HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2016 August 03.

Published in final edited form as:

Nat Rev Cancer. ; 12(1): 68–78. doi:10.1038/nrc3181.

BRCA1 and BRCA2: different roles in a common pathway of genome protection

Rohini Roy, **Jarin Chun**, and **Simon N. Powell**

Molecular Biology Program and Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, USA

Abstract

The proteins encoded by the two major breast cancer susceptibility genes, *BRCA1* and *BRCA2*, work in a common pathway of genome protection. However, the two proteins work at different stages in the DNA damage response (DDR) and in DNA repair. BRCA1 is a pleiotropic DDR protein that functions in both checkpoint activation and DNA repair, whereas BRCA2 is a mediator of the core mechanism of homologous recombination. The links between the two proteins are not well understood, but they must exist to explain the marked similarity of human cancer susceptibility that arises with germline mutations in these genes. As discussed here, the proteins work in concert to protect the genome from double-strand DNA damage during DNA replication.

The greatest risk factor for breast and ovarian cancer is inheritance of a mutation in one of the breast cancer susceptibility genes, *BRCA1* or *BRCA2*. *BRCA1* and *BRCA2* are tumour suppressor genes, the coding regions of which show no homology to previously described proteins or to each other. If one copy of either gene is mutated in the germ line, the result is hereditary breast and ovarian cancer (HBOC) syndrome, which is inherited in an autosomal-dominant manner. This syndrome is associated with not only early-onset breast cancer but also an increased risk of ovarian, pancreatic, stomach, laryngeal, fallopian tube and prostate cancer. HBOC syndrome accounts for 5–7% of all cases of breast cancer, and individuals with HBOC syndrome have a lifetime risk of developing breast cancer of 50–80%, and of 30–50% for ovarian cancer (TABLE 1). The estimated frequency of developing other common malignancies associated with a mutation in *BRCA2* is only 0.1% for prostate cancer and 0.5% for pancreatic cancer, although the relative risk is significantly increased (up to 20-fold for prostate cancer and 10-fold for pancreatic cancer)[1].

In addition to having similar disease phenotypes, both proteins are known to function in homologous recombination (HR), a vital DNA repair process that uses the undamaged sister chromatid to carry out high-fidelity repair of predominantly replication-associated DNA double-strand breaks (DSBs). HR appears to be the major mechanism for protecting the integrity of the genome in proliferating cells, because other DSB repair pathways are error-

Correspondence to S.N.P. powells@mskcc.org.

Competing interests statement

The authors declare no competing financial interests.

prone and generate chromosome deletions and translocations[2]. A curious feature of HBOC syndrome is that BRCA1-associated breast cancer is more often oestrogen-receptor (ER) negative, whereas BRCA2-associated breast cancers have the same distribution of cancer subtypes as found sporadically. In hereditary breast cancer for which there is no evidence of a *BRCA1* or *BRCA2* mutation, mutations in the DNA damage response (DDR) kinases *CHK2* or ataxia-telangiectasia mutated (*ATM*) might account for the breast cancer predisposition[3,4]. Additional inherited mutations may also occur in other members of the BRCA1–BRCA2–HR pathway, such as partner and localizer of BRCA2 (*PALB2*) and BRCA1-interacting protein C-terminal helicase 1 (*BRIP1*; also known as *BACH1* or *FANCJ*), but currently the frequency of mutations in these genes in hereditary breast cancer is low. In the absence of known germline predisposition for breast cancer, mutations in *BRCA1* and *BRCA2* are uncommon in sporadic breast cancer.

Linking the biochemistry of BRCA1 and BRCA2 function to a common pathway of genome protection often creates more questions than answers. This Perspective discusses how the BRCA1 and BRCA2 proteins function biochemically and how this is related to their observed roles. We ask from what type of DNA damage are these proteins protecting the cell? How do the two proteins communicate in a common pathway of genome protection? And why are breast and ovarian epithelial cells preferentially susceptible to tumorigenesis? We also discuss the association of breast cancer subtypes with BRCA1 and BRCA2 deficiency.

Maintaining genome integrity

One aspect of maintaining genomic integrity is mediated by a cellular network of signalling events (the DDR) that is triggered in response to genotoxic stress. The DDR to DSBs involves sensors that can detect broken ends, effectors that execute repair and mediators that facilitate interactions between sensors and effectors (FIG. 1). The DDR also includes the activation of checkpoints that delay the cell cycle before or during replication (G1/S or intra-S-phase checkpoints) or before cell division (G2/M checkpoint) to ensure that genetic errors are not transmitted to subsequent generations by allowing time for DNA repair. DSBs are considered to be the most threatening form of DNA damage, as the integrity of both strands of the DNA duplex is compromised simultaneously. DSBs can occur as by-products of DNA replication or during exposure to ionizing radiation and other genotoxic compounds. In mammalian cells, DSBs are repaired by HR (which is mostly error-free), or by non-homologous end-joining (NHEJ; which is error-prone). The genome is particularly susceptible to DNA damage during replication because damage on a single strand can be converted to double-strand damage and lead to replication fork collapse. In the absence of an intact HR pathway, these replication-associated DSBs can result in chromosome rearrangements and hence genomic instability.

