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Unsolved mystery: the role of BRCA1 in DNA end-joining

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ABSTRACT

Heritable mutations in the tumor suppressor gene *BRCA1* increase a woman's lifetime risk of developing breast and ovarian cancer. BRCA1's tumor suppressor function is directly linked to its myriad of functions in the cellular response to DNA double-strand breaks (DSBs). BRCA1 interacts with an extensive array of DNA damage responsive proteins and plays important roles in DSB repair, mediated by the homologous recombination pathway, and in the activation of cell cycle checkpoints. However, the role of BRCA1 in the other two DSB repair pathways, classical non-homologous end-joining (C-NHEJ) and alternative NHEJ (A-NHEJ), remains unclear. In this review, we will discuss the current literature on BRCA1's potential role(s) in modulating both C-NHEJ and A-NHEJ. We also present a model showing that BRCA1 contributes to genomic maintenance by promoting precise DNA repair across all cell cycle phases via the direct modulation of DNA end-joining.

KEYWORDS: BRCA1, NHEJ, HR, Ku70/80, DNA-PKcs, breast cancer

BRCA1 and breast cancer

Breast cancer is one of the deadliest and most common cancers affecting women. Although ~90% of breast cancers occur sporadically, ~2% are genetically linked to heritable mutations in the breast cancer associated gene 1 (*BRCA1*) [1]. Germline mutations in *BRCA1* confer increased susceptibility to developing breast cancer, with a lifetime risk of ~80% [2]. Mutations in the *BRCA1* gene also result in an elevated risk for various other types of cancer in women, including ovarian, fallopian tube and peritoneal cancer. In addition, these mutations are associated with an increased risk of pancreatic cancer in women and men, and surprisingly an elevated risk for prostate and breast cancer in men [2–6]. The human *BRCA1* gene is located on chromosome 17, specifically at 17q21; it consists of 24 exons and encodes for a protein of 1863 amino acids (1812 in mice) [7]. *BRCA1* is also known to encode at least two additional smaller-sized variant proteins due to alternate splicing [8–11]. The mature, full-length protein is located in the nucleus and comprises multiple functional domains, including an N-terminal RING finger domain, two nuclear localization signals, an 'SQ' cluster containing several serine and threonine residues that can be phosphorylated, a coiled-coiled domain, and C-terminal tandem BRCA1 C-terminus (BRCT) domains (Fig. 1) [12, 13]. The majority of BRCA1's functions are mediated by its zinc-binding RING finger motif that forms an enzymatically active E3 ubiquitin-protein ligase when it heterodimerizes with BRCA1-Associated RING Domain 1 (BARD1) and the tandem BRCT domain, which facilitates numerous protein–protein interactions via binding to phosphorylated serines. The importance of these two domains is underscored by the fact that a significant number of breast cancer predisposition mutations are located in these two domains [14].

BRCA1 in the cellular response to DNA double-strand breaks

Following its discovery, research has focused on identifying and characterizing BRCA1's function(s). To this end, multiple functions have been ascribed to BRCA1, including a role in transcription-coupled DNA repair, transcription regulation, chromatin remodeling, apoptosis, and ubiquitin ligation [12, 13]. Despite contributing to a diverse array of cellular pathways, BRCA1's function as a tumor suppressor is likely due to its role in promoting genomic stability. This was initially proposed with the discovery that tumor cell lines and mouse embryonic

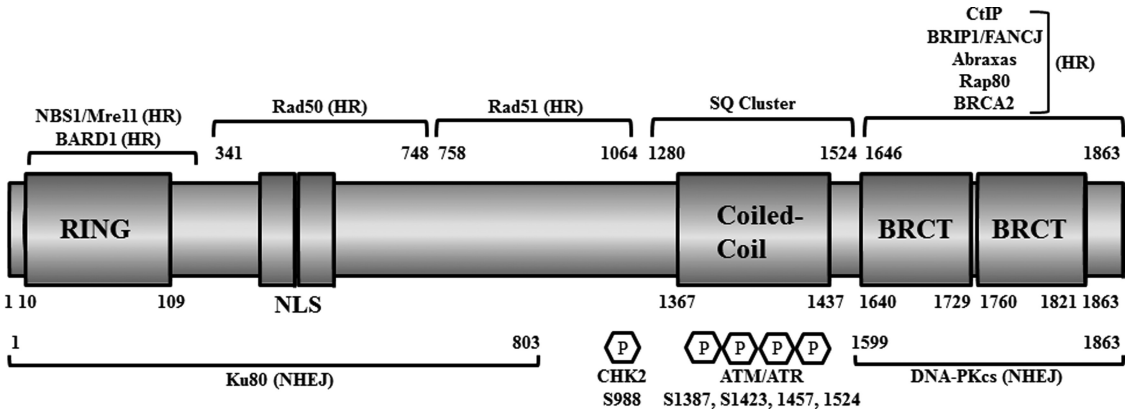

Fig. 1. Functional domains of BRCA1. Interacting proteins and phosphorylation sites required for regulating homologous recombination (HR) and non-homologous end-joining (NHEJ).

