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The antitumorigenic roles of BRCA1–BARD1 in DNA repair and replication

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Abstract

The tumour suppressor breast cancer type 1 susceptibility protein (BRCA1) promotes DNA double-strand break (DSB) repair by homologous recombination and protects DNA replication forks from attrition. BRCA1 partners with BRCA1-associated RING domain protein 1 (BARD1) and other tumour suppressor proteins to mediate the initial nucleolytic resection of DNA lesions and the recruitment and regulation of the recombinase RAD51. The discovery of the opposing functions of BRCA1 and the p53-binding protein 1 (53BP1)-associated complex in DNA resection sheds light on how BRCA1 influences the choice of homologous recombination over non-homologous end joining and potentially other mutagenic pathways of DSB repair. Understanding the functional crosstalk between BRCA1–BARD1 and its cofactors and antagonists will illuminate the molecular basis of cancers that arise from a deficiency or misregulation of chromosome damage repair and replication fork maintenance. Such knowledge will also be valuable for understanding acquired tumour resistance to poly(ADP-ribose) polymerase (PARP) inhibitors and other therapeutics and for the development of new treatments. In this Review, we discuss recent advances in elucidating the mechanisms by which BRCA1–BARD1 functions in DNA repair, replication fork maintenance and tumour suppression, and its therapeutic relevance.

The integrity of our genome is continually challenged by environmental agents such as high-energy radiation and mutagenic chemicals and also by reactive intermediates of cellular metabolism, such as free radicals and aldehydes[1–3]. Furthermore, DNA replication is replete with perils, including obstruction of the DNA polymerase ensemble by secondary DNA structures and by transcription-associated R-loops, which could lead to replication fork

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stalling or collapse. Failure to remove DNA lesions or to restart stalled replication forks can cause mutations or catastrophic genome rearrangements, leading to cell transformation and disease, in particular neurological disorders and cancer[4–8].

Of the myriad of continually occurring DNA lesions, the DNA double-strand break (DSB), which is formed through direct chemical assault or from the collapse of stalled replication forks, poses the greatest threat to genomic stability. Several conserved, mechanistically distinct pathways of DSB repair have evolved to repair DSBs, including homologous recombination (HR), non-homologous end joining (NHEJ), alternative end joining and single-strand annealing[9,10] (BOX 1). NHEJ, alternative end joining and single-strand annealing often entail deletion or insertion of several nucleotides and can also give rise to chromosome translocations. By contrast, HR is the most accurate DSB repair mechanism and is capable of faithfully restoring the original configuration of the broken DNA molecule. HR is also the default mechanism for replication fork repair[11–15]. However, since the HR machinery prefers to engage the sister chromatid over the homologous chromosome as a template for DSB repair, it is most active in S phase and G2 phase of the cell cycle, when sister chromatids become available[16,17] (BOX 1).

Studies of DSB repair mechanisms and pathway choice have garnered intense interest owing to their relevance for the maintenance of genome integrity and cancer origin. In this Review, we focus on two key factors of the HR pathway in humans: breast cancer type 1 susceptibility protein (BRCA1) and its obligatory partner BRCA1-associated RING domain protein 1 (BARD1). Mutations in *BRCA1* lead not only to familial breast and ovarian cancers but are also the likely driver of a variety of sporadic cancers[18–21]. There is compelling evidence that BARD1 is also a tumour suppressor[22–27]. We first introduce the original studies that led to the identification of BRCA1 as a suppressor of familial breast cancer and how BARD1 was isolated as an interactor and partner of BRCA1. We discuss how the BRCA1-BARD1 heterodimer, through its E3 ubiquitin ligase activity and the ability to interact with DNA and DNA damage response factors, helps channel DSBs, including at damaged replication forks, into the HR pathway for repair. We also discuss recent studies that implicate BRCA1-BARD1 in multiple stages of the HR process and a novel function of this tumour suppressor complex in the protection of stalled replication forks against deleterious attrition by cellular nucleases.

The recent approval of poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi) by the US Food and Drug Administration and the European Medicines Agency as cancer therapeutic agents adds to the arsenal of drugs to treat HR-deficient tumours[28] and also accords credence to the applicability of synthetic lethality in future drug development efforts[29–31]. However, most patients undergoing PARPi therapy ultimately develop resistance to these drugs, and we discuss the known mechanisms of PARPi resistance and the prospects for developing new therapeutics that further exploit the DSB repair deficiency of HR-deficient tumours.

The activity and interactions of BRCA1

BRCA1 and BARD1 together fulfil complex roles in DNA repair, replication fork protection, transcription and tumour suppression. Mouse studies have established that the *Brca1* and *Bard1* genes are essential for survival, as embryonic lethality is observed between 6.5 and 8.5 embryonic days in single-knockout or double-knockout mice. Cells derived from the mutant embryos exhibit chromosome structural abnormalities and aneuploidy[32–36]. Importantly, mammary-specific ablation of either *Brca1* or *Bard1* in combination with p53 deficiency leads to a high frequency of basal-like breast carcinomas with a propensity for being triple-negative tumours (that is, lacking the oestrogen, progesterone and HER2 (also known as ERBB2) receptors). The striking similarities between the mammary carcinomas of single-knockout and double-knockout mice again emphasize that BRCA1 and BARD1 function together in tumour suppression[37–40]. It is interesting that although heterozygous $Brca1^{+/-}$ and $Bard1^{+/-}$ mice are not particularly tumour-prone, women with one mutant *BRCA1* or *BARD1* allele exhibit tumour predisposition. In this section, we discuss the studies that led to the identification of BRCA1 and BARD1 as tumour suppressors and the functional domains and biochemical characteristics of BRCA1-BARD1 that are germane for its role in the maintenance of genome stability.

The characterization of BRCA1 as a familial breast cancer gene.

The *BRCA1* gene encodes a large protein of 1,863 amino acids[41] with several functional domains[42–44] (FIG. 1; see later). *BRCA1* was discovered as an early-onset breast cancer susceptibility gene in afflicted families by linkage analysis[45]. Subsequently, *BRCA1* mutations were also linked to familial ovarian cancers[46,47]. Among carriers of *BRCA1* mutations, the estimated lifetime risk of breast cancer is more than 80%, and that of ovarian cancer is 40–65%. This represents a sevenfold risk increase for breast cancer and a more than 20-fold increase for ovarian cancer compared with individuals without mutations in *BRCA1* (REF.[48]). Typically, tumorigenesis in carriers of *BRCA1* mutations entails loss of heterozygosity and the elimination of the wild-type *BRCA1* allele. Therefore, BRCA1 fits the definition of a classical tumour suppressor. Importantly, BRCA1-deficient breast tumours can develop also with no familial linkage[49], and many of these cancers may be explained by epigenetic silencing of the wild-type allele through hypermethylation of the *BRCA1* gene promoter[50]. A substantial fraction of breast tumours arising from BRCA1 deficiency are triple negative, representing one of the most aggressive forms of the disease[51].

BARD1 is the obligatory partner of BRCA1 and a tumour suppressor.

A screen based on yeast and mammalian two-hybrid analyses led to the isolation of BARD1, which has 777 amino acids and is the obligatory partner of BRCA1 (REF.[52]). Like BRCA1, BARD1 has a zinc-chelating RING (really interesting new gene) domain, and the two proteins assemble into a stable heterodimer through the association of their RING domains (FIG. 1). As discussed later, mutations that impair the BRCA1-BARD1 interaction, including those derived from tumours, result in deleterious proteolytic degradation of both proteins.

Compelling evidence that BARD1 is a tumour suppressor has been obtained from cancer association studies, which link *BARD1* mutations to breast, ovarian and other tumour types[22–26]. For unclear reasons, *BARD1* mutations are rarer than *BRCA1* mutations in familial breast cancer and are generally associated with a lower breast cancer risk[27]. A number of single-nucleotide polymorphic variants of *BARD1* result in differential expression of BARD1 isoforms, most notably the β-isoform, which lacks RING domain-encoding exons 2 and 3. RING-less BARD1 is thought to confer a dominant-negative effect on wild-type BARD1 and to contribute to the tumorigenesis of neuroblastoma and nephroblastoma[53,54]. Thus, not only is BARD1 a bona fide tumour suppressor but certain BARD1 isoforms appear to have an oncogenic effect as well[55].

The domains of BRCA1 and BARD1 and their functions.

A solution NMR structure of the BRCA1-BARD1 RING dimer has been solved[56], and a number of cancer-causing mutations in the BRCA1 RING domain have been described, which affect BRCA1-BARD1 heterodimer formation[57–59]. The C termini of BRCA1 and BARD1 each harbour two copies of the BRCA1 C-terminal (BRCT) repeat, which mediate interactions with different partner proteins. The BRCA1 BRCT repeats associate with the phosphorylated isoforms of interacting proteins[60–64], whereas the BARD1 BRCT repeats specifically recognize poly(ADP-ribose) and other factors[65–67] (FIG. 1). Substantial structural and functional information emphasizes the importance of the RING dimer and the BRCT domains for the tumour suppression activity of BRCA1-BARD1 (REFS[68,69]). The BRCA1 coiled-coil domain mediates complex formation with partner and localizer of BRCA2 (PALB2)[70–72], itself a tumour suppressor[73,74]. BARD1 possesses four tandem ankyrin motifs that specifically recognize unmethylated histone H4 Lys20 (K20) (REF.[75]). Biochemical analyses have identified protein domains that mediate DNA binding and interaction with the recombinase RAD51 in both BRCA1 and BARD1 (REF.[76]) (FIG. 1).