HR repairs DSBs during the S and G2 phases of the cell cycle, when an intact sister chromatid can serve as a template for repair; it is also pivotal in maintaining replication fidelity. The protection of the genome by HR involves damage recognition by the kinases ATM and ataxia telangiectasia and Rad3-related (ATR), signal mediation by CHK2 and BRCA1, and initiation of repair by the effectors BRCA2 and RAD51. There are also several

facilitators of the HR pathway, such as PALB2 and BRIP1, and each of these facilitators is a predisposing factor for HBOC syndrome when mutated, which suggests that it is the BRCA1–BRCA2–HR pathway that suppresses tumorigenesis.

BRCA1 functional domains and binding partners

BRCA1 is a versatile protein that links DNA damage sensing and DDR effectors. BRCA1 interacts with tumour suppressors, DNA repair proteins and cell cycle regulators through its various functional domains and thereby has diverse roles in multiple DNA repair pathways (particularly HR, NHEJ and single-strand annealing (SSA)) and in checkpoint regulation[5,6]. BRCA1 contains an amino-terminal RING domain that has E3 ubiquitin ligase activity (which catalyses protein ubiquitylation) and a BRCT domain that facilitates phospho-protein binding (FIG. 2a). Many inherited cancer-associated BRCA1 mutations have been found within the RING and BRCT domains, indicating that both domains are involved in suppressing breast and ovarian cancer[7–9]. BRCA1 E3 ubiquitin ligase activity is enhanced when associated with the RING domain of its partner protein, BRCA1-associated RING domain protein 1 (BARD1)[10]. The BRCA1–BARD1 heterodimer generates polyubiquitin chains at unconventional K6 linkages that do not appear to signal for protein degradation, but may instead mediate downstream signalling events through mechanisms that are still unclear[10–13]. The tumour suppressor function of the E3 ubiquitin ligase activity has been questioned recently by the observation that in a knock-in mouse model expressing an E3-ligase defective mutant of BRCA1, the development of tumours was suppressed to the same extent as when wild-type BRCA1 was expressed. BRCA1 ubiquitylation of CtBP-interacting protein (CtIP; also known as RBBP8), a protein involved in DNA DSB resection through its association with the MRN complex (which is comprised of MRE11, RAD50 and Nijmegen breakage syndrome protein 1 (NBS1; also known as nibrin)), may have a role in DSB repair pathway choice, as CtIP-dependent resection promotes HR and inhibits NHEJ[14]. Why the E3-ligase function appears to be dispensable for tumour suppression has not yet been satisfactorily answered, as many tumour-producing mutations are located in the RING domain, suggesting that there may be another function of the protein associated with the RING domain that has yet to be defined.

The BRCT phosphopeptide-binding motif, which is conserved in multiple DDR proteins, is responsible for the association of BRCA1 with proteins phosphorylated on serine in SXXF motifs by ATM. The BRCA1- interacting proteins include abraxas, BRIP1 and CtIP. The binding of these proteins make up separate BRCA1 macro-protein complexes that have distinct and overlapping functions in the DDR[15] (FIG. 1; TABLE 1). A fourth BRCA1-containing complex mediated through the BRCA1 coiled-coil domain is composed of PALB2 and BRCA2 and is specifically involved in DSB repair by HR[16–18]. How these multiple BRCA1 complexes work in a coordinated manner is still unclear. It will be interesting to uncover whether one BRCA1-containing complex is replaced with a different BRCA1-containing complex during the DDR, or whether BRCA1 functions as a platform on which initial DNA damage sensing proteins assemble and disassemble and subsequent repair proteins associate. As many of the current investigations have been performed using global DNA damaging agents, disentangling which BRCA1 complex functions at different steps in the pathway will be difficult. The study of a single, site-specific damage locus may help to

shed light on the crosstalk between the various BRCA1 macro-complexes and their assembly and disassembly.