fibroblasts (MEFs) deficient in *BRCA1* exhibit evidence of extensive genomic instability, including patterns of aneuploidy, centrosomal amplification, and chromosomal aberrations [1, 11, 15, 16]. BRCA1 primarily promotes genomic stability via its numerous functions in the cellular response to DNA double-strand breaks (DSBs). DSBs are deleterious DNA lesions that may lead to gene mutations, senescence, apoptosis, mitotic cell death, genomic instability, and tumorigenesis if misrepaired or left unrepaired [17]. An immediate and complex cellular response to DSBs drives multiple processes, including modulation of the cell cycle, a number of signaling cascades collectively known as the DNA damage response (DDR), and repair pathways that correct the DNA lesion [18]. DSBs are repaired by three major pathways in mammalian cells: the error-free and accurate homologous recombination (HR) pathway, the error-prone but relatively precise classical non-homologous end-joining (C-NHEJ) pathway, and the error-prone alternate non-homologous end-joining (A-NHEJ) pathway [19]. HR repairs DSBs by utilizing a DNA template, typically via a homologous sister chromatid in the S or G2 phases of the cell cycle, to drive repair. C-NHEJ mediates the direct re-ligation of the broken DNA molecule, and is active throughout the cell cycle, partly due to lack of constraints such as the requirement of a DNA template for repair completion [20]. A-NHEJ is active in all phases of the cell cycle, backing up the other two pathways [21]. It is an error-prone process due to its propensity to utilize microhomologies distant from the DSB to mediate repair, which results in deletions. Collectively, these DNA repair mechanisms are responsible for fixing the countless insults our genomes are exposed to, including those induced both by fault (ionizing radiation (IR)-induced DSBs, replication errors, etc.) and by design (V(D)J recombination).

BRCA1 plays a multifaceted role in the cellular response to DNA damage, including modulation of DSB repair and activation of cell cycle checkpoints. The first evidences of BRCA1 involvement in DSB repair came from studies showing that upon irradiation (IR), BRCA1 is hyperphosphorylated and forms discrete nuclear foci that co-localize with the HR factors Rad51 and BRCA2 [22, 23]. BRCA1 primarily functions as a mediator in the cellular response to DNA damage; it serves as a scaffold protein that recruits multiple repair proteins to the DSB via the formation of multiprotein complexes [24]. These distinct multiprotein complexes function in specific processes in the DNA damage response, in particular those that influence DSB repair. BRCA1-specific complexes include the following: (i) a complex with the BRCA1 interacting protein C-terminal helicase 1 (BRIP, also called FANCJ or BACH1) that inhibits C-NHEJ and promotes HR; (ii) a complex with the CtBP-interacting protein (CtIP) and MRE11-RAD50-NBS1 (MRN) that promotes DNA end resection, a prerequisite for the onset of HR; (iii) a complex with BRCA2, PALB2 and Rad51 that is required for DNA strand invasion for HR; and (iv) a complex with Rap80 and Abraxas that blocks aberrant DNA end resection to promote genomic stability [24]. Collectively, these BRCA1 complexes indicate a role for BRCA1 in promoting high-fidelity repair of DSBs, in particular by promoting HR. The positive influence of BRCA1 on HR is supported by studies showing that BRCA1-deficient cells are sensitive to IR and DNA cross-linking agents, which both produce DNA damage that is repaired by HR. Additionally, BRCA1-deficient cells also display increased frequency of chromatid breaks, which are frequently observed in HR-deficient cells [16]. Finally, BRCA1 deficiency leads to a reduction in HR repair, as evaluated by reporter assays, in both human and mouse cells [25, 26]. The role of BRCA1 in the HR pathway has been thoroughly dissected and properly reviewed by a number of groups [12, 13, 27]. BRCA1 also influences the cellular response to DNA damage by modulating the cell-cycle checkpoints in response to DSBs. Following DSB induction, BRCA1 is phosphorylated by the ataxia telangiectasia mutated (ATM) kinase at serine residues 1387 and 1423, and these phosphorylation events are required for activation of the S and G2/M checkpoints, respectively [28, 29]. Furthermore, the BRCA1–BARD1 interaction is important for ATM and ataxia telangiectasia and Rad 3-related (ATR)-mediated phosphorylation of p53 at serine 15 following IR- or UV-induced DNA damage to activate the G1/S checkpoint [30]. Although it is well established that BRCA1 plays an important role in the cellular response to DSBs, in particular HR, the role of BRCA1 in C-NHEJ and A-NHEJ remains a conundrum [31, 32]. Over the years, conflicting evidence pertaining to BRCA1's role in each pathway has added to the mystery. These aspects will be presented and discussed in detail in this review.

