Subject Review

The Role of the BRCA1 Tumor Suppressor in DNA Double-Strand Break Repair

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Abstract

The tumor suppressor gene *BRCA1* was cloned in 1994 based on its linkage to early-onset breast and ovarian cancer. Although the BRCA1 protein has been implicated in multiple cellular functions, the precise mechanism that determines its tumor suppressor activity is not defined. Currently, the emerging picture is that BRCA1 plays an important role in maintaining genomic integrity by protecting cells from double-strand breaks (DSB) that arise during DNA replication or after DNA damage. The DSB repair pathways available in mammalian cells are homologous recombination and nonhomologous end-joining. BRCA1 function seems to be regulated by specific phosphorylations in response to DNA damage and we will focus this review on the roles played by BRCA1 in DNA repair and cell cycle checkpoints. Finally, we will explore the idea that tumor suppression by BRCA1 depends on its control of DNA DSB repair, resulting in the promotion of error-free and the inhibition of error-prone recombinational repair. (Mol Cancer Res 2005;3(10):531–9)

Introduction

Hereditary breast cancers account for 5% to 10% of all breast cancers and are largely attributable to germ line mutations in either *BRCA1* or *BRCA2*. *BRCA1* was the first identified and cloned breast cancer susceptibility gene (1). Germ line mutations of the *BRCA1* gene account for ~40% to 45% of hereditary breast cancers and 80% of the families whose members have a high incidence of both breast and ovarian cancers (2-4). Although *BRCA1* gene mutations are rare in sporadic breast and ovarian cancers (5), BRCA1 protein expression is frequently reduced or absent in sporadic cases (6), suggesting a much wider role in both hereditary and sporadic mammary carcinogenesis. Why mutations of this gene strongly predispose to breast and ovarian cancers remains an unsolved mystery.

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Note: We apologize to those investigators whose important work we have not cited because of space restrictions.

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Recent studies have suggested that multiple functions of BRCA1 may contribute to its tumor suppressor activity, including roles in cell cycle checkpoints, transcription, protein ubiquitination, apoptosis, and DNA repair. Recently, a function of BRCA1 in DNA decatenation has been shown, which suggests that regulation of chromosome segregation may be another function of BRCA1 (7). Cancer-related BRCA1 mutations occur throughout the entire gene with studies of mutational spectra not revealing any significant mutational hotspots. Whether all these mutations share a common general mechanism is not established, but the protection of the genome by BRCA1 facilitating DNA repair is perhaps the most likely candidate mechanism.

BRCA1 Deficiency in Mice and Cultured Cells

In hereditary breast cancers, loss of the second wild-type allele is observed (8, 9), fitting the profile of a classic tumor suppressor gene. However, mouse embryos with a BRCA1 deficiency were found to die during early embryogenesis due to a proliferation defect (10-12). These findings seem incompatible with a tumor suppressor function and results in the "tumor suppressor paradox": how can a proliferative defect lead to tumorigenesis? The answer must involve some form of genetic cooperation (i.e., additional genetic changes allow BRCA1-deficient cells to grow in spite of the deficiency). Whether these additional changes develop while still heterozygous, or after the loss of function of both alleles, is not yet determined. BRCA1 deficiency results in the accumulation of DNA damage, and the inability to respond appropriately to genomic damage is thought to be the likely mechanism by which loss of BRCA1 results in genomic instability.

The response of cells to DNA damage can be divided into at least two pathways: DNA repair and cell cycle checkpoint activation. BRCA1-deficient cells show sensitivity to ionizing radiation and drugs that produce double-strand breaks (DSB) or interstrand cross-linking agents, such as mitomycin C (13-15). Sensitivity to these agents, coupled with genetic instability, was also observed in cells with an exon 11 isoform of BRCA1 (16) and BRCA1-deficient embryonic stem cells (17). Multiple, clinically observed, missense mutations arising throughout the entire *BRCA1* gene were found to be nonfunctional in assays of DNA DSB repair (18). Collectively, these findings suggest that the efficient repair of DSB by BRCA1 is linked to its tumor suppression activities. However, BRCA1 is also reported to participate in nucleotide excision repair, so the role of BRCA1 in DNA repair may ultimately be more complex (19). Cell cycle arrest in response

to DNA-damaging agents, such as ionizing radiation or mitomycin C, has shown that both acute S-phase checkpoint and G_2-M transition are dependent on the function of the BRCA1 protein (20, 21). As has been observed with other proteins, the checkpoint responses of BRCA1 can be genetically dissociated from the repair responses by the use of single amino acid mutations. The S988A mutation of BRCA1 (see Fig. 1) has normal S-phase checkpoint responses but shows a severe defect in homologous recombination (22). Conversely, the S1423A mutant has normal DNA repair but a defective G_2 checkpoint (22).