BRCA1 and HR

BRCA1 is directly involved in HR-mediated repair of DSBs[19–21]. BRCA1 binds to DSBs through its association with the abraxas–RAP80 macro-complex, which associates with ubiquitylated histones at DNA DSBs[19] (FIG. 1). Next, BRCA1 is involved in processing DSBs through its interaction with CtIP and the MRN complex (FIG. 1). The BRCA1–CtIP complex promotes CtIP-mediated 5′-end resection of DSBs[14], which is abrogated by three independent tumour-associated mutations in the BRCT domain of BRCA1 (REFS 22,23). BRCA1 is also required for RAD51 recruitment to the sites of DNA damage through its interactions with PALB2 and BRCA2 (FIG. 1). This interaction appears to be dependent on CHK2-mediated phosphorylation of S988 on BRCA1 (S.N.P., unpublished observations). Importantly, knock-in mice expressing an S971A mutant (in mice, S971 corresponds to human S988) develop mammary and endometrial tumours after treatment with DNA damaging agents[24]. BRCA1-deficient human cells expressing BRCA1-S988A have defects in HR but retain normal checkpoint function and resistance to ionizing radiation, implying that the HR function of BRCA1 is distinct from its other functions in the DDR and that this mutation causes a dissociation of function[25]. *Brca1*-null embryonic stem (ES) cells from mice expressing the human BRCA1-S988A mutant also displayed no difference in cell cycle profiles or sensitivity to DNA damaging agents compared to those expressing wild-type human BRCA1 (REF. 26). In this study, HR function was not analysed, and sensitivity to DNA damaging agents was measured by growth assay rather than clonogenic survival. The extent to which deficiency in HR contributes to cell survival after ionizing radiation, even for cells in S phase, remains a point of debate — HR is likely to be more important for surviving replication errors than for surviving exogenous DNA damage resulting in DSBs, in which case other repair pathways can be used. Similarly, tumour suppression is likely to be related to fixing replication errors rather than repairing DSBs, which is why HR and tumour suppression are closely linked.

BRCA1 and other DNA repair pathways

BRCA1 may also function in other DNA repair pathways, including NHEJ and SSA. The role of BRCA1 in NHEJ is somewhat controversial, as BRCA1 has been observed to facilitate[27,28], suppress[29,30] or have no effect on NHEJ[31]. These wide-ranging observations may be attributed to the variety of assays used to measure NHEJ and the possibility that BRCA1 has different roles in the various subtypes of NHEJ. BRCA1 may be involved in HR, NHEJ and SSA through its interaction with the MRN complex, which is required for DNA end resection before all three repair processes[32,33]. BRCA1 recruitment to DSBs is facilitated by a DNA damage-induced interaction of the BRCA1 N terminus with the NHEJ protein KU80, thereby providing another mechanism for BRCA1 accumulation at DSBs[34]. A recent study has proposed that a critical function of BRCA1 is to remove NHEJ proteins such as p53-binding protein 1 (53BP1) from DSBs[35] to prevent aberrant end-joining and to regulate the choice between HR and NHEJ. A *BRCA1* exon 11 deletion mutant exhibited decreased SSA in addition to decreased HR, providing further evidence that BRCA1 functions in both SSA and HR[36]. The relevance of SSA in genome stability and tumour

suppression is not known, but is likely to be limited. Like NHEJ, SSA is also mutagenic, producing deletions and insertions at sites of long repeat sequences.

BRCA1 and checkpoint activation

The BRCA1–BARD1 complex is involved in the activation of G1/S, S-phase and G2/M checkpoints (TABLE 1). The G1/S-checkpoint requires phosphorylation of BRCA1 by ATM or ATR, which facilitates phosphorylation of p53 on S15. p53-S15 phosphorylation is necessary for transcriptional induction of the cyclin dependent kinase (CDK) inhibitor p21 and ionizing radiation-induced G1/S checkpoint activation[37]. BRCA1–BARD1 depletion compromises the induction of p21 and activation of the G1/S checkpoint in response to ionizing radiation[38]. The exact mechanism of BRCA1–BARD1 control of the S-phase and G2/M checkpoints is not well characterized[39]. The BRCA1–BRIP1–DNA topoisomerase 2-binding protein 1 (TOPBP1) macro-complex appears to be necessary for the S-phase checkpoint in response to stalled or collapsed replication forks[40], whereas the BRCA1–abraxas–RAP80 macro-complex appears to be involved in the G2/M checkpoint in response to ionizing radiation-induced DNA damage (TABLE 1).

The G2/M checkpoint is defective in cells lacking functional BRCA1, BARD1, RAP80 or abraxas[20,21] (TABLE 1). Interestingly, partial defects in G2/M checkpoint activation and p53 stabilization were observed in mouse embryonic fibroblasts (MEFs) from knock-in mice expressing the cancer-associated BRCA1-S971A mutation (which is the equivalent to human BRCA1-S988A)[26]. The checkpoint defect was mild and disappeared 4 hours after treatment with ionizing radiation. Furthermore, after treatment with an alkylating agent, but not ionizing radiation, the levels of p53 were reduced in MEFs expressing BRCA1-S971A[24], whereas in human HCC1937 cells expressing BRCA1-S988A, no detectable defect in the G2/M checkpoint was observed (S.N.P., unpublished observations). The stability of p53 has not been tested in BRCA1-deficient human cells and may need to be measured to verify whether, in human cells, BRCA1-S988A is a true separation-of-function mutation. These additional functions of BRCA1 in checkpoints and in stabilizing p53 may contribute to genomic stability, which is primarily determined by the role of BRCA1 in HR. Likewise, this broader range of BRCA1 functions may account for the severity and pattern of genomic instability found in BRCA1-deficient tumours relative to BRCA2-deficient tumours.