BRCA1 in C-NHEJ

Significant effort has also been directed at uncovering a role for BRCA1 in C-NHEJ. One study showed that extracts derived from BRCA1-null MEFs exhibit significantly reduced end-joining activity

compared with MEFs with wild-type BRCA1, providing initial insight into BRCA1's function in C-NHEJ [33]. Similarly, whole-cell extracts from the human *BRCA1*-defective cell line HCC1937 had significantly reduced C-NHEJ activity compared with control cell extracts [34]. However, a number of studies suggest that BRCA1 is not required for C-NHEJ. For example, no discernible defect in DNA DSB rejoining was observed in HCC1937 cells when assayed by pulsed-field gel electrophoresis (PFGE) [35, 36]. Furthermore, *in vitro* NHEJ assays performed on a host of sporadic breast cancer and *BRCA1*-deficient cell lines revealed no major overall repair deficiency and demonstrated similar end-joining efficiencies and accuracies [37]. This glut of conflicting data has made it difficult to clearly define a role for BRCA1 in C-NHEJ. The contradictory results are likely due to variations in the assays used, differences in cell lines, and/or differences in the cell cycle phases when the assays were performed [31].

Closer analyses of the end-joining events revealed that BRCA1 is required for precise end-joining [34, 38, 39]. This was supported by a study showing that siRNA-mediated knockdown of BRCA1 reduced the frequency of precise ligation by C-NHEJ in chromosomally induced DSBs [40]. The decrease in precise repair in the *BRCA1*-deficient cells was similar to that observed when the C-NHEJ components Ku70, XRCC4 and Ligase IV were knocked down [40]. Furthermore, BRCA1 knockdown increased the proportion of deletions relative to knockdown of the NHEJ components, indicating that BRCA1 not only promotes precise end-joining, but may also divert some DSBs away from end-joining by precise C-NHEJ. Expression of the BRCA1Δ14–15 splice variant or downregulation of wild-type BRCA1 in the breast cancer cell line MCF-7 led to a reduction in the overall, as well as precise, end-joining efficiency, indicating that expression of this splicing variant has a dominant negative effect on the efficiency and fidelity of C-NHEJ [41]. Overexpression of the other BRCA1 splicing variant, termed BRCA1Δ17–19, lacking a portion of the BRCT domain that enables its interaction with critical DNA end-processing factors including CtIP and Abraxas, resulted in delayed dynamics of IR-induced BRCA1 foci formation, impaired HR, and undermined C-NHEJ activity [41]. Finally, decreased fidelity in DNA end-joining was observed in lymphoblastoid cell lines from breast cancer patients harboring a *BRCA1* missense mutation [38, 42].

To follow up on the intriguing results of BRCA1 regulating precise end-joining, several studies were directed at determining how BRCA1 mediates/influences C-NHEJ. Phosphorylation of BRCA1 by Chk2 at serine 988 plays a role in promoting precise end-joining [43, 44]. BRCA1 is also phosphorylated by ATM kinase in response to DSBs, and ATM-mediated phosphorylation of BRCA1 at serine 1423 and serine 1524 was found to be important for precise end-joining activity by C-NHEJ [43]. It was also reported that the N-terminal fragment of BRCA1 (1–304 aa), containing the RING finger domain, accumulates and dissociates rapidly after laser irradiation–induced damage and that this fast association with DSBs is dependent on the C-NHEJ factor Ku80 [45]. The BRCA1–Ku interaction and the rapid recruitment of BRCA1 to DSBs were abolished via cancer causing missense mutations in the RING finger domain. This finding suggests that this interaction is important in BRCA1's ability to promote genomic stability [45]. However, another study reported that amino acids 262–803, but not amino acids 1–200 of BRCA1, mediates the interaction between BRCA1 and Ku80 [7]. The authors found that BRCA1 stabilizes Ku80 binding to chromosomal breaks in G1 phase. In addition, knockdown of BRCA1 resulted in a significant reduction in C-NHEJ in G1 phase cells, with no effect in G2/S phase cells. Collectively, the data in the literature suggests that interaction of BRCA1 with the C-NHEJ factor Ku80 stabilizes the Ku heterodimer at DSBs and that this is required for precise end-joining repair by C-NHEJ in G1 phase of the cell cycle.