BRCA1 and DNA DSB Repair

Current studies suggest that BRCA1 plays a major role in the cellular response to DNA damage, mediating between the sensors of damage to the effectors of repair. BRCA1 becomes hyperphosphorylated after exposure to the DNA-damaging agents and is rapidly (within 1 hour) relocated to sites of replication forks marked by proliferating cell nuclear antigen (23). In addition, BRCA1 has been found in a large nuclear protein complex, named BRCA1-associated surveillance complex, which is believed to be an important sensor to monitor the genome for damage and to signal to downstream proteins (24).

There are two major types of DSB repair: homologous recombination (Fig. 2) and nonhomologous end-joining (NHEJ; Fig. 3). Homologous recombination is typically error-free, because an extensive nondamaged homologous sequence is used to repair the damaged duplex by gene conversion. NHEJ uses no sequence homology and usually involves a change in sequence at the break point. Perhaps, more importantly, the extent of sequence modification varies from loss or gain of 1 or 2 bp to large-scale deletions. The mutagenic potential of this pathway is determined as much by the quality of the repair rather than just whether the break is rejoined. If there is loss of sequence at the site of the break, the resulting junction is often mediated by a region of microhomology, up to 8 bp in size. Homologous recombination can also occasionally introduce errors in sequence by crossover events, but crossover products are actively suppressed in mitotic mammalian cells (25, 26). An intermediate type of repair, known as single-strand annealing that is independent of the Rad51 protein, is primarily involved in homologous recombination and can use sequence homology within a single chromosome. The likeliest source of single-strand annealing–driven events are the numerous regions of repeat sequence within the mammalian genome. Single-strand annealing typically involves significant lengths of sequence homology, up to kilobases, but whether it has a similar or different genetic basis compared with microhomology-mediated annealing is not clear. The molecular engine that drives single-strand annealing seems to require Rad52 and Rad50 in yeast, but it is not precisely defined in mammalian cells (27).

BRCA1 and Homologous Recombination

There are two likely roles for homologous recombination in mammalian cells: it is one method for repairing DNA DSB and it is the major pathway for restarting collapsed replication forks caused by a single-strand break, when there is no option for fork reversal or translesion synthesis by a specialized polymerase (Fig. 4). Loss of wild-type BRCA1 in mouse embryonic stem cells or human breast cancer cells, HCC1937, leads to reduced homology-mediated chromosomal plasmid integration or leads to decreased homology-directed repair of chromosomal DSBs induced by the site-specific I-*Sce*I endonuclease (15, 22, 28, 29). Stalled replication forks are the major source of DSBs in proliferating cells. BRCA1-containing nuclear foci are observed in untreated S-phase cells in addition to the cells exposed to DNA-damaging agents (23), where BRCA1 colocalizes with BARD1, Rad51, and proliferating cell nuclear antigen. It is suggested that the genetic instability in cells with a deficiency of BRCA1 is due to the defective response to replication fork stalling. Although it was originally suggested that the protection of the genome by BRCA1 could be indirect through cell cycle control (30), genetic dissociation of the repair defect from cell cycle effects makes this idea unlikely. BRCA1 plays a direct role in

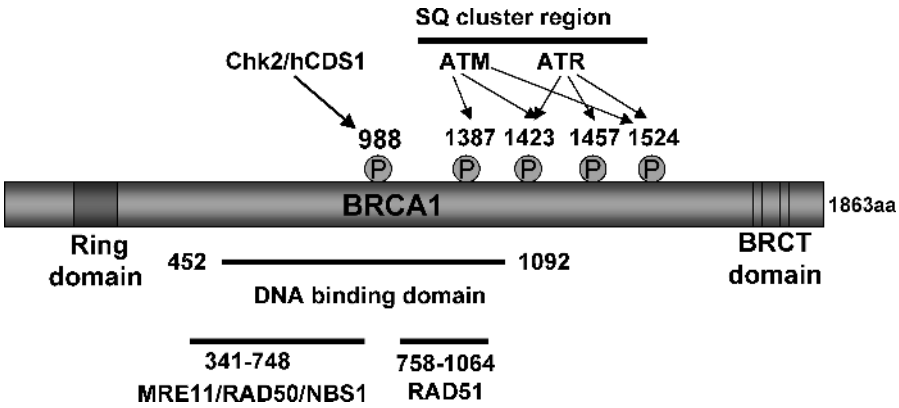

FIGURE 1. A schematic diagram of BRCA1 showing the functionally important sites, including the sites of BRCA1 protein phosphorylation. The BRCT domain was defined by being in the COOH terminus of BRCA1 and seems to be an important protein interaction domain for many DNA repair proteins. The ring domain at the NH_2 terminus of the protein has the features of an E3 ubiquitin ligase, but ubiquitylated targets of BRCA1 have not yet been identified. BRCA1 may function in cooperation with BARD1 as a ubiquitin ligase. The DNA-binding domain of BRCA1 has been mapped to a central region of the protein (amino acids 452-1092). There is a Chk2-dependent phosphorylation site at Ser^{988} within this domain. A SQ cluster region adjacent to the DNA-binding domain with multiple ATM and ATR target sites of phosphorylation is shown. The sites of interaction with Rad50 and Rad51 are shown.