BRCA2 functional domains and binding partners

In contrast to the multifunctional activities of BRCA1, the primary function of BRCA2 is in HR (TABLE 1). BRCA2 mediates the recruitment of the recombinase RAD51 to DSBs; RAD51 recruitment is not only essential for HR but is also responsible for the tumour-suppressive function of this repair process[41] (FIG. 1). BRCA2 contains a DNA-binding domain (DBD) that binds single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) and eight BRC repeats that bind RAD51 (FIG. 2b). The DBD contains five components: a 190-amino-acid α-helical domain, three oligonucleotide binding (OB) folds that are ssDNA-binding modules, and a tower domain (TD) that protrudes from OB2 and binds dsDNA[42]. The helical domain, OB1 and OB2 also associate with deleted in split-hand/split-foot syndrome (DSS1), which has been linked to BRCA2 protein stabilization[42–45]. *Ustilago maydis* Brh2, the BRCA2 homologue in corn smut, binds to DNA at the resected

ends of a DSB, where both dsDNA and ssDNA exist[46], presumably facilitating RAD51 filament formation at that site[47]. This implies that BRCA2 mediates RAD51 filament formation at the appropriate sites of ssDNA and prevents it from binding to dsDNA, as supported by recent biochemical data using purified BRCA2 (REF. 48).

Point mutations within BRC repeats that compromise interactions with RAD51 are found in individuals with HBOC syndrome[49]. The BRC repeats have subtle differences in sequence and bind RAD51 with varying affinity by mimicking the structure of RAD51 monomers[50]. In addition to facilitating the recruitment of RAD51 to ssDNA, the BRC repeats accelerate replication protein A (RPA)-displacement from ssDNA by RAD51 (REF. 51), block RAD51 nucleation at dsDNA and facilitate RAD51 filament formation on ssDNA by maintaining the active ATP-bound form of RAD51 on ssDNA[52]. The binding of RAD51 by the C terminus of BRCA2 has been shown to be dependent on CDK activity[53,54]. This association, however, appears to be dispensable for HR *in vivo* and may be important for the disassembly of RAD51 complexes to facilitate mitotic entry[2]. Whether this association is important for suppression of breast and ovarian tumorigenesis is unknown.

Much of our knowledge of BRCA2 comes from studying portions of the BRCA2 protein or investigating BRCA2 orthologues, such as Brh2 in *U. maydis* and BRC-2 in *Caenorhabditis elegans*[46,52,55–57]. Insight into the details of mammalian BRCA2 function had been hampered by the inability to isolate the full-length protein, which is 3,418 amino acids in humans. Recently, however, three independent groups have successfully purified and functionally validated full-length human BRCA2 (REFS 48,58,59). These studies showed that the stoichiometry of RAD51 binding was 6–7 to 1, and that the protein could indeed catalyse many steps of the RPA to RAD51 transition, as had been predicted by the genetic studies. Future studies using the full-length BRCA2 protein will help to further our understanding of the structure and function of BRCA2.

BRCA2, HR and replication fidelity

Overwhelming evidence suggests that the primary function of BRCA2 is to facilitate HR (TABLE 1). BRCA2-deficient cells are defective in recruiting RAD51 to sites of DSBs and in repairing DSBs by HR[60]. Although human and mouse cells expressing a BRCA2 loss-of-function truncation mutant display some defects in replication and checkpoint control, BRCA2 is not essential for these processes[61,62]. Recently, BRCA2-deficient hamster cells treated with hydroxyurea (which causes replication fork stalling and collapse) were shown to have defects in maintaining the length of the nascent strand of DNA, perhaps because BRCA2 protects the nascent strand from degradation at stalled replication forks[2]. Thus, in addition to its role in repair by HR, these results imply a second role for BRCA2 in protecting the replication fork. The critical evidence supporting these two functions of BRCA2 was a dissociation-of-function mutant, S3291A, the expression of which allows normal DSB-induced HR but results in defective protection of replication forks. Without the dissociation-of-function mutant, the reduced length of the nascent strand after replication fork stalling could be secondary to deletions in the sister chromatid arising either from defective HR repair of the collapsed replication fork or from defective daughter-strand gap (DSG) repair by HR. We favour the view that the activity of BRCA1 and BRCA2 can occur

at DSBs created behind the replication fork (in addition to replication fork collapse) as a consequence of gaps on the nascent strand that are created by single-strand lesions on the parental strand (FIG. 3).