In 2001, BRCA1 was shown to bind DNA[77], thereby providing key evidence of a direct involvement of BRCA1-BARD1 in DNA repair and replication fork maintenance. Recently, a DNA-binding domain was found in BARD1 as well[76] (FIG. 1). This study also revealed that both BRCA1-BARD1 and BARD1 bind a variety of DNA substrates, with the highest affinity being for the displacement loop (D-loop) structure, which is a DNA intermediate generated by RAD51 during HR, followed by the affinities for the replication fork structure, double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA)[76].

The E3 ubiquitin ligase activity of BRCA1-BARD1.

Like many other RING domain proteins, BRCA1 and BARD1 can interact with E2 ubiquitin-conjugating enzymes and function as E3 ubiquitin ligases[78,79]. There is compelling evidence that optimal E3 ligase activity requires complex formation between the two tumour suppressors[56,80–82]. BRCA1-BARD1 interacts with a number of E2 enzymes to assemble K6-linked, K48-linked or K63-linked ubiquitin chains[83,84]. BRCA1-BARD1 is unique among E3 ubiquitin ligases in being capable of assembling K6-linked ubiquitin chains.

Initial studies in mouse breast and pancreatic tumour models involving the BRCA1-I26A mutant protein, which is impaired in ubiquitin ligase activity, concluded that ubiquitin conjugation by BRCA1-BARD1 is dispensable for the suppression of tumorigenesis[69,85]. Characterization of another E3 ligase-deficient BRCA1-BARD1 complex (harbouring the *BARD1*^{R99E} mutation) revealed an intriguing pattern of cellular sensitivities to genotoxic agents[86]. Specifically, mutant cells are hypersensitive to inhibitors of topoisomerase I and topoisomerases II, to ionizing radiation and to the PARPi olaparib, but unlike BARD1-depleted cells, they are resistant to cisplatin (a DNA crosslinking agent), aphid-icolin (a DNA polymerase inhibitor) and hydroxyurea (another inhibitor of DNA replication). Moreover, the mutant cells show defects in DNA end resection, which is a crucial early step in the repair of DSBs by HR[86]. These observations suggest that the BRCA1-BARD1 E3 ligase activity is important for DNA repair but not for DNA replication fork maintenance.

Several physiological substrates of BRCA1-BARD1 have been identified (TABLE 1). Specifically, histone H2A (H2A) ubiquitylation may lead to the eviction of p53-binding protein 1 (53BP1), which is a reader of histone modifications and an antagonist of DNA end resection, to allow access to DNA ends to the HR machinery[86] (see below). Mutations in BARD1 that impair H2A ubiquitylation, but do not destabilize the BRCA1-BARD1 heterodimer, have been identified in families afflicted with breast cancer[87]. There is evidence that BRCA1-BARD1-mediated H2A ubiquitylation is important for transcriptional repression of satellite repeats and that this function is relevant for tumour suppression[88,89].

Considerable controversies remain about the involvement of the BRCA1-BARD1 E3 ligase activity in HR and in tumour suppression. Specifically, the BRCA1-I26A mutant protein that was tested in mouse models of tumorigenesis[69] possesses residual capacity to interact with certain E2 enzymes and in ubiquitin conjugation when tested in vitro[90]. On the other hand, even though the *BRCA1*^{R99E} mutation has been reported to impair HR and confer sensitivity to genotoxic agents[86], another study found that the expression of this mutant allele in BARD1-depleted cells restores cellular resistance to PARPi[75]. Clearly, more work is needed to critically evaluate the BRCA1-BARD1 E3 ligase activity and its roles in tumour suppression in humans.

Protein interactions involving BRCA1-BARD1.

Numerous protein interactors of BRCA1-BARD1 have been described, including factors that function in HR, in transcription and in the avoidance of transcription-replication conflicts (TABLE 1). Some of the protein-protein interactions that are important for HR are mediated by the BRCT repeats of BRCA1 or BARD1. Complex formation with other factors that directly influence HR efficiency is dependent on the ankyrin repeats of BARD1, on the coiled-coil domain of BRCA1 and apparently on unstructured regions of both BRCA1 and BARD1 (FIG. 1). As we discuss in the following sections, the functional importance of some of the protein complexes involving BRCA1-BARD1 has been delineated.

The role of BRCA1-BARD1 in HR

Early studies revealed HR defects in *Brca1*$^{-/-}$ mouse embryonic stem cells, manifested as impaired gene targeting and diminished repair of an induced, site-specific DSB[91]. The

observation that BRCA1 interacts with the recombinase RAD51 and that they colocalize to ionizing radiation-induced nuclear foci[92] provided the first evidence for the involvement of BRCA1 in HR repair. Furthermore, BRCA1 abrogation in mouse and human cells impairs DNA damage-induced RAD51 focus formation[93,94]. Likewise, ablation of the *BARD1* gene in mouse and human cells also decreases the formation of damage-specific RAD51 foci and causes a defect in HR that is equivalent to the loss of BRCA1 (REFS[36,76]). These findings thus reveal an important function of BRCA1-BARD1 in RAD51 recruitment to DSBs, which is a crucial early event in HR. In this regard, BRCA1-BARD1 likely functions as part of a higher-order 'HR mediator' complex with two other tumour suppressors, BRCA2 and PALB2 (REFS[4,95,96]) (fig. 2; see later). In addition to RAD51 recruitment, there is evidence that BRCA1-BARD1 has a role in the nucleolytic resection of DSB ends (FIG. 2), as first proposed on the basis of the cell cycle-dependent association of BRCA1 with the MRE11-RAD50-NBS1 nuclease complex (MRE11 being the catalytic subunit) and with the end resection factor CtBP-interacting protein (CtIP)[64,97].

A central step in HR is the assembly of a RAD51 nucleoprotein filament on the 3′ ssDNA tail, which is capable of catalysing pairing with homologous DNA[11,95,98] (FIG. 2). BRCA1 inactivation triggers a modest reduction in ssDNA levels compared with the abrogation of an essential resection factor such as CtIP[99–102]. Moreover, ablation of the BRCA1-CtIP complex affects resection efficiency only mildly[102–104]. Thus, it would appear that BRCA1-BARD1 is an accessory factor of DNA end resection, but is not a component of the core resection machinery[105,106]. Since resection can still occur in BRCA1-deficient cells, attenuated formation of RAD51 foci cannot be explained solely by lack of ssDNA formation. A more likely scenario is that BRCA1 not only influences the efficiency of DNA end resection but also helps recruit RAD51 to resected DNA ends (see later).

The antagonistic activities of BRCA1 and 53BP1 in DNA end resection.

Whereas nullizygous *Brca1* mice die in utero[32–35], mice carrying the *Brca1* exon 11 deletion (*Brca1*$^{\Delta 11/\Delta 11}$) are partially viable or show embryonic lethality later in embryogenesis[37,107]. BRCA1-deficient mice die embryonically due to the accumulation of endogenous DNA damage, which activates DNA damage signalling through the kinase ataxia telangiectasia mutated (ATM) (FIG. 3a). It was therefore anticipated that abolishing DNA damage sensors would increase tolerance and promote survival on the occurrence of DNA damage in these mice. This assumption was proved correct, as deletion of *Atm* or the gene encoding its effector checkpoint kinase 2 (*Chk2*) rendered *Brca1*$^{\Delta 11/\Delta 11}$ mice viable[108]. Two research groups introduced the *Brca1*$^{\Delta 11/\Delta 11}$ deletion into mice lacking the *Trp53bp1* gene (orthologue of the gene encoding human 53BP1), which encodes a p53 interacting protein[109,110] that functions as a signal transducer in the ATM-dependent DNA damage checkpoint[111–113]. Deletion of *Trp53bp1* restored viability in *Brca1*$^{\Delta 11/\Delta 11}$ mice[114].