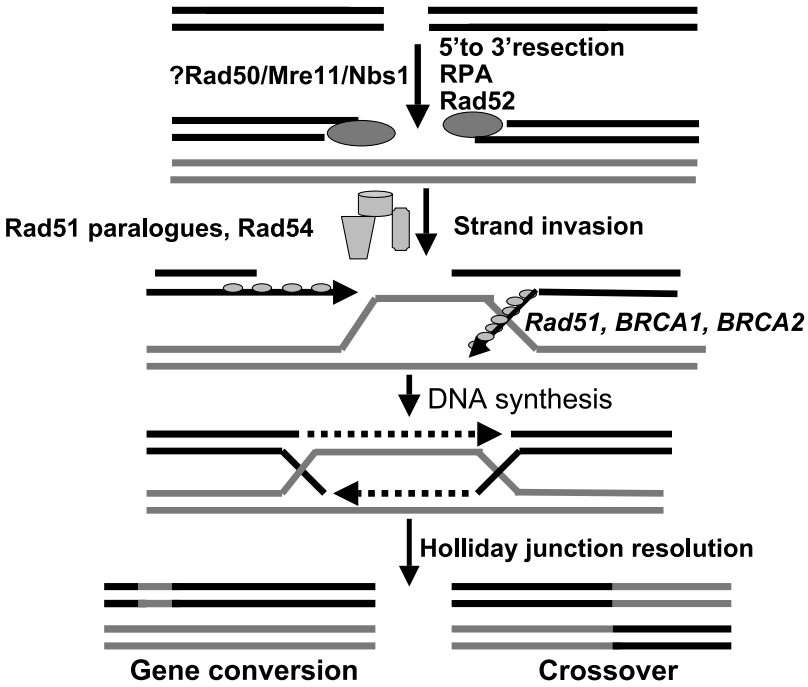

FIGURE 2. The key steps in the repair of a DSB by homologous recombination. The DNA gap is processed to develop 3′ tails of ssDNA. A large number of proteins are required for converting RPA bound to ssDNA into a Rad51 filament, which is then capable of invading an homologous region of dsDNA, usually the sister chromatid. The previous gapped region in the damaged duplex now has a template strand from the undamaged duplex, which can then be repaired by gap repair synthesis and ligation. Resolution of the Holliday junction can result in either nonreciprocal or reciprocal exchange and the patch or splice outcomes as shown.

responding to DSBs and, in ways still to be defined, recruits BRCA2, which facilitates Rad51 filament formation (31, 32). BRCA1 is observed to colocalize with Rad51, a DNA recombinase related to the bacterial RecA protein, which is required for the strand invasion of homologous recombination. BRCA1 is also required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin (33). Direct interactions between BRCA2-Rad51 and BRCA2-BRCA1 proteins have been reported; however, the association of Rad51 with BRCA1 at the site of DNA damage is most likely indirect through BRCA2 (34).

Curiously, BRCA2 was not found to be part of the large BRCA1-associated surveillance complex. The explanation may be that BRCA1 interacts with BRCA2 only transiently at sites of damage or stalled replication forks, whereas most of BRCA1 is found in other protein complexes. The fact that the only common feature shared by BRCA1 and BRCA2 is their role in homologous recombination suggests that homologous recombination is the important determinant preventing tumor susceptibility. However, BRCA1 has multiple roles in response to DSBs, whereas BRCA2 seems to have a single function in homologous recombination. BRCA1-deficient cells have defects in homologous recombination, NHEJ, and S- and G_2-M-phase checkpoints (20, 21). BRCA2 acts downstream of BRCA1 and has a more direct role in the process of homologous recombination via its interaction with Rad51.