Links between BRCA1 and BRCA2

Although germline *Brca1* or *Brca2* heterozygous mutations in mice do not have a strong phenotype, most homozygous mutations are embryonically lethal[63,64]. Both *Brca1* and *Brca2* homozygous mouse embryos are hypersensitive to ionizing radiation and have widespread chromosome and chromatid aberrations, which indicates error-prone repair of chromatid breaks. Interestingly, the phenotype of BRCA-deficient mouse embryos mimics the phenotype of mice with inactivating mutations of *Rad51* (REF. 65). Therefore, these mouse models were the initial evidence to indicate that BRCA1 and BRCA2 function in a common pathway of RAD51-mediated HR[63,64].

In humans, the tumours that develop in patients with germline heterozygous mutations in *BRCA1* or *BRCA2* are defective in HR-mediated repair. The germline heterozygous cells must be haplo-insufficient, but this functional defect has so far been difficult to identify or investigate at the cellular level. Defective HR can also be found in sporadic breast cancers despite the absence of a germline mutation in one of the crucial members of the HR pathway, as shown by array-comparative genomic hybridization (aCGH) studies[66] and functional evaluations of human breast cancer cells[67]. However, the nature of how these functional defects in HR occur is not yet clear. Nevertheless, the common defect in HR indicates that the function of the BRCA1–BRCA2 pathway in mediating HR is important for tumour suppression in inherited and sporadic breast cancer.

PALB2 connects BRCA1 and BRCA2

PALB2 binds directly to both BRCA1 and BRCA2 and thereby provides a physical link between the two proteins[16–18] (FIG. 1). The N-terminal coiled-coil domain of PALB2 interacts with the coiled-coil domain of BRCA1, and the C terminus of PALB2 interacts with the N terminus of BRCA2 (REF. 68) (FIG. 2b). The interaction of PALB2 with BRCA2 was shown to be essential for loading RAD51 onto RPA-bound ssDNA[69]. Furthermore, the BRCA1–PALB2 interaction is a prerequisite for the recruitment of BRCA2 and RAD51 to the site of DNA damage and for HR, but had no impact on BRCA1-mediated S-phase checkpoint activation[17,18]. Depletion of PALB2 expression in cells phenocopied BRCA2 deficiency and abrogated the interaction between BRCA1 and BRCA2. Additionally, BRCA1 phosphorylation on S988 by CHK2 promotes formation of the BRCA1–PALB2–BRCA2 complex (FIG. 1), which may explain why mutating this site abrogates HR[25]. It is unknown whether there are other regulators of the BRCA1–PALB2–BRCA2 complex.

BRCA1 and BRCA2 function in a common pathway

Repair by HR can be triggered at ionizing radiation-induced two-ended DSBs or at one-ended DSBs that are generated by the cleavage of replication forks that have been stalled secondary to a blocking lesion. In some instances, single-strand lesions that do not produce a strand break are bypassed by the replication machinery and replication is restarted

downstream of the lesion, leaving behind a region of ssDNA or a DSG in which no DSB end is present[70] (FIG. 3). Replication-associated one-ended DSBs or DSGs recruit BRCA1, PALB2, BRCA2 and RAD51. *Brca1*-mutant mice exhibit telomere dysfunction, chromosome translocations and chromatid aberrations[71], and *Brca2*-mutant mice accumulate chromatid breaks and aberrant chromatid exchanges[61], providing further evidence in support of a BRCA1–BRCA2-mediated HR response to replication-associated DNA damage. Similarly, *Brca1*- and *Brca2*-mutant mice develop thymic lymphomas[61,71], which is a common tumour that arises in mice owing to defective DSB repair. Moreover, conditional expression of homozygous *Brca1* or *Brca2* mutants in mammary epithelium was sufficient to generate mammary cancers[72].

BRCA1 is involved in DDR signalling, checkpoint activation and HR and may also play a role in other DNA repair processes, such as NHEJ and SSA. Conversely, BRCA2 is primarily involved in HR. The human syndromes associated with *BRCA1* or *BRCA2* germline mutations are almost identical, and the only common functional link between the two BRCA proteins is the HR pathway. Therefore, it seems reasonable to conclude that the HR pathway is crucial for protecting the genome and that this pathway is disrupted in tumours arising in these mutation carriers. The Fanconi anaemia pathway — which includes BRCA2 — has some functional overlap with the BRCA1–BRCA2 pathway, but the human syndrome Fanconi anaemia (which is caused by defects in members of the Fanconi anaemia pathway) is markedly different to HBOC syndrome and is characterized by anaemia, skeletal abnormalities and predisposition to squamous cell carcinomas. In addition, the inheritance of Fanconi anaemia is autosomal recessive (owing to the inheritance of two hypomorphic alleles), whereas HBOC syndrome shows autosomal-dominant inheritance, with loss of the second allele (loss of heterozygosity (LOH)) occurring in the cancers that arise in mutation carriers. The effect of a defective *BRCA1* or *BRCA2* allele in the germ line must cause haploinsufficiency of HR to trigger the subsequent genetic alterations that result in cancer. Presumably, haplo-insufficiency from a single defective allele has different biological impacts compared with biallelic inheritance of hypomorphic alleles. However, the total number of families in the world that have been diagnosed with Fanconi anaemia caused by defects in BRCA1–BRCA2 pathway genes (*BRCA2* (also known as *FANCD1*), *BRIP1* (also known as *FANCJ* and *BACH1*), *PALB2* (also known as *FANCN*), *RAD51C* (also known as *FANCO*) or *SLX4* (also known as *FANCP*)) is small, and whether these patients show all the characteristic features of Fanconi anaemia has been debated[73].