The protein 53BP1 interacts with epigenetically modified histones to nucleate the formation of a multisubunit complex, which includes the telomere-associated proteins RIF1, REV7 (also known as MAD2L2 or MAD2B), shieldin complex subunit 1 (SHLD1), SHLD2 and SHLD3 and prevents access of the resection machinery to DNA ends in G1 phase of the cell cycle (BOX 2). The removal of the 53BP1-associated protein complex from DNA ends

occurs in S phase and G2 phase of the cell cycle and requires BRCA1-BARD1. Abrogation of 53BP1 renders *BRCA1*-mutant cells proficient in the repair of DNA damage induced by camptothecin, ionizing radiation and olaparib[100]. This discovery has provided the generally accepted model, in which 53BP1 ablation allows *BRCA1*-mutant cells to process DSBs for HR repair (FIG. 3b). Consequently, BRCA1 and 53BP1 double-mutant cells and tumours are resistant to PARPi such as olaparib, which induce the formation of DSBs that can be channelled into the HR pathway for repair (FIG. 3b). This has important clinical implications (discussed later). However, an important yet often overlooked aspect of these results is that 53BP1 abrogation in cells lacking BRCA1 does not restore HR and genome stability to wild-type levels[76,100,114,115]. For example, targeted DNA integration mediated by HR at some specific loci occurs at only 10–50% efficiency in *Trp53bp1* depleted *Brca1*$^{-/-}$ mouse embryonic stem cells relative to the BRCA1-proficient counterparts[115]. Likewise, even though formation of RAD51 foci in response to ionizing radiation is upregulated in BRCA1-deficient and 53BP1-deficient cells relative to the BRCA1-deficient and 53BP1-proficient cells, it does not reach the wild-type level[100,115]. It is also important to note that 53BP1 deletion fails to suppress the meiotic defects of *Brca1*$^{\Delta 11/\Delta 11}$ mice[116,117]. Notably, however, the DNA-damage sensitivity and tumour susceptibility caused by the RING-less *Brca1*$^{\Delta 2}$ allele can be suppressed by 53BP1 ablation[118,119]. Emerging evidence suggests that a functional BRCA1 protein is in fact needed to mediate HR steps that occur downstream of DNA end resection in order to make possible the observed cellular resistance to genotoxic agents and partial restoration of HR in 53BP1-null cells[120,121].

Roles of BRCA1 and the 53BP1-associated complex in DSB repair pathway choice.

The discovery of how 53BP1 may antagonize BRCA1-dependent DSB resection prompted certain predictions about the interplay between BRCA1 and 53BP1 in engaging HR or NHEJ, respectively, for DSB repair (BOX 2; FIG. 3a). The most important prediction is that, by counteracting DNA end resection activity, 53BP1 helps channel DSBs into repair by NHEJ. Conversely, by promoting end resection, BRCA1 favours HR repair. According to this model, competition between BRCA1 and 53BP1 at DSBs is the defining factor in DSB repair pathway selection (FIG. 3b). This model is supported by extensive experimental evidence[99,100,122.] For example, whereas wild-type cells are able to assemble BRCA1 foci only in S phase and G2 phase of the cell cycle, such foci form in G1 phase on 53BP1 ablation[99].

Immunostaining and high-resolution microscopy[123] have revealed progressive, BRCA1-dependent exclusion of 53BP1 from DNA damage sites during S phase. Other studies have begun to provide mechanistic insights into how BRCA1-BARD1, through its E3 ubiquitin ligase activity and the ATPase-helicase SMARCAD1, contribute to the eviction of 53BP1, and presumably of its associated RIF1-shieldin complex, from the vicinity of DSB ends to facilitate DNA end resection and HR repair[86]. It has been known for quite some time that SMARCAD1 and its yeast orthologue Fun30 have a crucial role in the resection of DSB ends[124,125]. A recent study has provided tantalizing evidence that BRCA1-BARD1, through its ability to ubiquitylate three lysine residues of H2A (K125, K127 and K129)[126], contributes to the recruitment of SMARCAD1 through its ubiquitin-binding CUE domains[86]. Specifically, introduction of the E3 ligase-inactivating BARD1-R99E mutant

protein into the BRCA1-BARD1 complex leads to impairment of DNA end resection and, as mentioned earlier, causes hypersensitivity to genotoxic agents such as olaparib. Moreover, the expression of a ubiquitin-H2A fusion protein in cells lacking BARD1 helps to restore DNA end resection resistance to genotoxic agents in a SMARCAD1-dependent manner[86]. Thus, the E3 ligase activity of BRCA1-BARD1 likely contributes to SMARCAD1-dependent 53BP1 eviction from DSBs and helps initiate DNA end resection through H2A ubiquitylation. It remains to be determined whether BRCA1-BARD1 also participates in the ubiquitylation of other factors important for the removal of 53BP1, RIF1 and shieldin from DSB ends and for the end resection process itself (FIG. 3c).

Role of BRCA1-BARD1 in RAD51-mediated DNA strand invasion.

As discussed already, early studies showed that BRCA1 co-immunoprecipitates with RAD51 from mammalian cell extracts[92]. Additionally, BRCA1 binds DNA and is required for the assembly of DNA damage-induced RAD51 nuclear foci[76,77,86,127,128], suggesting a direct role of BRCA1-BARD1 in the enhancement or regulation of the recombinase activity of RAD51. Recent studies using highly purified BRCA1-BARD1 complex and RAD51 directly support this idea. Specifically, both BRCA1 and BARD1 were shown to bind DNA and interact directly with RAD51 (REF.[76]). The interaction of BRCA1-BARD1 with RAD51 is species specific, because yeast Rad51 and *Escherichia coli* RecA (the bacterial orthologue of RAD51) fail to associate with BRCA1-BARD1. In DNA-binding assays, both BRCA1-BARD1 and the DNA-binding domain of BARD1 show the highest affinity for the D-loop structure — the DNA structure formed by RAD51-catalysed pairing of homologous DNA molecules (FIG. 2). Importantly, in a reconstituted in vitro system of D-loop formation, BRCA1-BARD1 strongly enhanced the RAD51-mediated reaction but had no effect on either yeast Rad51 or *E. coli* RecA[76].

As a prerequisite for D-loop formation, the RAD51-ssDNA complex must capture a duplex DNA molecule, conduct a search for DNA homology and align the recombining ssDNA and dsDNA in homologous registry. The three-stranded aligned nucleoprotein intermediate is referred to as the ‘synaptic complex’[76,95] (FIG. 2). Biochemical and single-molecule biophysical analyses have provided evidence that BRCA1-BARD1 works in conjunction with RAD51 to assemble the synaptic complex[76].

The functional relevance of the BRCA1-BARD1-RAD51 complex was established by isolation of a BARD1 mutant (BARD1-AAE (F133A, D135A and A136E)) that compromises the ability of BARD1 and BRCA1-BARD1 to associate with RAD51 but that has no effect on DNA binding by these protein species[76]. Importantly, the BRCA1-BARD1-AAE mutant complex is greatly impaired in its ability to enhance RAD51-mediated synaptic complex assembly and D-loop formation in vitro. Cells that are devoid of endogenous BARD1 but express the BARD1-AAE mutant exhibit a deficiency in DSB repair by HR and hypersensitivity to mitomycin C and olaparib. However, these mutant cells are still competent in the assembly of DNA damage-induced RAD51 foci. Taken together, the results from this study provide evidence that BRCA1-BARD1 functions in synergy with RAD51 in synaptic complex assembly and D-loop formation[76].

Postulated role of BRCA1-BARD1 in the assembly of the RAD51 repair complex.

One of the strongest phenotypes associated with BRCA1 deficiency is impaired assembly of DNA damage-induced RAD51 foci[76,93]. That BRCA1-BARD1 likely has an important role in the assembly of the RAD51 repair complex to initiate HR relies on the following findings: BRCA1 and BARD1 physically interact with RAD51 (REFS[76,92]); mutations in BARD1 that affect its interaction with RAD51 cause a defect in the RAD51-dependent protection of stalled DNA replication forks against nucleolytic attrition[129]; BRCA1 interacts with PALB2, which functions to recruit the RAD51 loader BRCA2 to DNA lesions for assembling RAD51 repair complexes, and mutations that abrogate the interaction between BRCA1 and PALB2 cause strong HR defects[71,72]; and the suppression of HR defects associated with BRCA1 deficiency by 53BP1 depletion is far from complete[100,115].

In its postulated role in RAD51-ssDNA complex assembly, BRCA1-BARD1 likely functions as part of a larger protein ensemble that includes PALB2 and BRCA2-DSS1 (REFS[95,96]) (FIG. 2). BRCA2, by binding both DNA and RAD51, nucleates the assembly of RAD51 filaments on ssDNA occupied by replication protein A (RPA), which is an abundant factor with high affinity for ssDNA[130–132] (FIG. 2). The exchange of RPA with RAD51 on ssDNA is enhanced by the BRCA2-associated factor DSS1, which is a small, highly acidic protein that weakens the grip of RPA on ssDNA[130]. Thus, BRCA2-DSS1 is a ‘mediator’ of HR[11,130,133,134]. In summary, aside from their roles in the recruitment of BRCA2-DSS1 to sites of DNA damage[95,96], BRCA1-BARD1 and PALB2 likely also enhance the HR-mediator activity of BRCA2-DSS1[95,96]. In the future, it will be important to isolate protein complexes that include these factors and to test them for HR-mediator activity to reveal the mechanism of function of BRCA1-BARD1 and PALB2 in RAD51 repair complex assembly.