BRCA1 is also found associated with another DNA damage response protein, Rad50, which forms a tight complex with Mre11 and Nijmegen breakage syndrome gene 1. This complex of Mre11/Rad50/Nijmegen breakage syndrome gene 1 (MRN) is implicated in both homologous recombination and NHEJ. Interestingly, the protein foci of Mre11 or Rad50 are recruited to the sites of DSB, but Rad51 foci are never seen in the same cell at the same time (35, 36). Rad50 and Rad51 can both colocalize with phosphorylated H2AX (γ-H2AX) foci after DNA damage. BRCA1 can also colocalize with γ-H2AX and is recruited to these sites before Rad50 or Rad51 (37), suggesting that BRCA1 may determine the recruitment of Rad50 or Rad51. However, MRN is also recruited early to sites of DSB in the presence or absence of H2AX (38). It is tempting to speculate that BRCA1 is involved in the response to DSB or stalled replication forks and directs the subsequent recruitment of repair proteins, either MRN or BRCA2/Rad51. Although it is not clear whether the MRN complex is involved in homologous recombination in human cells, it has been suggested that the MRN complex might function in the resolution of stalled replication forks (39) and Nijmegen breakage syndrome gene 1 was reported to facilitate homologous recombination in DT40 cells (40). Disruption of the *Nijmegen breakage syndrome gene 1* gene led to reduced gene conversion and sister chromatid exchanges, but the formation of Rad51 foci induced by γ-radiation (6 Gy) was not affected. Thus, one of the major unknowns to solve is how BRCA1 recruits BRCA2 and Rad51 while also influencing the behavior of the MRN complex. The relationship between BRCA1 function and the appearance of Rad50 nuclear protein foci has been controversial, with reports showing that BRCA1 was required for the formation Rad50 nuclear protein foci and other reports saying there was no such relationship (41-43). We reported that loss of function of BRCA1 resulted in more Mre11 foci after homologous recombination and that complementation of BRCA1 inhibited their formation (22). However, whether this observation results from Mre11 being recruited to sites of residual damage, presumably increased when BRCA1 is inactive, or whether BRCA1 is actively preventing the formation of the MRN complex at sites of damage is not yet resolved.

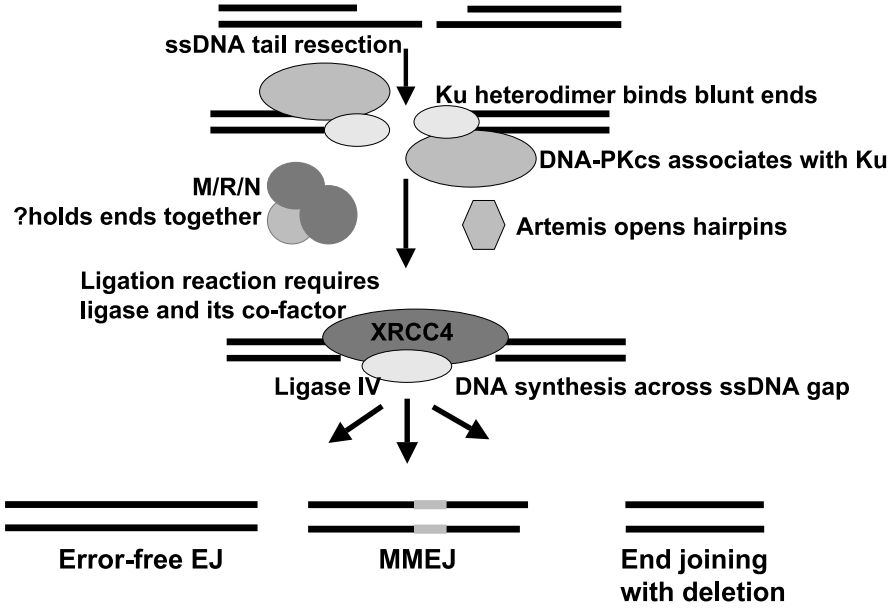

FIGURE 3. Key steps in the repair of a DSB by NHEJ. Here, end-binding proteins (Ku heterodimer) prefer a blunt end. Once bound, the catalytic subunit of DNA-PK is recruited and results in phosphorylation of the Ku proteins and itself, resulting in dissociation of DNA-PKcs and translocation of the Ku proteins. The "hand-off" mechanism to recruit XRCC4 and ligase IV is not fully characterized. Additional proteins, such as Artemis, are recruited for resolving covalent loops or hairpin ends. The role of the MRN complex in this process is still being defined. Repair usually results in loss of sequence at the repaired ends, although small insertions are also seen.