In the BRCA1–BRCA2-mediated HR pathway, BRCA1 functions upstream of BRCA2, the function of which is dependent on BRCA1. In mammalian cells, HR can also occur through an alternative, BRCA1–BRCA2-independent, RAD52-dependent pathway. When BRCA2 function is disrupted in a tumour cell, RAD52 helps the cell to stay viable. Indeed, cells that are simultaneously depleted of RAD52 and BRCA1, RAD52 and PALB2 (S.N.P., unpublished observations) or RAD52 and BRCA2 (REF. 74) exhibit synthetic lethality. Taken together, these results support the hypothesis that BRCA1 and BRCA2 are connected in a common HR pathway that functions to repair DSBs, collapsed DNA replication forks or DSGs. Germline mutations in genes involving this common HR pathway are all associated with HBOC syndrome, suggesting that this pathway is the crucial tumour suppressor activity. Mutations in *BRCA1* and *BRCA2* are the predominant cause of HBOC syndrome,

with *ATM* and *CHK2* mutations being less common. Mutations in *PALB2* that lead to HBOC syndrome are very rare compared to mutations in *BRCA1* and *BRCA2* (REF. 75).

BRCA1 and BRCA2 in tumorigenesis

Common genetic alterations are associated with heterozygous *BRCA1* or *BRCA2* mutations, and these include loss of the wild-type *BRCA1* or *BRCA2* allele (LOH), loss of *TP53* (which encodes p53), and loss of ATM or CHK2 function. These additional alterations may allow cells to bypass checkpoint controls and evade apoptosis, and thereby initiate tumorigenesis. The fact that both *BRCA1* and *BRCA2* mutation carriers display these similar somatic alterations further confirms that their role in HR-mediated repair is important for tumour suppression.

Loss of the wild-type BRCA allele

When a tumour develops in patients with HBOC syndrome caused by BRCA mutation, loss of the wild-type BRCA allele (LOH) was initially always reported, as would be expected of a tumour suppressor. However, more recently this dogma has been questioned. Initial studies of *Brca1*$^{+/-}$ and *Brca2*$^{+/-}$ mice showed no increase in tumour formation compared with wild-type mice[76], but subsequent studies using *Brca1*$^{+/-}$ *Trp53*$^{+/-}$ mice showed a slight increase in mammary carcinoma incidence compared with *Trp53*$^{+/-}$ mice[77]. All tumours in this study retained BRCA1 protein expression, which rules out epigenetic silencing. Similar results were also observed in *Brca2*$^{+/-}$ mice: mammary tumours developed in a p53-deficient background[78]; however, in this study, epigenetic silencing cannot be ruled out because levels of BRCA2 protein were not measured. A study of breast cancer tissue samples from patients with HBOC syndrome caused by BRCA mutation showed that out of 18 cases in which LOH was observed, 11 patients showed loss of the mutant rather than the wild-type allele, suggesting that loss of the wild-type BRCA allele is not required for tumorigenesis[79]. By contrast, all ovarian cancer tissue samples tested showed LOH of the wild-type BRCA allele[79]. LOH was also not observed in a small study of pancreatic cancer tissue samples from carriers of the Icelandic founder mutation *BRCA2*-999del5, which produces a truncated form of BRCA2 (REFS 80,81). However, it is important to keep in mind the methodological issues about measuring LOH in microdissected tumour samples by PCR, because a small amount of contaminating normal tissue could produce a wild-type allele. In addition, epigenetic silencing of the wild-type allele, which cannot be detected by PCR, must also be considered. In the absence of LOH, haploinsufficiency of BRCA activity may cause enough genomic instability to promote tumorigenesis. Although some data suggest that loss of the wild-type allele may not be required for all BRCA-associated tumorigenesis, in most cases LOH does occur. However, whether BRCA heterozygosity promotes loss of the wild-type allele or whether loss occurs randomly is still unclear.

BRCA LOH is thought to occur by either deletion or gene conversion. When LOH does occur, a germline mutation in *BRCA1* results in loss of the wild-type *BRCA1* allele but not the wild-type *BRCA2* allele, and vice versa. The similar pattern of LOH observed in patients carrying a *BRCA1* or a *BRCA2* mutation and the fact that only one of the BRCA genes is affected in an individual (and not both genes in one individual) suggests that tumorigenesis

in *BRCA1* and *BRCA2* mutation carriers is primarily caused by functional inactivation of either BRCA protein, and we suggest that it is the deficiency in HR that leads to tumorigenesis (as discussed above).

