from the other BRCA1-deficient cells, suggesting that both BRCA1 and BRCA2 might be involved in preventing these events. It is unclear, however, whether an increased frequency of inversions is due to an NHEJ defect or rather due to general chromosomal instability in BRCA1- and BRCA2-deficient cells as a result of impaired HR. Although AID-induced breaks are believed to be repaired during the G1 cell-cycle phase by NHEJ, some of these breaks may persist to the S/G2 phases and are expected to be repaired by HR (45–47). It is therefore possible that an HR defect in these cells contributes to an elevated level of unrepaird DSBs, which might increase the chance for unconventional joining through inversions.

Several recent studies have suggested a central function for BRCA1 in the choice of repair pathways, through promotion of resection and HR during the S/G2 cell-cycle phases (35–37). Thus, it might seem contradictory that BRCA1 could also inhibit resection and promote NHEJ during the G1 cell cycle. However, several DNA repair proteins have opposing functions during HR and NHEJ, including ATM, H2AX, BLM, and RIF1 (48, 49). Furthermore, by studying RNF168-deficient cells, we showed that combined deficiency of 53BP1 and BRCA1 did not rescue the NHEJ defect, as it did for HR. Our results highlight the complexity of the DNA repair machinery. With new cancer therapies, such as poly(ADP ribose) polymerase inhibitors (50), targeting specific DNA repair pathways, it is important to delineate the functional properties of each DDR/repair factor in different contexts, including cell-cycle stages.

The tumor-suppressing activity of BRCA1 has mainly been attributed to its roles in HR and checkpoint control (9, 10). In light of our findings here, the involvement of BRCA1 in regulating the NHEJ pathway may also contribute to its cancer-preventing function. The importance of efficient repair through NHEJ, especially in lymphocytes, becomes evident when studying mice double deficient for p53 and any of the c-NHEJ factors and most of them develop aggressive lymphomas that often harbor translocations involving the Ig loci (51). Moreover, we have previously shown that DNA repair genes are frequently mutated in human B-cell lymphomas, and NHEJ mutations are associated with IgH translocations in these tumors (52). With the contribution of BRCA1 to both HR and NHEJ, two repair pathways that are important for the maintenance of genome stability and resolution of AID-induced DSBs during CSR, BRCA1 could function as a tumor suppressor in mature B cells. Accordingly, one of the most common tumors observed in BRCA1-deficient mouse models is lymphoma (20). Furthermore, by going through our recently published coding genome sequencing data on 31 diffuse large B-cell lymphomas (53) as well as data from an additional 22 germinal center-related B-cell lymphoma cases, we have observed a number of somatic and germ-line mutations or rare SNPs in BRCA1 in these samples, including a germ-line, pathogenic frame shift mutation (p.Q1111fs) (SI Appendix, Table S4). Notably, CSR junctions from several of these patients showed a similar skewed repair pattern as those from the BRCA1-deficient patients with one common lymphoma (SI Appendix, Table S5). Furthermore, somatic and germ-line mutations were also found in BRCA2, BRIP1, PALB2, UIMCI (RAP80), and FAM175A (Abraxas) in a number of samples, including nonsense mutations in BRCA2 (p.P3063X) and BRIP1 (p.R162X). BRCA1, BRCA2, and related FA pathway genes might thus also be good candidates for cancer-susceptibility genes in B-cell lymphomas, although further screening of variations in these genes in a larger cohort of patients will be required. Taken together, BRCA1 and its interacting proteins, through their functions in HR and NHEJ, may play an important role in maintaining the chromosome/genome stability and thus in preventing tumorgenesis in mature B lymphocytes.

Materials and Methods

BRCA1, BRCA2, BRIP1, and RNF168-Deficient and CtIP-Compromised Patient Samples. Fifteen individuals carrying heterozygous mutations in BRCA1 (P1–P15) (Fig. 1), two Fanconi anemia patients (P16 and P17), two previously described Seckel syndrome patients (P18 and P19) (54), and one RNF168-deficient patient (P22) presented with ataxia, microcephaly, and immunodeficiency (39) were included in the study. P16, who carried compound heterozygous mutations in BRCA2, presented with intrauterine growth retardation, short stature, and developed acute myelocytic leukemia at 21 mo of age (55). P17 carried homozygous mutations in BRCA1 (p.S56) (56). P18 and P19 presented with dwarfism, microcephaly, and café au lait spots and carried homozygous SCKL2 mutations, which consist of a splice mutation in the RBBP6 gene, encoding CtIP. In addition, the hematopoietic cells of P17 were also CtIP deficient (56). The details of mutations, age at sampling, and cancer status for P1–P22 are shown in SI Appendix, Table S1. Four lymphoma patients with mutations in BRCA1 (described in SI Appendix, Table S4, and Fig. 1) were furthermore
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