BRCA1 in Alt-NHEJ

BRCA1, as a regulator of genomic stability, has been shown to mostly influence DSB repair processes, such as HR and precise end-joining, involved in precise ligation/repair of the broken DNA strand. However, a few reports have suggested that this may not be entirely true, as BRCA1 has been shown to positively influence the inherently error-prone A-NHEJ pathway. First, BRCA1 null MEFs exhibited a 50–100-fold deficiency in microhomology-mediated end-joining (MMEJ)/A-NHEJ of a defined chromosomal DSB [42]. Furthermore, a recent study found that BRCA1 in conjunction with CtIP are required for telomeric fusions in TRF2-depleted cells (uncapped telomeres), independent of Ku80/Ligase IV (C-NHEJ), but dependent on PARP1/Ligase III, well known components of A-NHEJ [10]. BARD1, an interaction partner of BRCA1, was shown to mediate the rapid recruitment of BRCA1 to DNA damage sites [9]. The tandem BRCT domains of BARD1 were found to be a poly ADP-ribose (PAR) binding module, and binding of the BARD BRCTs to PAR targets the BRCA1/BARD1 heterodimer to DNA damage sites. PARs are linked to proteins via PAR polymerases (PARPs), with PARP1 being a factor required for A-NHEJ [21]. PARP1 inhibition suppresses the early recruitment of the BRCA1/BARD1 complex to DNA lesions, suggesting that the A-NHEJ pathway may influence BRCA1's recruitment to DSBs.

In contrast, a significant amount of data suggests that BRCA1 blocks A-NHEJ. Knockdown or loss of BRCA1 protein resulted in an increased frequency of overall plasmid DNA mutagenesis and microhomology-mediated end-joining (MMEJ)/A-NHEJ following DSB induction [46]. Furthermore, inhibition of the exonuclease activity of the DNA end processing and A-NHEJ factor, Mre11, with the specific inhibitor mirin significantly decreased the occurrence of A-NHEJ/MMEJ, but did not considerably affect the overall mutagenic frequency of plasmid DSB repair [46]. These results suggest that BRCA1 protects DNA from mutagenesis during nonhomologous DSB repair. BRCA1 is also known to interact with BRIP1, and disruption of the BRCA1–BRIP1 complex through mutation in *BRIP1* compromised C-NHEJ and accelerated error-prone A-NHEJ/MMEJ [47]. Furthermore, disruption of the integrity of the BRIP1 helicase domain resulted in a modest decrease in extrachromosomal (but not intrachromosomal) A-NHEJ/MMEJ. We propose that BRCA1 typically blocks A-NHEJ, but may promote error-prone repair when C-NHEJ is absent and/or inhibited, like at clustered or complex lesions and telomeric DSBs, circumstances when repair of the broken DNA ends takes precedence over restoring sequence accuracy.

BRCA1 influences C-NHEJ in a cell cycle–specific manner

We propose that BRCA1 promotes genomic stability by modulating multiple DSB repair pathways in a cell cycle–specific manner. First,