The BLM helicase, which is defective in Bloom's syndrome, has been shown to have a role in stalled replication forks and preventing excessive amounts of homo-logous recombination. Biochemically, BLM can reverse the formation of Holliday junctions (44). In the absence of BLM, sister chromatid exchanges are seen more frequently, leading to the idea that BLM is preventing Rad51-mediated recombination. The relationship between BLM function and BRCA1 is not fully elucidated, but one model is that BLM and BRCA1 are two different pathways for resolving stalled replication forks (Fig. 4). The stalled fork can result in the formation of a "chicken-foot" structure (reversal of the replication fork, such that the newly synthesized strands anneal to each other forming a four-strand structure). BLM is a helicase with specific activity to reverse this chicken-foot structure (45). Thus, a lesion that blocks progression of the replication fork can potentially be by-passed by fork reversal and template switching. Then, BLM plays the role of restarting replication by reversing the fork structure. Alternatively, the stalled replication fork can be cleaved by a specific endonuclease (such as Mus81) to form a double-stranded DNA end, which can then initiate a strand exchange reaction (46, 47). We hypothesize that BRCA1 facilitates the conversion of the stalled replication fork into a cleaved structure thereby initiating Rad51-dependent strand exchange. The precise mechanistic details of these processes are still to be elucidated, but in this model both BRCA1 and BLM would be expected to be present in a damage surveillance complex and BLM is found as part of the BRCA1-associated surveillance complex. Whether BRCA1 inhibits BLM function, perhaps predicted by this model, is not clear. Similarly to BRCA1, BLM is phosphorylated by ATM (48, 49) and forms foci with Rad51 after ionizing radiation exposure (50). The functional consequence of the ATM-dependent phosphorylation of BLM is not known, although a BLM phosphorylation mutant does not complement the sister chromatid exchange defect in Bloom's syndrome cells (51).

A Functional Link between BRCA1 and Fanconi Anemia Proteins

Early reports suggested that monoubiquitination of the FANCD2 protein is a key step in the Fanconi anemia pathway and is dependent on BRCA1 function. BRCA1-deficient cells are associated with a loss of DNA damage-inducible FANCD2 subnuclear foci and monoubiquitination of FANCD2 (52). Several lines of evidence suggested that FANCD2 might be a target of BRCA1 E3 ubiquitin ligase activity (53, 54). However, none of these studies have shown that FANCD2 was a direct substrate of the BRCA1/BARD1 ubiquitin ligase. Conversely, recent studies found that small interfering RNA–mediated knockdown of BRCA1 in human cells results in defective targeting of FANCD2 to sites of DNA damage but not to a defect in FANCD2 ubiquitination (55). Similarly, mutant DT40 cells containing a knockout of both BRCA1 and BARD1 are clearly sensitive to DNA damage, but they are not defective in FANCD2 ubiquitination (56). Thus, the purpose of BRCA1-mediated ubiquitination, the target protein, and whether this ubiquitin ligase activity is important for the roles of BRCA1 in homologous recombination or tumor suppression remain unknown. A recent report showed that the BRCA1-associated helicase, BACH1, is involved in homologous recombination and seems to be the Fanconi anemia gene product FANCJ (50). The identification of BACH1 as FANCJ and FANCD1 as BRCA2 suggests that there are multiple links between BRCA1 and the Fanconi anemia pathway (57, 58).

BRCA1 and NHEJ

There is a growing body of evidence suggesting that a component of NHEJ is regulated by BRCA1, which may also be important in maintaining genomic stability. Genotypic polymorphisms of the genes involved in NHEJ have been reported to be associated with a higher risk of developing breast cancer (59). The observation that breast cancer risk and DNA DSB

end-joining capacity are affected by BRCA1 provides support for the contribution of NHEJ to BRCA1-related breast cancer development (60). However, the reports addressing the function of BRCA1 in NHEJ have described somewhat conflicting observations (22, 28, 29, 61-65), but these results may be a reflection of the different assays used to measure NHEJ and the likelihood that there are different subpathways of NHEJ (see Fig. 3). It is known that cells have end-joining activity that is independent of Ku70/Ku80/DNA-PK/XRCC4/ligase IV, and recent unpublished evidence from our laboratory has suggested that BRCA1 stimulates a Ku-independent NHEJ pathway.

Subtypes of NHEJ must exist in mammalian cells, including error-free and error-prone end-joining. Error-free end-joining is detected as religation activity that is seen when linearized plasmid is converted back to circular DNA either by cell extracts or by cell-mediated assays *in vivo*. Most physiologic end-joining is error-prone, such as the coding joints of V(D)J rejoining. There can be either small changes in sequence or larger changes (which are typically deletions) when there is a deficiency in one or more components of the Ku-dependent repair pathway. Microhomologies may or may not be involved in the formation of the junction (66). Recently, there is evidence that error-free and error-prone end-joining have different genetic requirements (67), further supporting the idea that there is more than one pathway of NHEJ. Current evidence has supported the idea that BRCA1 is required for the error-free form of NHEJ (63, 68), but the mechanism involved in this regulation of NHEJ is not known. The Ku-DNA-PK–dependent pathway was initially thought to be the likely connection, but given the lack of effect of BRCA1 inactivation on either DSB repair, as measured by pulsed-field gel electrophoresis or on V(D)J recombination, both of which are medicated by the Ku70/Ku80/DNA-PKcs/XRCC4/ligase IV proteins, it is perhaps unlikely that BRCA1 promotes NHEJ via this pathway (22, 65).