Functions of BRCA1-BARD1 in DNA replication

In addition to its well-documented DSB repair function, BRCA1-BARD1 has crucial roles in the repair and restart of stalled and damaged DNA replication forks and in their protection from nucleolytic attack and attrition (FIG. 4). As we discuss in the following sections, the involvement of BRCA1-BARD1 in the preservation of replication fork integrity is complex and likely also involves bypass of potentially pathogenic secondary DNA structures — R-loops (which arise following transcription perturbation) — and G-quadruplexes.

The multifaceted role of BRCA1-BARD1 in replication fork repair and protection.

The first indication of a role of BRCA1 in DNA replication came from a study on the response of BRCA1-mutant cells to hydroxyurea, which causes replication stress by inhibiting ribonucleotide reductase, leading to depletion of nucleotides needed for DNA synthesis[135]. In hydroxyurea-treated cells, BRCA1 colocalizes with RAD51 at S phase-specific foci containing the DNA polymerase accessory factor PCNA, which likely correspond to stalled replication forks[135]. Later studies showed colocalization of BRCA1 with other factors that act on stalled replication forks, including the MRE11-RAD50-NBS1 nuclease complex and the helicase Bloom syndrome protein[136].

The aforementioned studies were performed in the presence of hydroxyurea and thus in conditions of replication stress. The question remains whether BRCA1 has a role in DNA replication in physiological settings. Early on, analysis of mouse embryonic fibro-blasts derived from *Brca1*$^{\Delta 11/\Delta 11}$ mice revealed spontaneous complex chromosomal aberrations in the form of radial chromosomes[37], which are a molecular signature of misrepaired replication-associated DNA damage. Importantly, *BRCA1* haploinsufficiency is associated with defects in replication fork restart[137], even though HR-mediated DSB repair capacity remains unaffected. This suggests BRCA1 has a primary function in DNA replication, for which the normal level of fully functional BRCA1 is required; alternatively, mutant BRCA1 might exert a dominant-negative effect on the wild-type protein[66]. Replication stress stemming from a defect in the timely resolution of stalled replication forks and the resulting genomic instability are hallmarks of BRCA1-deficient cells and tumours[96,138].

In addition to its role in the stabilization, repair and restart of stalled DNA replication forks, BRCA1 is also very important for the protection of stressed forks from nucleolytic degradation by MRE11 (REFS[139,140]) and other nucleases[141–145]. The newly identified biorientation of chromosomes in cell division protein 1-like 1 (BOD1L) appears to function with BRCA1 and BRCA2 to protect stressed replication forks from the destructive activity of the nuclease DNA2 (REF.[146]) (FIG. 4a).

Several members of the SNF2 family of helicases, namely SMARCAL1, ZRANB3 and HLTF, remodel stalled replication forks in a process termed ‘fork regression’ into a branched structure that harbours a single DNA end, which becomes prone to degradation by the nuclease MRE11 when BRCA1 is absent[147] (FIG. 4a). This is mediated by PTIP, a component of the histone methyltransferase complex MLL3 (KMT2C)-MLL4 (KMT2B) and an effector of 53BP1 in DSB end protection, which recruits MRE11 to stalled replication forks in BRCA1-deficient cells to trigger fork attrition[148]. Whether the recruitment of the helicase-like proteins SMARCAL1, ZRANB3 and HLTF is also PTIP dependent has not been determined.

Another mechanism of fork protection is BRCA1 independent and instead relies on CtIP[149]. Unlike in BRCA1-deficient cells, in which stalled forks are degraded by MRE11, CtIP deficiency renders replication forks susceptible to DNA2. Thus, loss of CtIP in BRCA1-deficient cells further increases the vulnerability of replication forks to nucleolytic attrition.

The precise mechanisms by which BRCA1 protects and restarts stalled replication forks are unknown, but RAD51 loading at the ssDNA that accumulates at the stalled fork or at the free end of a regressed fork is likely required (FIG. 4a). In *Xenopus laevis* egg extracts, Rad51 associates with replication intermediates in a Brca1-dependent manner to protect and restart stalled forks[150,151]. A recent study has implicated the proline isomerase PIN1 in BRCA1-dependent replication fork protection against the destructive activity of MRE11. Specifically, PIN1 converts the BRCA1-BARD1 complex into a form that has higher affinity for RAD51. This PIN1 function requires cyclin-dependent kinase 1 (CDK1)-mediated or CDK2-mediated BRCA1 phosphorylation[129].

Replication fork collapse and misrepair are major sources of mutagenesis and chromosomal rearrangements and are likely relevant to tumorigenesis. However, whether the activity of BRCA1-BARD1 at replication forks contributes to its tumour suppressor function is unclear. Better mechanistic understanding of how BRCA1-BARD1 functions at replication forks versus at DSBs and the development of separation-of-function mutants that affect DNA repair or replication fork maintenance will be necessary to answer this question.

BRCA1 facilitates the progression of replication through specific obstructions.

During DNA replication, the DNA polymerase ensemble may stall at genomic sites that are difficult to replicate. One such replication barrier is the R-loop, an RNA-DNA hybrid with an appended displaced ssDNA strand. R-loops accumulate in sites where strong DNA secondary structures, such as the G-quadruplexes (see later), are formed on perturbation of transcription or of transcription-coupled processes such as mRNA splicing[152,153]. Head-on collisions between the DNA replication machinery and RNA polymerase-associated R-loops lead to replication fork collapse and DSB formation[154]. BRCA1 interacts with senataxin (SETX), which is a putative RNA-DNA hybrid helicase and partner of RNA polymerase II involved in transcription termination and R-loop resolution[155] (FIG. 4b). BRCA1 and SETX resolve conflicts between transcription and replication at transcription termination sites[156]. Consistent with this function, BRCA1-deficient tumours show high mutation rates in transcription termination regions. However, the manner by which BRCA1-BARD1 regulates the activity of SETX in R-loop resolution remains to be determined. SETX may also function to resolve RNA-DNA hybrids at DSB sites[157] created as a result of de novo transcription at the DNA lesions[158–161]. This activity of SETX is believed to suppress DSB-induced chromosome translocations[162].

G-quadruplex DNA structures can form at G-rich sequences when the DNA becomes single stranded during DNA replication or transcription[163]. Such G-quadruplex structures present a strong impediment to replication fork progression. Cells and tumours deficient in BRCA1 are highly sensitive to chemicals that interact with and stabilize the G-quadruplex structure[164,165]. These observations are consistent with the premise that BRCA1-BARD1 promotes the restart of replication forks arrested at G-quadruplex sites.

By-products of cellular metabolism (for example, acetaldehyde) can cause interstrand crosslinks (ICLs), which interfere with DNA replication. BRCA1-deficient cells and tumours are exquisitely sensitive to ICL-inducing agents, which underlies their vulnerability to certain chemotherapeutic drugs such as platinum salts and mitomycin C. Abrogation of detoxification pathways results in accumulation of endogenous acetaldehyde and replication stress in BRCA1-deficient cells[166,167]. These observations highlight the role of BRCA1 in the resolution of ICL lesions during DNA replication through the Fanconi anaemia pathway of DNA damage response[168–171]. Diseases resembling Fanconi anaemia were identified in two individuals homozygous for *BRCA1* mutations[172,173], and consequently the *BRCA1* gene was classified as the Fanconi anaemia gene *FANCS*.

The clinical impact of *BRCA1* mutations

The deficiency of BRCA1-mutant cells and tumours in DNA repair and in replication fork restart and protection renders them prone to oncogenic genome alterations (FIG. 3b). Importantly, BRCA1 deficiency is also a vulnerability that can be therapeutically targeted[28,29,174,175] with chemical compounds (such as platinum salts, DNA crosslinking agents and PARPi) that increase the load of DNA damage or generate replication stress[176,177]. It is unfortunate that the mutator phenotype of HR-deficient tumours can give rise to drug resistance through secondary mutations that either allow the expression of functional BRCA1 or inactivate or downregulate HR repressors such as 53BP1. Overall, tumour cells possess surprising plasticity in circumventing the effects of drugs. We now discuss studies that are beginning to elucidate the underlying basis of innate and acquired drug resistance in animal models and in clinical settings.

Targeting BRCA1-deficient tumours and the emergence of PARPi resistance.

BRCA1-deficient tumours are hypersensitive to drugs that exacerbate their intrinsic DNA replication defects[178]. DNA crosslinking and alkylating agents, such as platinum salts, mitomycin C, cyclophosphamide and melphalan, impair replication fork progression by inducing ICLs, whereas topoisomerase I and topoisomerase II inhibitors such as camptothecin, irinotecan and etoposide covalently trap these enzymes on the DNA and generate a strong DNA replication block. More recently, inhibitors of PARP1 and PARP2 (PARPi) were shown to ‘trap’ these proteins on DNA to interfere with DNA replication. PARPi such as olaparib are generally well tolerated by normal cells and tissues but potently induce the demise of BRCA1-deficient cells and tumours[28]. PARPi have been approved by the US Food and Drug Administration and the European Medicines Agency as therapeutic agents for advanced-stage or recurrent ovarian[179] and breast[180,181] cancers with germline *BRCA1* or *BRCA2* mutations. In addition, some of the inhibitors mentioned are used in the clinic for maintenance treatment of patients with ovarian cancer who have successfully completed platinum-based chemotherapy[182,183].