BRCA1 promotes HR, the prominent DSB repair pathway in S/G2 phases [12, 13]. As BRCA1 positively influences HR, BRCA1 is believed to primarily be biologically active in S and G2 phases of the cell cycle. This is supported by early studies showing that BRCA1 expression is extremely low in G1 phase, but high in S and G2 phases [48, 49]. However, BRCA1 expression is normal in G1 in cycling cells, suggesting that BRCA1 expression is only low in G1 phase when cells are contact inhibited [50]. It was found that two circuits, the pro-HR factors BRCA1/CtIP and the pro-C-NHEJ factors 53BP1/RIF1, influence each other antagonistically, with BRCA1/CtIP displacing 53BP1/RIF1 from DSBs in S phase to allow DNA end resection to proceed to initiate HR, whereas 53BP1/RIF1 blocks localization of BRCA1 to DSBs in G1 [50, 51]. The inability of BRCA1 to form detectable DSB-induced foci in G1 suggests that BRCA1 should not be able to modulate C-NHEJ in this cell cycle phase. However, we propose that BRCA1 differentially influences specific DSB repair processes by either directly interacting with proteins at the sites of DSBs or in the vicinity of the DSB site in a cell cycle phase–dependent manner. This is supported by a recent study demonstrating that BRCA1 is recruited to both the DSB site and to regions surrounding the DSB [52]. BRCA1 is recruited directly to the vicinity of the DSB through an interaction with the MRN complex factor, Nijmegen Breakage Syndrome 1 Protein (NBS1), and this interaction is required for precise re-ligation of genomic DSBs by C-NHEJ in G1. We hypothesize that BRCA1 modulates C-NHEJ directly at the DSB site in G1 by influencing the dynamics of Ku70/80 at DSB sites (Fig. 2) [7]. We predict that the fraction of BRCA1, which is localized in the flanking regions of the DSB and not at the DSB site, is disrupted by 53BP1/RIF1. Thus, 53BP1/RIF1 does not influence the ability of BRCA1 to regulate precise end-joining by C-NHEJ. However, Ku80's function in C-NHEJ in G1 may only be partially regulated by BRCA1, because silencing of BRCA1 does not completely disrupt Ku80 binding to DSB ends; also, the effect of Ku80 loss on C-NHEJ is superior to that of BRCA1 loss [7]. We speculate that C-NHEJ may contain subset/multiple pathways, those that are inherently precise or error-prone. If BRCA1 modulates only a subset of C-NHEJ in G1 phase that drives precise end-joining, this raises a number of questions. For example, the factors required for this repair process are still unclear. Also, it is still unknown if BRCA1 regulates specific enzymes required for this subset of C-NHEJ, and if this is dependent on the nature of the DSB (i.e. 'easily ligatable ends' vs 'ends that require processing'). The requirement for BRCA1 in a subset of C-NHEJ, which is inherently precise, may also explain the contradictory reports in regards to BRCA1's role in C-NHEJ. Last, it is also possible that BRCA1 does not actually modulate C-NHEJ directly, but promotes precise end-joining by blocking A-NHEJ in G1 phase. BRCA1 inhibits the nuclease activity of MRE11 and the MRN complex *in vitro* [53]. As MRE11 nuclease activity is required for the formation of microhomology in A-NHEJ, BRCA1 may attenuate MRE11-dependent DNA end processing to drive DSB repair towards the more precise C-NHEJ pathway in G1 phase. It will be of great interest to determine if and how BRCA1 promotes precise rejoining of DSBs in G1 directly.

In S phase, BRCA1 plays a number of roles that promote HR [12, 13]. One key role is the stimulation of HR by attenuating C-NHEJ in this cell cycle phase to block inappropriate repair of replication-associated DSBs. This is supported by studies indicating that the embryonic lethality, HR deficiency, and genomic instability associated with loss of BRCA1 in mouse models is driven by C-NHEJ in S phase, and that this can be rescued by genomic deletion of the pro-NHEJ factor 53BP1 [54, 55]. Furthermore, the Serine–Glutamine (SQ) cluster between the N- and C-terminal domains of BRCA1 contains residues phosphorylated by ATM and ATR that are critical for HR. Mutations at these sites (S1387A, S1423A, S1457A and S1524A) shifted DSB repair from HR to NHEJ and abrogation of the G2/M checkpoint, leading to increased chromosomal

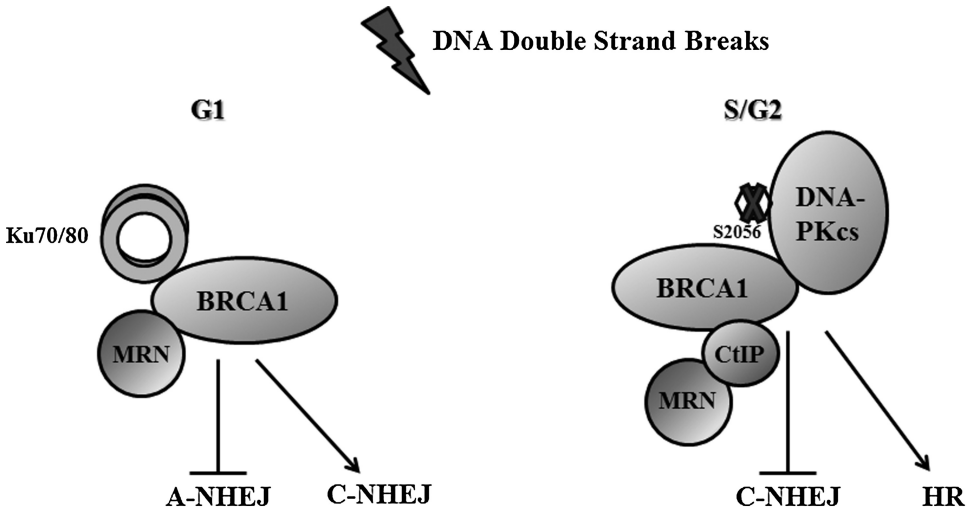

Fig. 2. In G1 phase, BRCA1, through its N-terminal interaction with Ku80, stabilizes Ku80 binding to DNA ends (favoring C-NHEJ) and inhibits end-processing activity of the MRN complex through its interaction with NBS1, preventing DSBs from being repaired by mutagenic A-NHEJ. In S/G2 phases, BRCA1 blocks the autophosphorylation of DNA-PKcs at S2056 through its C-terminal interaction with DNA-PKcs, preventing DSBs from being funneled to the C-NHEJ pathway. BRCA1's interaction with the CtIP and MRN complex accelerates end processing, thus driving the repair of DSBs to the HR pathway.