In addition to error-free NHEJ, evidence from both yeast and mammalian cells strongly supports the notion that Ku-independent NHEJ is an error-prone process (69-72). BRCA1 may also regulate error-prone end-joining: BRCA1 inactivation was associated with large deletions at the site of the DSB (63). A physical interaction between BRCA1 and human Rad50 has been observed (43), but the functional consequences of this interaction remain unclear. Recently, Hopfner et al. (73) published crystallographic evidence that Rad50 forms a hook-like structure in its coiled region. Thus, the globular heads of the Rad50 protein, which interact with the DNA-binding region

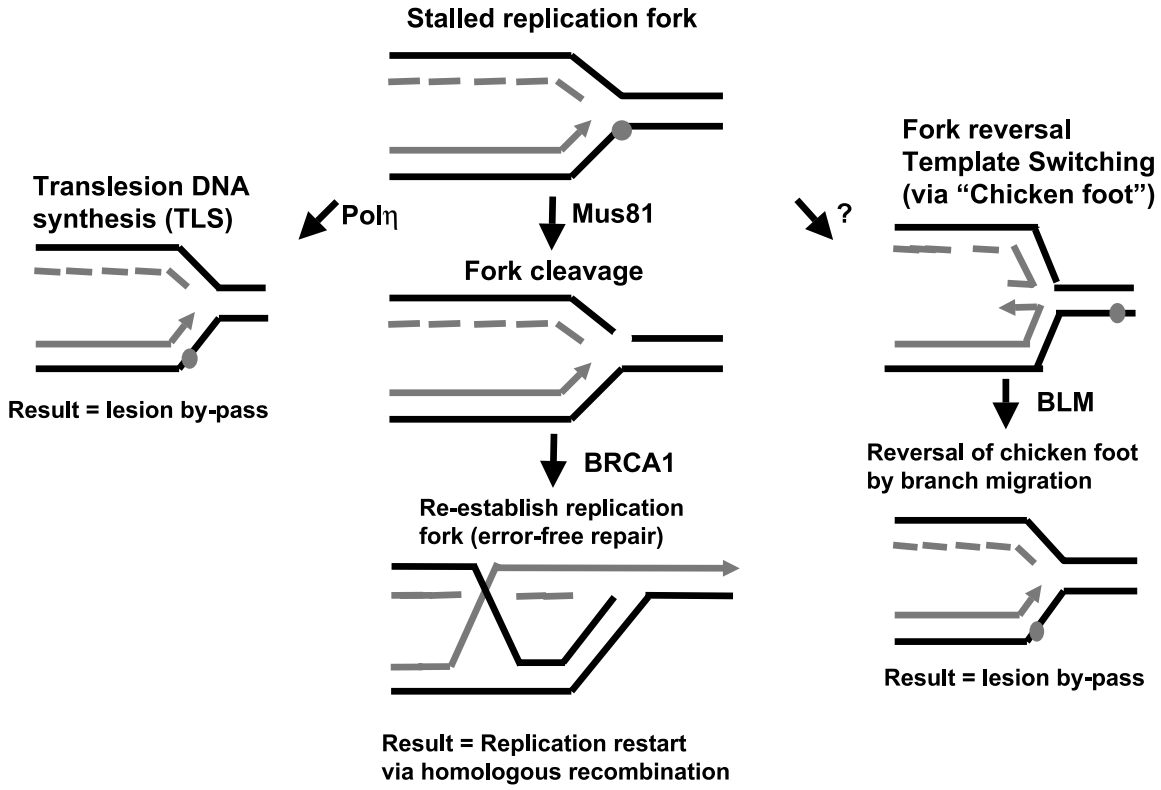

FIGURE 4. Proposed model for the roles of BRCA1 and BLM in blocked replication forks. The stalled replication fork can be resolved by at least three different pathways. The simplest is translesion synthesis, in which base damage sufficient to stall the replicative DNA polymerase can be by-passed by substituting a specialized TLS polymerase. The polymerase switching is mediated by ubiquitylation of proliferating cell nuclear antigen. Alternatively, the stalled replication fork can be reversed to form a chicken-foot structure, which then allows the extension of the blocked polymerase by synthesis on the alternate template of the newly synthesized lagging strand or allows the removal of the blocking lesion by direct repair mechanisms. The BLM protein seems to be the specific helicase involved in reversing the chicken-foot Holliday junction. The third pathway of restarting a stalled replication fork involves fork cleavage and then recombination to restart the replication fork. If the lesion blocking the replication fork results in a single-strand gap, fork cleavage is produced. Different blocking lesions may result in different preferences for the three pathways for recovering the stalled replication fork.