Loss of p53 expression

p53 plays a vital part in maintaining genomic integrity by regulating the transcription of target genes that are involved in cell cycle arrest, apoptosis and DNA repair[82]. Multiple studies suggest that the loss of p53 cooperates with the loss of BRCA1 or BRCA2 in tumorigenesis[78,83–86]. *TP53* mutations are present in 30–50% of human cancers, and they occur in tumours with *BRCA1* or *BRCA2* mutations with greater frequency than in sporadic tumours with wild-type *BRCA1* and *BRCA2* (REFS 83,87). A susceptibility to develop breast carcinomas is also a clinical feature of Li–Fraumeni syndrome, which is caused by germline mutation of *TP53* and is associated with predisposition to develop several types of cancer. Loss of *Trp53* delays embryonic lethality by 2 to 3 days in *Brca1*$^{-/-}$ or *Brca2*$^{-/-}$ mice[88]. Loss of *Trp53* also partially rescues the embryonic lethality of *Palb2*$^{-/-}$ mice[16,17,69,89]. Presumably, chromosome breaks caused by loss of BRCA function activate p53-dependent checkpoint controls and/or apoptosis to prevent tumour formation. Selective pressures then favour the proliferation of cells with loss of p53 function.

In support of this idea, T cells from T cell lineage-specific BRCA2-deficient mice have an accumulation of chromosome aberrations that result in increased p53-mediated apoptosis[90]. However, p53 mutants (such as T150I, G199R and R202S) that were identified specifically in tumours from *BRCA1* or *BRCA2* mutation carriers retain the transactivation, checkpoint and apoptotic activities of wild-type p53, but they still fail to suppress transformation and exhibit gain of function transforming activity in rat embryo fibroblasts[91,92]. Future studies may reveal p53 functions that are uniquely impaired in BRCA-deficient cells. The rarity of these mutants in human cancer and their multiple occurrences in BRCA-associated breast tumours suggests that these novel p53 mutants are selected for during malignant progression in the unique genetic background of *BRCA1*- or *BRCA2*-mutation-associated tumours. Therefore, the common HR defect in both BRCA1- and BRCA2-deficient cells may be responsible for the selection of these specific p53 mutants.

Loss of ATM or CHK2 function

Consistent with being in the same signalling pathway as p53, loss of *Atm* or *Chek2* also rescues the embryonic lethality of *Brca1* mutant mice and leads to the development of multiple tumours, although at a lower frequency compared to mice with *Brca1* and *Trp53* mutations[93]. In addition, ATM expression can be aberrantly reduced or lost in tumours expressing BRCA1 or BRCA2 mutants compared with sporadic tumours without *BRCA1* or *BRCA2* mutations[94]. These data suggest that the genomic instability caused by heterozygous BRCA mutations may lead to the selection of ATM-deficient cells. Although ATM, BRCA1 and BRCA2 are in a common signalling pathway, additional loss of ATM activity may contribute to a selective growth advantage[95]. This apparently paradoxical finding makes sense when one considers that ATM functions in multiple DDR signalling pathways. Alternatively, in genetically unstable BRCA-deficient tumours, random gains and

losses occur across the genome — secondary to genetic instability — and ATM loss could occur secondary to global instability.

Breast and ovarian tissue tropism

Why breast and ovarian cancer?

Given that BRCA1 and BRCA2 protect the genome from errors that arise during DNA replication, it is logical that cells driven to replicate would develop potentially oncogenic genetic alterations in the absence of BRCA1 or BRCA2 function. However, most cancers are driven to grow and divide, so this feature alone does not determine why there is a major predisposition to breast and ovarian cancer in individuals who lack functional BRCA1 or BRCA2. One common feature is that breast and ovarian epithelial cells are subject to strong growth signals by hormonal stimulation during the normal menstrual cycle. The question then becomes: what features of hormonally triggered growth make it vulnerable to genetic instability in the context of BRCA1 or BRCA2 deficiency? A number of theories have surfaced to explain the tissue specificity of HBOC syndrome, but as yet none is definitive.

One hypothesis relates to the connection between BRCA1 function and the regulation of ER signalling[96], whereby BRCA1 represses the transcription of hormone-mediated signalling factors and therefore functions in growth control. Early in BRCA1 research, many transcriptional effects of BRCA1 were described, including co-activation and co-repression of target genes, but the importance of these effects has lessened over time, suggesting that some of the initial observations were a product of overexpression studies[97]. A recent report has suggested that the effect of BRCA1 is to maintain heterochromatin, and the loss of *Brca1* in mice could be reversed by expressing histone 2A fused to ubiquitin[98]. Some of the reported effects of BRCA1 on transcription may actually be due to effects on chromatin structure. However, these findings do not explain why germline *BRCA2* mutations have the same tissue predisposition.