aberrations and mitotic catastrophe [56]. Our recent data suggest that BRCA1 may also modulate C-NHEJ directly in S phase [57]. We found that DNA-PKcs interacts with BRCA1 independently of DNA damage, but that this interaction is specific for S phase of the cell cycle. DNA-PKcs autophosphorylation at serine 2056 is cell cycle–regulated with phosphorylation at this site, high in G1 but markedly attenuated in S phase. This finding suggests that attenuating 2056 phosphorylation may modulate NHEJ specifically in this cell cycle phase. We demonstrated that autophosphorylation of DNA-PKcs at serine 2056 is attenuated in S phase by BRCA1 [57]. Blocking phosphorylation of DNA-PKcs at the serine 2056 cluster resulted in an increase in the DNA end processing required for HR, as observed via increased RPA and Rad51 focus formation. Our data suggest that BRCA1 attenuates NHEJ specifically by abrogating DNA-PKcs autophosphorylation. This is different from BRCA1's role in NHEJ in G1 because the interactions of BRCA1 with Ku80 and DNA-PKcs are distinct, with BRCA1 interacting with Ku80 in G1 phase and with DNA-PKcs in S phase. Furthermore, Ku80 interacts with the N-terminus of BRCA1 and DNA-PKcs interacts with the C-terminal region of BRCA1, and this interaction occurs even in the absence of Ku70/80. Hence, BRCA1 influences C-NHEJ in a cell cycle phase–dependent manner via differential interaction with Ku80 (G1 phase) and DNA-PKcs (S phase). We believe these interactions play important roles in driving precise repair in all cell cycle phases, promoting precise C-NHEJ and blocking erroneous A-NHEJ in G1 and driving HR and attenuating C-NHEJ in S/G2 phases (Fig. 2).

CONCLUSION

We propose that BRCA1 promotes precise DSB repair across all cell cycle phases. In the absence of HR in G1, BRCA1's interactions with Ku80 and the MRN complex positively influences the relatively precise C-NHEJ pathway, while negatively regulating mutagenic A-NHEJ. In S phase, the interaction of BRCA1 with DNA-PKcs inhibits C-NHEJ and drives pathway choice towards the accurate HR pathway (Fig. 2). BRCA1 is thus a critical factor driving repair pathway choice and is required for a cell's ability to maintain genomic stability. In conclusion, BRCA1 drives precise DSB repair while negatively regulating the error-prone pathways. It is this function that promotes BRCA1's roles as a tumor suppressor and a driver of genomic stability. The role of BRCA1 in DNA damage response, with emphasis on accurate repair of genomic insults, provides a better understanding of the etiology of BRCA1-associated tumorigenesis. It also provides a solid platform for the development of therapeutic approaches, some of which have already entered the clinical setting.