It is important to note that mutations in other HR genes also increase the vulnerability of cells to PARPi; this is known as the BRCAness concept[178,184]. For example, patients with prostate cancer with mutations in *BRCA2, PALB2, FANCA, RAD51* or *CHK2* are responsive to olaparib[178,185]. As such, PARPi may be efficacious for a much wider variety of tumours than those specifically lacking BRCA1 function.

The early discovery that 53BP1 inhibition in BRCA1-deficient cells leads to partial HR reactivation and resistance to PARPi[100] was subsequently corroborated by in vivo data. In a mouse *Brca1*-mutant cancer model, tumours that are sensitive to olaparib acquire resistance following prolonged exposure to the drug. Silencing of 53BP1 expression is a major cause of drug resistance and tumour regrowth[186]. This discovery suggests that the expression level of 53BP1 could be a predictor of the response of BRCA1-deficient tumours to PARPi. Consistent with this premise, analysis of two large cohorts of patients with breast cancer (the Yale study and the Helsinki study)[115] revealed an association of low 53BP1 expression with poor prognosis. Moreover, early-stage triple-negative breast tumours with reduced 53BP1 levels exhibited a higher risk of metastasis[115]. In congruence with these observations, REV7

was found to be downregulated in a significant fraction of triple-negative human breast carcinomas[101]. Moreover, the REV7-interacting protein and shieldin complex component SHLD2 is absent in the *BRCA1*-mutated breast cancer-derived HCC1937 cell line, which shows abnormal resistance to PARPi treatment[187].

Studies of tumour xenografts derived from patients with triple-negative breast cancer with germline *BRCA1* mutations have led to the characterization of specific *53BP1* (also known as *TP53BP1*) and *REV7* (also known as *MAD2L2)* mutations that likely give rise to PARPi resistance in tumours. In addition, loss of *SHLD1* or *SHLD2* expression has been observed to cause PARPi resistance[188]. These studies were conducted with tumour xenografts derived from patients who had received prior therapy and, as such, it was not possible to determine whether the secondary mutations and gene silencing events arose spontaneously (innate resistance) or during treatment (acquired resistance). To answer this question, patients who receive PARPi as monotherapy at the onset of treatment will need to be examined. Such efforts would also shed light on whether the expression status of the 53BP1, RIF1 and shieldin axis might serve as a reliable biomarker for predicting drug response. BRCA1-deficient cells and tumours that have become olaparib resistant through abrogation of 53BP1 or shieldin components remain susceptible to ionizing radiation[188,189] and to chemotherapeutic treatments, including G-quadruplex ligands[164,165], chlorambucil[190] and cisplatin[117,188,191]. In a recent clinical trial, patients with *BRCA1*-mutated or *BRCA2*-mutated, PARPi-resistant ovarian cancers were found to respond favourably to platinum-based therapies[192], suggesting that the level of HR restoration in these tumours is insufficient for the timely removal of drug-induced DNA damage[117]. In addition, abrogation of 53BP1-dependent NHEJ may be dispensable for the repair of olaparib-induced DNA damage, but not for that induced by other cancer therapeutics[117].

PARPi resistance mechanisms that are independent of HR restoration have been reported in *Brca1*-deficient mouse cells and tumours. In *Brca1*$^{-/-}$ *Trp53*$^{-/-}$ mouse mammary tumours, olaparib resistance, as well as resistance to doxorubicin and docetaxel, is mediated by activation of the P-glycoprotein drug efflux transporter[193,194]. Studies in vitro or using patient-derived tumour xenografts have identified inhibition of the poly(ADP-ribose)-degrading enzyme poly(ADP-ribose) glycohydrolase[195] and abrogation of NEDD8 (REF. [196]) as additional mechanisms of resistance in the context of BRCA1-deficiency. PTIP abrogation in BRCA-deficient tumours leads to restoration of replication fork stability by preventing access ofMRE11 to stalled forks[148] (FIG. 4a). This, in turn, causes resistance not only to PARPi but also to cisplatin. Of note, PTIP interacts with artemis, a nuclease that trims DNA ends and promotes NHEJ[197]. Thus, PTIP abrogation also leads to partial HR restoration and inhibition of PARPi sensitivity[198].

BRCA1 promoter methylation versus gene mutation in tumour response to therapy.

Tumours are classified as BRCA1 deficient on the basis of several criteria, including the presence of germline *BRCA1* mutations, spontaneously acquired deleterious mutations causing intragenic *BRCA1* deletions or epigenetic silencing of *BRCA1* through promoter methylation. Germline *BRCA1* mutations are the most reliable marker for BRCA1 deficiency in predicting tumour response to PARPi or platinum-based therapy. Most cases of

cisplatin resistance in these tumours are attributed to secondary mutations that restore the *BRCA1* open reading frame and the expression of a functional protein[199,200]. A consequence of these secondary mutations is that mutant cells regain the ability to eliminate ICLs or DSBs induced by cisplatin or PARPi.

A common feature of sporadic triple-negative breast tumours is *BRCA1* promoter methylation and silencing, which is a good predictor of PARPi sensitivity[183]. Consequently, treatment-induced loss of *BRCA1* promoter methylation could restore normal *BRCA1* expression levels or trigger a hypomorphic phenotype (low levels of *BRCA1* mRNA and protein expression) associated with PARPi resistance[201]. A study of tumour xenografts derived from patients with triple-negative breast cancer identified cases in which *BRCA1* promoter methylation was retained yet, surprisingly, BRCA1 mRNA and protein were still produced and caused resistance to olaparib and cisplatin treatments[202]. Next-generation sequencing revealed that genomic rearrangements had placed the *BRCA1* gene under the transcriptional control of a heterologous promoter. This is a unique example of the genomic plasticity of BRCA1-deficient tumour adaptation to treatment, which, unfortunately, allows tumour regrowth.

Recently, a panel of tumour xenografts assembled from patients with triple-negative and metastatic breast cancers and from patients with metastatic ovarian cancer carrying germline *BRCA1* mutations was assessed for *BRCA1* expression and response to therapy[191]. PARPi resistance emerged in a subset of these tumours even though germline *BRCA1* mutations and the loss of the second allele remained detectable by sequence analysis. *BRCA1* mRNA and protein analyses revealed the expression of a hypomorphic isoform in these tumours[191]. The same study identified a mutation in *SLFN11* in a *BRCA1*-mutated patient-derived tumour xenograft that likely gave rise to PARPi resistance. Studies have shown that *SLFN11*, which encodes the putative DNA and RNA helicase Schlafen family member 11, is needed for PARPi sensitivity irrespective of BRCA status[203]. A recent CRISPR-Cas9 screen in human cell lines identified multiple gene deletions that sensitize cells to PARPi treatment in the presence or absence of functional BRCA1, similarly to the *SLFN11* deletion[204]. It will be interesting to determine whether mutations in these genes are present in *BRCA1*-mutated tumours from human patients and whether they can be used as biomarkers for PARPi response regardless of *BRCA1* inactivation (TABLE 2).

Conclusion and future perspective

In this Review, we have analysed the multifaceted role of BRCA1 in the initial and later stages of the HR process, which is crucial for the timely elimination of DSBs and the repair and restart of damaged DNA replication forks. BRCA1, acting in concert with its obligatory partner BARD1, clearly fulfils an important function in DNA end resection, by overcoming the restriction imposed by 53BP1 and its associated complex. In addition, emerging evidence has implicated BRCA1-BARD1 in the RAD51-dependent assembly of the synaptic complex in D-loop formation. As we have discussed at length, BRCA1-BARD1 also very likely helps mediate the exchange of RPA with RAD51 on ssDNA. BRCA1-BARD1 may act alone (in DNA end resection) or likely functions with PALB2 (in synaptic complex assembly) and with PALB2-BRCA2-DSS1 (in the RAD51 for RPA exchange). Importantly,

recent evidence has revealed a role for BRCA1-BARD1 in the protection of stalled replication forks against deleterious attrition by cellular nucleases in a manner that is independent of its canonical DSB repair function.

During DNA end resection, BRCA1-BARD1 not only help overcome the various restrictive factors to allow access to the resection machinery but may also influence the activity of the resection machinery[75,205,206] (unpublished results from the P.S. laboratory). Moving forward, the major challenge in fully understanding the roles of BRCA1-BARD1 in HR and in replication fork protection will be to develop reconstituted systems consisting of purified factors to interrogate the functions of BRCA1-BARD1 and test mutants that are impaired in either DNA binding or protein-protein interactions. Likewise, it will be important to ascertain whether the E3 ligase activity of BRCA1-BARD1 modifies proteins that are relevant for the execution or regulation of HR repair and replication fork protection.