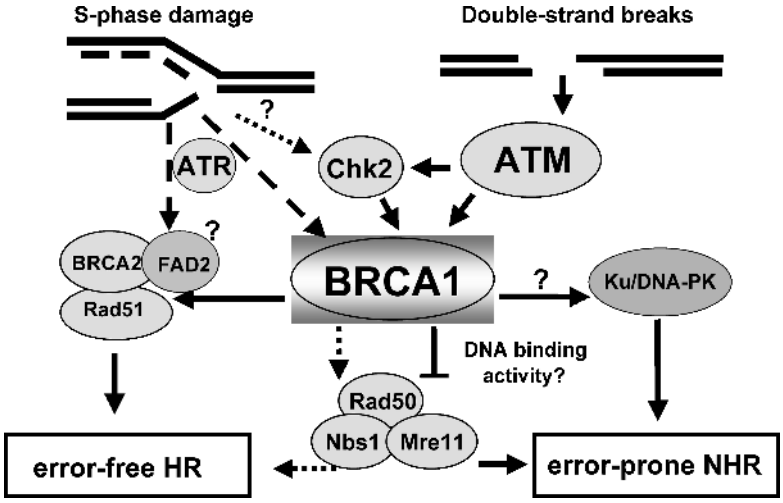

FIGURE 5. The schematic role of BRCA1 in DNA repair and S-phase arrest. Upstream damage signals via ATM and ATR plus Chk2 result in post-translational modifications of BRCA1 that affect its function in terms of DNA repair and cell cycle checkpoints. This review argues that BRCA1 functions to promote error-free recombination repair via BRCA2/Rad51 and functions to inhibit the MRN complex that promotes error-prone end-joining. The role of FancD2 is unclear in the error-free pathway. Other proteins, such as BACH1, likely play a role in this pathway. The role of BRCA1 in the Ku-dependent repair pathway is subject to debate, where the published reports are conflicting.

of two Mre11 proteins, can link (via this hook) two independent MRN complexes, thereby bringing together two DNA ends. This mechanism may play a role in holding sister chromatids together to allow homologous recombination or in holding together two unrelated broken ends to stimulate NHEJ. Therefore, the MRN complex can carry out both a structural holding function and the well-described nuclease function, which is associated with Mre11. Our previous studies had suggested that BRCA1 could inhibit the MRN complex, thereby reducing the associated error-prone NHEJ (22). In addition, we also observed that cells with a deficiency of BRCA1 preferred to use microhomologies to mediate end-joining, which was similar to the reported effect of Mre11 in yeast (72). The hypothesis that Mre11 activity is inhibited by wild-type BRCA1 to prevent error-prone end-joining was supported by the biochemical observation that BRCA1 inhibited the nucleolytic activity of Mre11 *in vitro* (74). It is possible that another unidentified protein, rather than Mre11, might be inhibited by BRCA1, because other nucleases with potential roles in end-joining have been suggested, such as WRN and Artemis (75-78).

Post-transcriptional Regulation of BRCA1 by Phosphorylation

BRCA1 has been identified as a target for several nuclear phosphatidylinositol 3-kinase relatives, such as ATM and ATR. BRCA1 is phosphorylated *in vitro* and *in vivo* on Ser^{1387}, Ser^{1423}, Ser^{1457}, and Ser^{1524} by ATM and is also phosphorylated by ATR on six serine or threonine residues, including Ser^{1423} *in vitro*, which is shared by ATM (refs. 79-82; see Fig. 1). Reported studies have suggested that ATM-mediated phosphorylations dominate the response to ionizing radiation, whereas ATR mediates phosphorylations that occur in response to UV-C irradiation (81). In addition, Ser^{1387} was specifically phosphorylated after ionizing radiation, whereas Ser^{1457} was phosphorylated predominantly after UV treatment. Recently, Chk2, which itself is thought to be a tumor suppressor (and mutations of which were found in Li-Fraumeni variants; ref. 83), was found to interact with BRCA1 in discrete nuclear foci after ionizing radiation and to phosphorylate BRCA1 on Ser^{988} (84, 85). However, there was little information about how these different phosphorylation events affected the functional repair pathway. Recently, our own studies have suggested that the Chk2-dependent phosphorylation site, Ser^{988}, is a major pathway to regulate the function of BRCA1 in homologous recombination and nonhomologous recombination (22). This established a relationship between a protein kinase (Chk2) and the regulation of DSB repair. The mechanism of how Chk2-mediated phosphorylation of BRCA1 subsequently regulates DSB repair is not fully understood. Biochemical studies have suggested that a DNA-binding domain of BRCA1 exists and maps to amino acids 452 to 1,079 (74). As a result of this DNA binding, BRCA1 inhibited the exonuclease activity of Mre11 *in vitro*. It has also been suggested that a Rad51 interaction domain was located in a segment of BRCA1 encompassing amino acids 758 to 1,064 (23). In our work, the Ser^{988} site of BRCA1 not only directed the control of homologous recombination but also was critical for the inhibition of error-prone end-joining. Thus, it is possible that the Ser^{988} mutation will regulate the function of the DNA-binding domain. In addition, this site mutation may also affect the interaction between BRCA1 and Rad51, but direct *in vivo* evidence for this hypothesis remains to be elucidated.