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REFERENCES

1. Tirkkonen M, Johannsson O, Agnarsson B-A, et al. Distinct somatic genetic changes associated with tumor progression in carriers of *BRCA1* and *BRCA2* germ-line mutations. *Cancer Res* 1997;57:1222–7.
2. Bau D-T, Fu Y-P, Chen S-T, et al. Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by *BRCA1*. *Cancer Res* 2004;64:5013–9.
3. Coupier I, Baldeyron C, Rousseau A, et al. Fidelity of DNA double-strand break repair in heterozygous cell lines harbouring *BRCA1* missense mutations. *Oncogene* 2004;23:914–9.
4. Zhong Q, Boyer T-G, Chen P-L, et al. Deficient nonhomologous end-joining activity in cell-free extracts from Brca1-null fibroblasts. *Cancer Res* 2002;62:3966–70.
5. Xu X, Weaver Z, Linke S-P, et al. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in *BRCA1* exon 11 isoform-deficient cells. *Mol Cell* 1999;3:389–95.
6. Davis A-J, Chi L, So S, et al. *BRCA1* modulates the autophosphorylation status of DNA-PKcs in S phase of the cell cycle. *Nucleic Acids Res* 2014;42:11487–501.
7. Jiang G, Plo I, Wang T, et al. BRCA1–Ku80 protein interaction enhances end-joining fidelity of chromosomal double-strand breaks in the G1 phase of the cell cycle. *J Biol Chem* 2013;288:8966–76.
8. Lin W-Y, Wilson J-H, Lin Y. Repair of chromosomal double-strand breaks by precise ligation in human cells. *DNA Repair* 2013;12:480–7.
9. Li M, Yu X. Function of *BRCA1* in the DNA damage response is mediated by ADP-ribosylation. *Cancer Cell* 2013;23:693–704.
10. Badie S, Carlos A-R, Folio C, et al. *BRCA1* and *CtIP* promote alternative non-homologous end-joining at uncapped telomeres. *EMBO J* 2015;34:410–24.
11. Dohrn L, Salles D, Siehler S-Y, et al. BRCA1-mediated repression of mutagenic end-joining of DNA double-strand breaks requires complex formation with BACH1. *Biochem J* 2012;441:919–26.
12. Huen M-S, Sy S-M, Chen J. *BRCA1* and its toolbox for the maintenance of genome integrity. *Nat Rev Mol Cell Biol* 2010;11:138–48.
13. Thompson E-G, Fares H, Dixon K. BRCA1 requirement for the fidelity of plasmid DNA double-strand break repair in cultured breast epithelial cells. *Environ Mol Mutagen* 2012;53:32–43.
14. Rebbeck T-R, Mitra N, Wan F, et al. Association of type and location of *BRCA1* and *BRCA2* mutations with risk of breast and ovarian cancer. *JAMA* 2015;313:1347–61.
15. Weaver Z, Montagna C, Xu X, et al. Mammary tumors in mice conditionally mutant for *Brca1* exhibit gross genomic instability and centrosome amplification yet display a recurring distribution of genomic imbalances that is similar to human breast cancer. *Oncogene* 2002;21:5097–107.
16. Riballo E, Critchlow S-E, Teo S-H, et al. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol* 1999;9:699–702.
17. Hanahan D, Weinberg R-A. Hallmarks of cancer: the next generation. *Cell* 2011;144:646–74.
18. Thompson L-H. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. *Mutat Res* 2012;751:158–246.
19. Goodarzi A-A, Jeggo P-A. The repair and signaling responses to DNA double-strand breaks. *Adv Genet* 2013;82:1–45.