On the clinical front, there is an urgent need to delineate different mechanisms that cause innate and acquired resistance of BRCA-deficient and HR-deficient tumours to drugs such as platinum-based compounds and PARPi. In this regard, the recent discovery of the HR inhibitory axis comprising the 53BP1-RIF1-shieldin and CST complexes, the mutational inactivation or silencing of which can help restore HR proficiency to HR mutant cells, can be expected to lead to refined understanding of these processes. Intense efforts are being expended on the development of new chemical inhibitors of DNA repair and of DNA damage checkpoint pathways for clinical applications. There can be little doubt that some of these inhibitors will ultimately make their way into the clinic as cancer prophylactics and/or for the treatment of incalcitrant tumours[207–209].

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R-loops

Three-stranded nucleic acid structures that arise during transcription, consisting of an RNA-DNA hybrid formed by annealing of the nascent transcript with its DNA template. The non-template DNA is displaced as single-stranded DNA.

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Synthetic lethality

Induction of cell death (lethality) by simultaneously inactivating two different biological pathways or genes, which normally do not affect cell viability inactivated individually.

Ionizing radiation-induced nuclear foci

Subnuclear domains into which factors needed for DNA damage signalling and repair concentrate on exposure to ionizing radiation. Their formation and resolution reflect the robustness of the cellular response to ionizing radiation.

G-quadruplexes

Non-canonical DNA (or RNA) structures consisting of stacks of two or more guanine quartets, each stabilized by a monovalent cation. G-quadruplexes form spontaneously on guanine-rich single-stranded DNA during DNA replication and transcription.

Radial chromosomes

Fused chromosomes that arise from aberrant repair of DNA double-strand breaks or stalled replication forks, or from incomplete resolution of repair intermediates.

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Fanconi anaemia

A multigenic disorder characterized by bone marrow failure and cancer predisposition, owing to an inability to properly process DNA interstrand crosslinks and other DNA lesions.

Box 1 |

Conservative and error-prone pathways of double-strand break repair

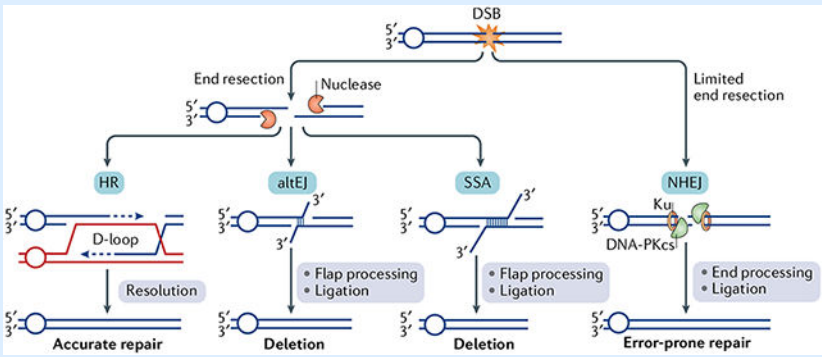
Four conserved pathways of DNA double-strand break (DSB) repair have been characterized. In non-homologous end joining (NHEJ), the DNA ends are engaged by a complex of proteins including the Ku70-Ku80 (Ku) complex and DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which further recruit other proteins to conduct nucleolytic trimming of the DNA ends, followed by DNA gap filling and ligation[210] (see the figure). NHEJ is a highly efficient DsB repair pathway, but it generates DNA products that typically harbour the deletion or insertion of a few nucleotides.

The ends of a DsB can undergo nucleolytic degradation, in a process known as end resection. DNA end resection typically channels the DsB into the homologous recombination (HR) repair pathway. end resection removes a few hundred or more bases of the 5′-terminated strand to yield a 3′ single-stranded DNA (ssDNA) tail, which serves as the template for the assembly of protomers of the recombinase RAD51 to form a helical nucleoprotein filament. The RAD51-ssDNA ensemble then conducts a search for DNA homology in either the sister chromatid (preferred partner) or the homologous chromosome and catalyses the formation of a displacement loop (D-loop; see the figure). DNA synthesis occurs within the D-loop, followed by the resolution of the extended structure through one of several pathways[211] to form different types of DNA products.

Occasionally, the 3′ tail originating from end resection can undergo annealing through sequences with microhomology, or through regions of more extensive homology (for example, DNA repeats) to yield an intermediate with 3′ DNA flaps. In both cases (microhomology or extensive homology), the 3′ flaps are trimmed and, following further processing, the DNA joint is sealed by ligation. The microhomology-mediated DNA repair process is termed ‘alternative DNA end joining’ (altEJ), whereas joining via the hybridization of DNA repeats is referred to as ‘single-strand annealing’ (SSA). Both altEJ and SSA require distinct cadres of factors, yet both pathways result in deletions[212] (see the figure).

DSB repair pathway choice is intimately linked with cell cycle progression. Specifically, NHEJ remains active throughout the cell cycle and becomes the predominant pathway in G1 phase. By contrast, HR is most active in S phase and G2 phase, as the ability of cells in G1 phase to conduct DNA end resection is greatly attenuated[75,213]. Given that altEJ and SSA also require DNA end resection, they are primarily operational in S phase and G2 phase. DNA repair pathway choice is intricate and entails the exclusion of the resection machinery from DNA ends and also the cell cycle-specific regulation of HR repair complex assembly and chromatin modifications[9,10].

HR is the only DSB repair pathway capable of restoring the original DNA sequence of the damaged site and is therefore conservative in nature. The mechanisms of different DSB repair pathways have been reviewed recently[212,214], whereas the cell cycle-specific regulation of DNA end resection is discussed in this article.



Box 2 |

Protein complexes that antagonize DNA end resection

Multiple protein interactors help p53-binding protein 1 (53BP1) to restrict DNA end resection (TABLE 1). RIF1 inhibits DNA double-strand break (DsB) resection and homologous recombination repair similarly to 53BP1. Much like 53BP1 abrogation, the loss of RIF1 restores ionizing radiation-induced RAD51 focus formation and triggers resistance to poly(ADP-ribose) polymerase inhibitors (PARPi) in breast cancer type 1 susceptibility protein (BRCA1)-deficient cells[99,122,215]. REv7 (also known as MAD2L2 or MAD2B) is another component of the 53BP1-RIF1-associated complex that helps restrict DNA end resection; REV7 was isolated in a screen for factors whose inactivation leads to PARPi resistance in a BRCA1-deficient background[101]. Recent efforts by multiple groups have led to the identification of three new 53BP1-interacting factors — shieldin complex subunit 1 (SHLD1), SHLD2 and SHLD3 — that form the stable shieldin complex with REV7, thus named because it acts in conjunction with 53BP1-RIF1 to ‘shield’ DSB ends from the DNA end resection machinery[187,188,216–219]. Independently, characterization of the REV7 interactome in B lymphocyte extracts led to the isolation of shieldin[220]. Studies involving CRISPR-Cas9-based genetic screens for factors that, when disrupted in a BRCA1-deficient background, confer resistance to DNA damaging agents also identified shieldin[188,217]. Finally, SHLD2 was isolated as a REV7 interactor in BRCA1-defective cells that exhibit abnormal PARPi resistance[187].

The CST complex (consisting of CTC1, STN1 and TEN1) functions in telomere replication to facilitate C-strand synthesis by recruiting the DNA polymerase-α (Polα)-primase complex[221]. CST inactivation leads to increased single-stranded DNA (ssDNA) at telomeres and at ionizing radiation-induced DSBs genome wide[218]. Importantly, CST interacts with shieldin, and its inactivation renders BRCA1-deficient cells resistant to olaparib. These results suggest that the 53BP1-RIF1-shieldin ensemble cooperates with CST and Polα to fill in resected DSBs, thereby adding a new dimension to the mechanism by which 53BP1 counteracts the formation of recombinogenic 3′ ssDNA overhangs[218]. A separate study isolated the CST complex as a resection antagonist and mediator of PARPi sensitivity in BRCA1-deficient cells[219]. It remains possible that the primary role of shieldin is to recruit and activate the protein ensemble comprising CST and Polα-primase.

In a loss-of-function CRISPR-Cas screen, dynein light chain 1 (DYNLL1) was found to be an antagonist of DNA end resection[222]. Like 53BP1 and its effectors, loss of DYNLL1 can suppress the DNA end resection and homologous recombination defects of BRCA1-mutant cells. Accordingly, DYNLL1 deletion results in resistance of BRCA1-deficient cells to platinum-based drugs and PARPi. DYNLL1 associates in cell extracts with components of the DNA end resection machinery, including the MRE11-RAD50-NBS1 complex, the helicase Bloom syndrome protein and the nuclease DNA2. Importantly, DYNLL1 interacts directly with MRE11 and attenuates its nuclease activity[222]. In addition to directly inhibiting the DNA end resection machinery, DYNLL1 promotes the

oligomerization of 53BP1 (REF.[223]), which is a prerequisite for the association of 53BP1 with DSB-harbouring chromatin[224–226].