In addition to DNA repair, BRCA1-mediated effects on cell cycle checkpoints can also be modulated by protein kinases. The S1423A mutation abolished the ability of BRCA1 to cause G_2-M arrest while retaining the ability to support DNA repair (20, 22). In contrast, the ATM-dependent ionizing radiation–inducible S-phase checkpoint required the Ser^{1387} site of BRCA1 but not the Ser^{1423} site (21). It is recognized that homologous recombination plays a predominant role during the S and G_2-M phases of the cell cycle, when the sister chromatid template is available. However, the ability to support the S and G_2-M cell cycle checkpoints seems to be independent of the role in homologous recombination (22). The S988A mutation of BRCA can prevent its role in homologous recombination but does not have an effect on the S-phase checkpoint; conversely, the S1423/1524A mutation has normal homologous recombination but a deficient G_2-M phase checkpoint. There is further evidence to support this idea of genetic dissociation from

recent work with Fanconi anemia cells. Blocking the sites of ATM modification of FANCD2 (S222A) resulted in a defective S-phase checkpoint but had no effect on mitomycin C sensitivity (86).

MDC1 is another important regulator of the ATM-dependent phosphorylation of BRCA1 (87-90). This protein is a nuclear factor with a NH_2-terminal forkhead-associated domain and a tandem repeat of BRCT domains. These domains are present in several proteins involved in DNA repair and/or DNA damage signaling pathways. MDC1 is required to activate Chk2 as part of the response of mammalian cells to DNA damage. A failure to induce BRCA1 foci after ionizing radiation was observed when MDC1 was down-regulated in cells (88). Thus, the role of MDC1 in BRCA1-dependent effects, such as homologous recombination and cell cycle checkpoints, remains to be determined.

The emerging picture is that BRCA1 lies at a critical intersection of the DSB response pathways. Post-translational regulation of BRCA1 by phosphorylation has established the relationship between BRCA1 and Chk2, ATR, and ATM and has allowed insight into the relationship of BRCA1 with Mre11 and Rad51. This raises the possibility that, like mutations in BRCA1 itself, changes in Chk2, ATR, or ATM activity may promote genetic instability and increase susceptibility to the development of breast cancer and other tumors. A growing body of evidence has suggested that ATM mutations confer increased susceptibility to breast cancer (91-95). Epidemiologic studies have put the Chk2 and BRCA1 in the same breast cancer development pathway (96, 97). Genetic mutations of the Mre11/Rad50/Nijmegen breakage syndrome gene 1 complex have also been identified in breast cancers (98). There remains the possibility that deficiencies of ATR or Rad51 might be linked to breast cancer predisposition.

Summary

BRCA1 has an important role in DNA repair by promoting homologous recombination, in concert with BRCA2 and Rad51, and inhibiting error-prone NHEJ processes to restrict the extent of deletion at the break site. The active regulation of both repair mechanisms by the Chk2-dependent phosphorylation of Ser^{988} shows that DSB repair is tightly regulated toward an error-free (and not error-prone) recombinational repair process. Therefore, the chromosomal instability and the risk of breast cancer promoted BRCA1 germ line mutations is a direct consequence of their defects in DNA repair (see Fig. 5). New research initiatives to exploit this defect in DSB repair in BRCA1-related cancers should be forthcoming.

Carcinogenesis is a multistep process. Starting with a BRCA1 germ line mutation, the additional genetic events that are required for the development of breast cancer remains unclear. For example, inactivation of the p53 gene is found in >90% of BRCA1-related cancers; the reason for this observation is not clear in terms of an effect on DNA repair. The usual explanation is that loss of damage-induced apoptosis allows cells to survive with damage and further promote mutations, implying that these effects are independent. Finally, the reason why BRCA1 mutations predispose individuals to only breast and ovarian cancers remains obscure but is the subject of intense interest.

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