20. Davis A-J, Chen D-J. DNA double strand break repair via non-homologous end-joining. *Transl Cancer Res* 2013;2:130–43.
21. Redon C-E, Dickey J-S, Bonner W-M, et al. γ-H2AX as a biomarker of DNA damage induced by ionizing radiation in human peripheral blood lymphocytes and artificial skin. *Adv Space Res* 2009;43:1171–8.
22. Wang H-C, Chou W-C, Shieh S-Y, et al. Ataxia telangiectasia mutated and checkpoint kinase 2 regulate *BRCA1* to promote the fidelity of DNA end-joining. *Cancer Res* 2006;66:1391–400.
23. Moynahan M-E, Chiu J-W, Koller B-H, et al. *Brca1* controls homology-directed DNA repair. *Mol Cell* 1999;4:511–8.
24. Pollard J-M, Gatti R-A. Clinical radiation sensitivity with DNA repair disorders: an overview. *Int J Radiat Oncol Biol Phys* 2009;74:1323–31.
25. Hendry J-H, Simon S-L, Wojcik A, et al. Human exposure to high natural background radiation: what can it teach us about radiation risks? *J Radiol Prot* 2009;29:A29–42.
26. Hall E-J, Brenner D-J. Cancer risks from diagnostic radiology. *Br J Radiol* 2008;81:362–78.
27. Girard P-M, Kysela B, Härer C-J, et al. Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. *Hum Mol Genet* 2004;13:2369–76.
28. Beels L, Werbrouck J, Thierens H. Dose response and repair kinetics of gamma-H2AX foci induced by *in vitro* irradiation of whole blood and T-lymphocytes with X- and gamma-radiation. *Int J Radiat Biol* 2010;86:760–8.
29. Sak A, Grehl S, Erichsen P, et al. γ-H2AX foci formation in peripheral blood lymphocytes of tumor patients after local radiotherapy to different sites of the body: dependence on the dose-distribution, irradiated site and time from start of treatment. *Int J Radiat Biol* 2007;83:639–52.
30. Fabbro M, Savage K, Hobson K, et al. BRCA1–BARD1 complexes are required for p53Ser-15 phosphorylation and a G1/S arrest following ionizing radiation–induced DNA damage. *J Biol Chem* 2004;279:31251–8.
31. Bau D-T, Mau Y-C, Shen C-Y. The role of BRCA1 in non-homologous end-joining. *Cancer Lett* 2006;240:1–8.
32. Durant S-T, Nickoloff J-A. Good timing in the cell cycle for precise DNA repair by BRCA1. *Cell Cycle* 2005;4:1216–22.
33. Redon C-E, Nakamura A-J, Gouliaeva K, et al. The use of gamma-H2AX as a biodosimeter for total-body radiation exposure in non-human primates. *PloS One* 2010;5:e15544.
34. Schmid T-E, Zlobinskaya O, Multhoff G. Differences in phosphorylated histone H2AX foci formation and removal of cells exposed to low and high linear energy transfer radiation. *Curr Genomics* 2012;13:418–25.
35. Ciccia A, Elledge S-J. The DNA damage response: making it safe to play with knives. *Mol Cell* 2010;40:179–204.
36. Roch-Lefevre S, Mandina T, Voisin P, et al. Quantification of γ-H2AX foci in human lymphocytes: a method for biological dosimetry after ionizing radiation exposure. *Radiat Res* 2010; 174:185–94.
37. Merel P, Prieur A, Pfeiffer P, et al. Absence of major defects in non-homologous DNA end joining in human breast cancer cell lines. *Oncogene* 2002;21:5654–9.
38. Plowman P-N, Bridges B-A, Arlett C-F, et al. An instance of clinical radiation morbidity and cellular radiosensitivity, not associated with ataxia-telangiectasia. *Br J Radiol* 1990;63:624–8.
39. Iliakis G-E, Cicilioni O, Metzger L. Measurement of DNA double-strand breaks in CHO cells at various stages of the cell cycle using pulsed field gel electrophoresis: calibration by means of 125I decay. *Int J Radiat Biol* 1991;59:343–57.
40. Zhong Q, Chen C-F, Chen P-L, et al. BRCA1 facilitates microhomology-mediated end joining of DNA double strand breaks. *J Biol Chem* 2002;277:28641–7.
41. Dikomey E, Dahm-Daphi J, Brammer I, et al. Correlation between cellular radiosensitivity and non-repaired double-strand breaks studied in nine mammalian cell lines. *Int J Radiat Biol* 1998;73:269–78.
42. Rothkamm K, Lobrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci U S A* 2003;100:5057–62.
43. Lobrich M, Rief N, Kuhne M, et al. In vivo formation and repair of DNA double-strand breaks after computed tomography examinations. *Proc Natl Acad Sci U S A* 2005;102:8984–9.
44. Kato T-A, Wilson P-F, Nagasaw H, et al. Variations in radiosensitivity among individuals: a potential impact on risk assessment? *Health Phys* 2009;97:470–80.
45. Wei L, Lan L, Hong Z, et al. Rapid recruitment of BRCA1 to DNA double-strand breaks is dependent on its association with Ku80. *Mol Cell Biol* 2008;28:7380–93.
46. Rothkamm K, Balroop S, Shekhdar J, et al. Leukocyte DNA damage after multi-detector row CT: a quantitative biomarker of low-level radiation exposure. *Radiology* 2007;242:244–51.
47. Burdak-Rothkamm S, Short S-C, Folkard M, et al. ATR-dependent radiation-induced gamma H2AX foci in bystander primary human astrocytes and glioma cells. *Oncogene* 2007;26: 993–1002.
48. Gonzalez J-E, Roch-Lefevre S-H, Mandina T, et al. Induction of gamma-H2AX foci in human exfoliated buccal cells after *in vitro* exposure to ionising radiation. *Int J Radiat Biol* 2010;86:752–9.
49. Azzam E-I, de Toledo S-M, Gooding T, et al. Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. *Radiat Res* 1998;150:497–504.
50. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, et al. A cell cycle–dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 2013;49:872–83.
51. Chapman J-R, Barral P, Vannier J-B, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol Cell* 2013;49:858–71.
52. Snouwaert J-N, Gowen L-C, Latour A-M, et al. BRCA1 deficient embryonic stem cells display a decreased homologous recombination frequency and an increased frequency of non-homologous recombination that is corrected by expression of a brca1 transgene. *Oncogene* 1999;18:7900–7.
53. Paull T-T, Cortez D, Bowers B, et al. Direct DNA binding by Brca1. *Proc Natl Acad Sci U S A* 2001;98:6086–91.
54. Bouwman P, Aly A, Escandell J-M, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and *BRCA*-mutated breast cancers. *Nat Struct Mol Biol* 2010;17: 688–95.
55. Bunting S-F, Callen E, Wong N, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 2010;141:243–54.

56. Woodbine L, Grigoriadou S, Goodarzi A-A, et al. An Artemis polymorphic variant reduces Artemis activity and confers cellular radiosensitivity. *DNA Repair (Amst)* 2010;9:1003–10.

57. Davis A-J, Chi L, So S, et al. BRCA1 modulates the autophosphorylation status of DNA-PKcs in S phase of the cell cycle. *Nucleic Acids Res* 2015;42:11487–501.