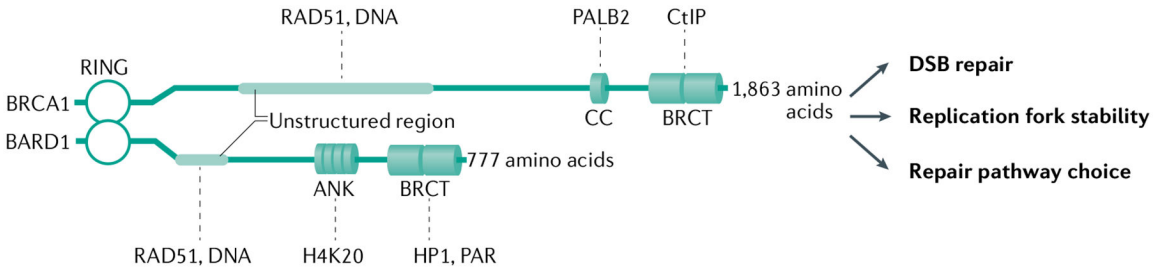


Fig. 1 |. The functional domains of BRCA1 and BARD1.

The RING (really interesting new gene) domain of breast cancer type 1 susceptibility protein (BRCA1) and BRCA1-associated RING domain 1 (BARD1) mediates their heterodimerization and is crucial for the E3 ubiquitin ligase activity of the BRCA1-BARD1 complex. Both proteins also harbour two BRCA1 C-terminal (BRCT) repeats arranged in tandem, which confer the ability to interact with various proteins involved in the DNA damage response, and unstructured regions that are involved in interactions with the recombinase RAD51 and with DNA. Unique features include a coiled-coil (CC) domain of BRCA1, which allows assembly of a higher-order complex with BRCA2-DSS1 through partner and localizer of BRCA2 (PALB2), and four ankyrin (ANK) repeats in BARD1, which serve to target the BRCA1-BARD1 complex to DNA lesions by interacting with unmethylated histone H4 Lys20 (H4K20), which is present in nucleosomes of newly replicated DNA[75]. The BRCT domain of BRCA1 interacts with CtBP-interacting protein (CtIP), which is involved in DNA end resection; the BRCT domain of BARD1 mediates its association with heterochromatin protein 1 (HP1), which is required for heterochromatin formation and maintenance, and with poly(ADP-ribose) (PAR), which is synthesized by poly(ADP-ribose) polymerase. PAR recognition is important for the recruitment of BRCA1-BARD1 to stalled replication forks and thus for maintaining the stability of these forks[66]. See TABLE 1 for a more complete list of protein interactors of this tumour suppressor complex. DSB, double-strand break.

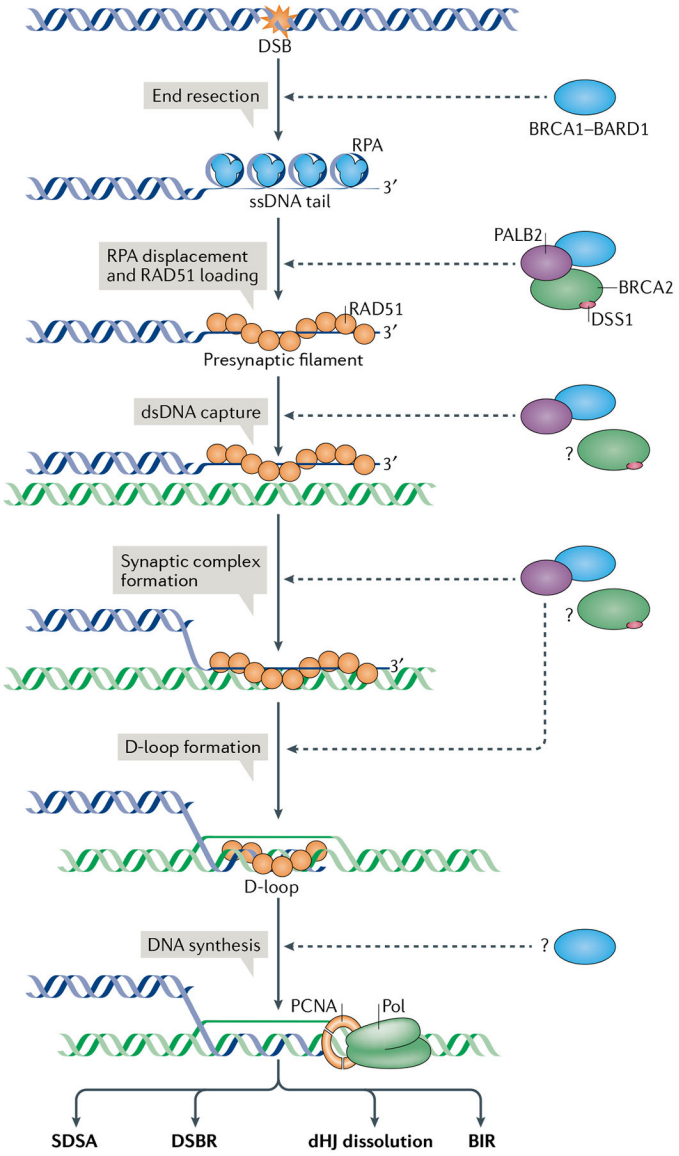


Fig. 2 |. The roles of BRCA1-BARD1 in DSB repair by homologous recombination.

The single-stranded DNA (ssDNA) tail generated by end resection, which is aided by the breast cancer type 1 susceptibility protein (BRCA1)-BRCA1-associated RING domain 1 (BARD1) complex, is occupied by the abundant ssDNA-binding factor replication protein A (RPA), which must be replaced by the recombinase RAD51 for downstream repair steps to occur. The exchange of RPA with RAD51 on DNA is mediated by BRCA2-DSS1, with the involvement of BRCA1-BARD1 and partner and localizer of BRCA2 (PALB2). Importantly, BRCA1-BARD1 also enhances the ability of the RAD51-ssDNA nucleoprotein complex to capture the homologous duplex DNA and assemble the synaptic complex, which gives rise to the displacement loop (D-loop). Following repair DNA synthesis by DNA polymerase-δ (Pol) associated with PCNA, the extended D-loop structure can be resolved by one of four mechanistically distinct pathways — synthesis-dependent single strand annealing (SDSA), canonical double-strand break (DSB) repair (DSBR), double Holliday junction (dHJ)

dissolution and break-induced DNA replication (BIR)[11,95] — that yield distinct products. Homologous recombination steps that are facilitated by BRCA1-BARD1 or BRCA2-DSS1 are indicated by dashed arrows. Whether BRCA1-BARD1 and its homologous recombination partners also function in other steps of the repair reaction (for example, in repair DNA synthesis), remains to be determined (denoted by question marks). For simplicity, only one of the two DNA DSB ends is depicted. dsDNA, double-stranded DNA.

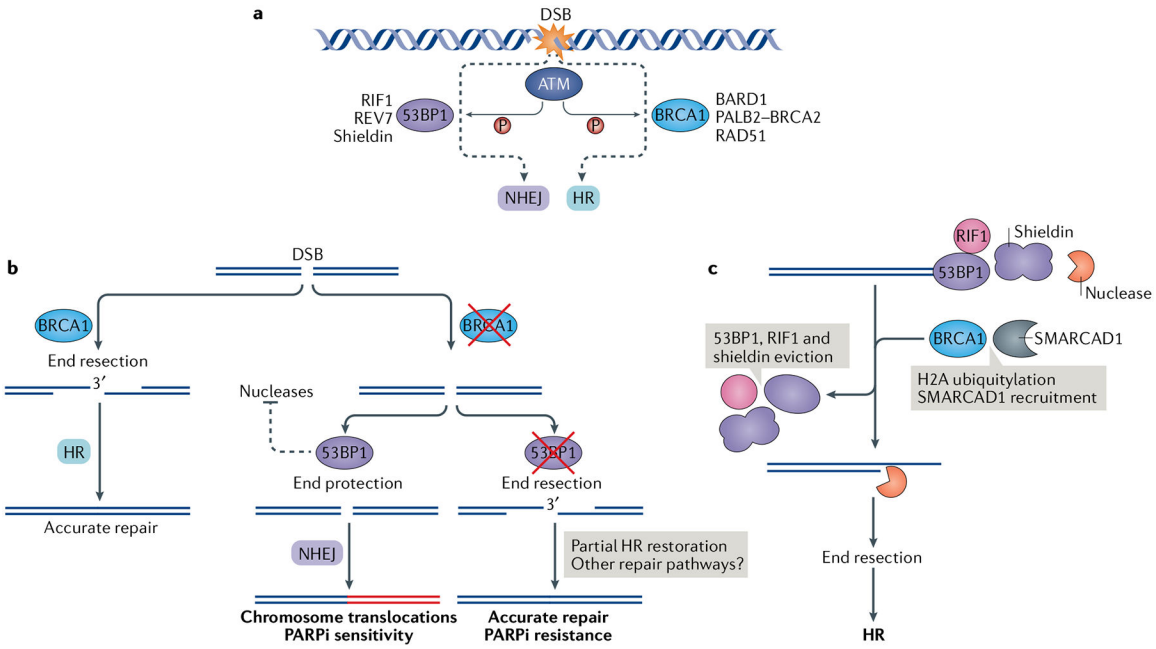


Fig. 3 |. Antagonistic roles of BRCA1-BARD1 and the 53BP1 complex.

a | A DNA double-strand break (DSB) elicits ataxia telangiectasia mutated (ATM)-dependent checkpoint responses, which activate and recruit breast cancer type 1 susceptibility protein (BRCA1)-containing and/or p53-binding protein 1 (53BP1)-containing complexes to the DNA lesion. BRCA1 and 53BP1 compete and perform opposing functions at broken DNA ends by engaging either homologous recombination (HR) or non-homologous end joining (NHEJ) for DSB repair, respectively. **b** | BRCA1 promotes DNA end resection and HR repair (left). If BRCA1 is abrogated, DSB ends are protected from resection by 53BP1-containing complexes and are channelled into NHEJ for repair (right). This process can lead to deleterious chromosome translocations, which underline the sensitivity of BRCA1-deficient cells to poly(ADP-ribose) polymerase inhibitors (PARPi). If, in addition to BRCA1, 53BP1 is concomitantly abrogated, the DNA ends are accessible to resection nucleases, leading to partial HR restoration and PARPi resistance. **c** | 53BP1-containing complexes (53BP1—RIF1—shieldin) protect DNA ends from nucleolytic digestion by nucleases. This end protection is overcome by BRCA1-mediated histone H2A ubiquitylation and recruitment of the helicase SMARCAD1. The eviction of the 53BP1-containing complexes leads to DNA end resection and HR. BARD1, BRCA1-associated RING domain 1; PALB2, partner and localizer of BRCA2.

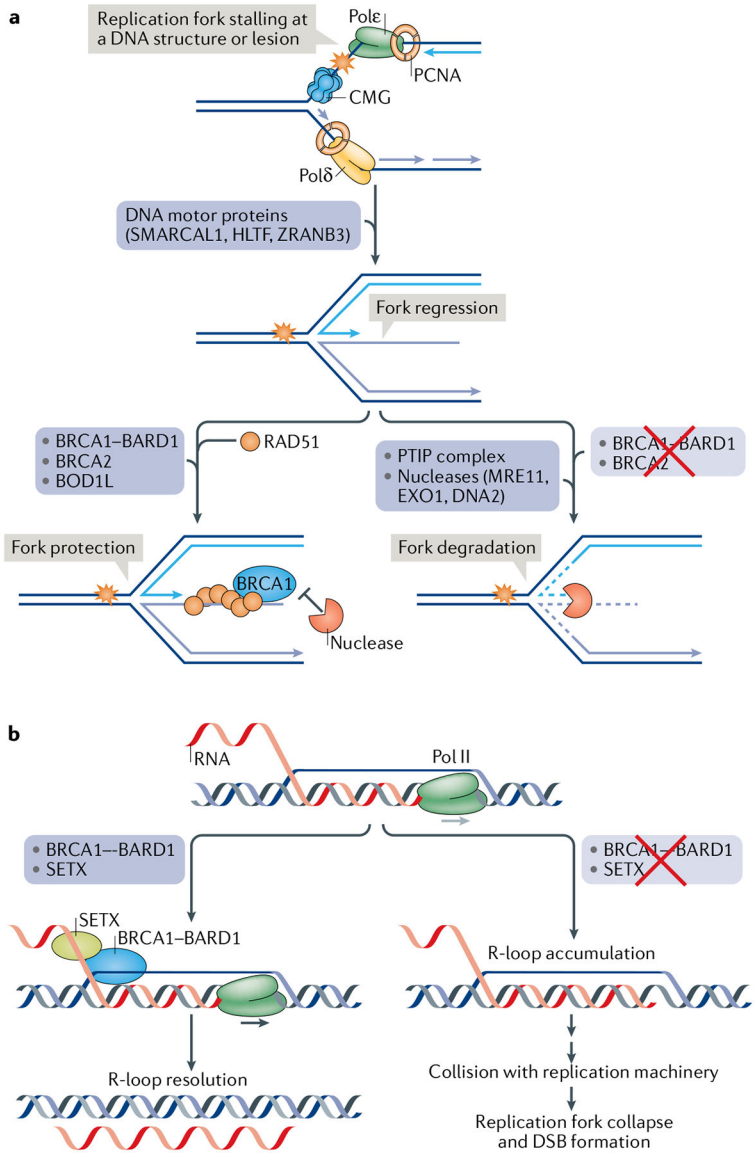


Fig. 4 |. Roles of the Brca1 complex in protecting regressed replication forks and resolving r-loops.

a | During DNA replication, when the DNA polymerase ensemble comprising DNA polymerase-ε (Polε), PCNA and the CDC45-MCM-GINS (CMG) helicase complex on the leading strand and Polδ-PCNA on the lagging strand is impeded by a DNA structure or lesion, one of the nucleic acid motor proteins SMARCAL1, HLTF or ZRANB3 can catalyse the reversal of the replication fork, a process often referred to as ‘replication fork regression’, to generate a four-armed DNA structure that harbours a free DNA end. Formation of the regressed fork allows a replicative mechanism of lesion bypass[15,227]. However, the free DNA end is prone to attack by MRE11 and other nucleases in conjunction with the PTIP complex[148], unless RAD51 is deposited on the regressed fork. The stability of the regressed fork is also dependent on breast cancer type 1 susceptibility protein (BRCA1)-BRCA1-associated RING domain 1 (BARD1), BRCA2 and biorientation of chromosomes in cell division protein 1-like 1 (BOD1L), which are postulated to stabilize the RAD51-DNA

interaction to prevent nucleolytic attrition[139,140,146]. Other pathways of replication fork repair and restart have been identified, and readers are referred to several comprehensive reviews on this topic[227–229]. **b** | Stalling of RNA polymerase II (Pol II) or delay in mRNA processing can lead to accumulation of R-loops (RNA-DNA hybrids). An R-loop can stall the DNA replication machinery, thereby causing fork collapse and the formation of a DNA double-strand break (DSB). BRCA1-BARD1 and the putative RNA-DNA helicase senataxin (SETX) facilitate R-loop resolution[155].

Table 1 |

Interactors and antagonists of BRCA1-BARD1

Direct interaction partners	Proteins	Refs
DNA repair machinery	PALB2	71,72
	RAD51	76,92
	CtIP	230
	FANCJ	61,231
	MRE11-RAD50-NBS1	64
	PIN1	129
	FANCA	232
	DNA-PKcs	233
	MSH2	234
	CSB	235
DNA damage signalling	ATM	236
	ATRIP	237
	ATR	238,239
	Histone H2A	126
	Histone H4	75
	Merlin (NF2)	240
	HP1	67
	ABRAXAS	241,242
	CDK-cyclin	243,244
	Protein phosphatase 1α	245
	TGFβ1 (SMAD3)	246
Transcription (or R-loop metabolism)	RNA polymerase II	247,248
	SETX	156
	CBP (p300)	249,250
	COBRA1 (NELFB)	251
	p53	249
Cell growth	BAP1	252
	RB	253
Centromere regulation	KIAA0101	254
	OLA1	255
Chromosome segregation	Topoisomerase 2α	256
Chromatin remodelling	BRG1	257
	BRD7	258
	HDAC1, HDAC2	259
Other	Acetyl-CoA carboxylase 1	260
	Importin-α subunit	261
E3 ligase substrates[a]	Merlin (NF2)	240

Direct interaction partners	Proteins	Refs
	Claspin	262
	Histone H2A	126
	Histone macroH2A	263
	RNA polymerase II	264,265
	Aurora kinase A, aurora kinase B	53
Antagonists	53BP1	100
	RIF1	99,122,215
	Shieldin complex (REV7-SHLD1-SHLD2-SHLD3)	187,188,216–219
	CST complex (CTC1-STN1-TEN1)	218,219
	DNA polymerase-α-primase	218
	DYNLL1	222,223
	USP48	266,267

BARD1, breast cancer type 1 susceptibility protein-associated RING domain 1; BRCA1, breast cancer type 1 susceptibility protein; CDK, cyclin-dependent kinase; CtIP, CtBP-interacting protein; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; TGFβ1, transforming growth factor-β1.

[a] Other E3 ligase substrates have been identified but are not listed.

Table 2 |

Mechanisms of resistance to PARP inhibitors in BRCA1-deficient cells and tumours

Mode of resistance	Mechanism	Refs
Restoration of BRCA1 function	Secondary mutations in *BRCA1*	199,200
	Promoter demethylation	201,202
	Promoter switch	202
Partial restoration of homologous recombination	Loss of 53BP1, RIF1, REV7, PTIP, DYNLL1 or the shieldin complex (BOX 2)	99–101,115, 122,186–189,197, 198,215,222
Restoration of replication fork stability	Loss of PTIP, SMARCAL1, ZRANB3 or HLTF	147,148
Restoration of PARP signalling	Loss of poly(ADP-ribose) glycohydrolase (PARG)	195
Other	Loss of SLFN11 (putative DNA/RNA helicase)	191,203

BRCA1, breast cancer type 1 susceptibility protein; PARP, poly(ADP-ribose) polymerase.