Our preferred theory, which is still speculative but reflects ongoing work in our laboratory, is that hormonally driven growth during each menstrual cycle produces reactive oxygen species, which cause measurable oxidative DNA damage[99–102]. The consequence of oxidative DNA damage is the production of a subset of lesions that cause DNA replication stress and result in one-ended DSBs or DSGs. In other words, oxidative DNA damage can produce replication stress that demands the use of the BRCA1–BRCA2–HR pathway. This explanation would account for the common features of BRCA1 and BRCA2 predisposing to breast and ovarian cancer.

Subtypes of breast cancer

An additional unsolved mystery is why *BRCA1* mutation carriers develop predominantly (but not exclusively) ER-negative tumours, whereas *BRCA2* mutation does not favour the development of any particular subtype of breast cancer[103] (TABLE 2). However, the breast cancers that arose in Ashkenazi Jewish women with founder mutations in *BRCA1* or *BRCA2* were mostly triple-negative (ER-negative, progesterone receptor (PR)-negative and *ERBB2*-non-amplified)[104], so the association with ER expression and BRCA genes is not

rigid. These observations pose the question of why there should be a difference in the biological subtypes of breast cancer when the two proteins appear to be working in a common pathway of DNA repair (TABLE 3).

One explanation is that there is no difference in the predisposition to genetic alterations between carriers of *BRCA1* or *BRCA2* mutations, but that the true connection to the ER-negative subtype is the occurrence of co-inherited mutations (or even polymorphisms) with the *BRCA1* mutant haplotype. This idea has been studied in some detail, and at present there is no candidate co-inherited mutation or polymorphism to support this hypothesis. A second explanation is that the role of BRCA1 in transcriptional co-activation (or co-repression), which is a function not shared by BRCA2, produces changes in gene expression that are sufficient to change the expression of the ER bio-marker. ER-negative tumours have a characteristic gene expression profile[105], and so it would be intriguing to determine whether the profile of BRCA1-associated ER-negative and sporadic ER-negative tumours is similar. However, for this hypothesis to be correct, a mechanistic connection between BRCA1-dependent transcription and the transcriptional profile of ER-negative tumours should be established. A third explanation is that a different mutational spectrum is induced by *BRCA1* heterozygosity compared with *BRCA2* heterozygosity, which could potentially be due to the differences in DNA repair found with BRCA1 deficiency (which could include defects in SSA and NHEJ). Analyses using aCGH show some similarities between BRCA1- and BRCA2-associated cancers, including large deletions and amplifications[66]. However, some differences are also detectable[66], such as the locus specificity of the alterations. The importance of these alterations in specific loci in terms of how they arise or the resulting cellular consequences is not understood. A final explanation is that the cell-of-origin of ER-negative tumours is more susceptible to alterations in *BRCA1*, whereas *BRCA2* haplo-insufficiency predisposes to loss of the second allele in all cell lineages, which would therefore result in the same biomarker profile as sporadic cancers. All of these explanations need more supportive evidence and should be the focus of future studies into the molecular genetics of breast cancer.

One intriguing clue regarding BRCA mutations and tissue tropism is that male breast cancer, which is almost always ER-positive, is more strongly associated with *BRCA2* mutations[106]. A second clue is that the range of cancer types observed in *BRCA2* mutation carriers is broader (*BRCA2* mutation carriers can develop prostate and pancreatic cancer, among others) than the range observed in *BRCA1* mutation carriers. The mechanistic explanation here could be linked to the cellular stresses to which the epithelial cells are exposed, although why this selects for differences in the repair pathway response to stress is not clear. BRCA2-deficient cells have been reported to be capable of carrying out SSA, whereas BRCA1-deficient cells cannot[36]. However, the role of SSA in the maintenance of genome integrity is not clear. In addition, recent work has suggested that 53BP1 mediates the genetic and chromosome rearrangements specifically in BRCA1-deficient cells, as loss of 53BP1 lessens the severity of the repair defect caused by loss of BRCA1 (but not loss of BRCA2)[35]. In this study, 53BP1 protected DSB ends from 5′-end resection, which initiates HR. If this was the entire explanation for the effect of 53BP1, this protein should also protect against the defective HR seen with BRCA2 defects, but this does not seem to be the case. Instead, the specificity towards BRCA1 would suggest that 53BP1 is protecting the

genome from defective SSA, which could be more important in genome stability than currently considered. In conclusion, although we have stressed the role of BRCA1 and BRCA2 functioning in a common pathway of DNA repair, there may be subtle differences between BRCA1- and BRCA2-deficient cells that account for this curious difference in the range of cancers that develop.

Sporadic breast and ovarian cancers

We and others have reported that HR defects occur in sporadic breast cancers as well as the cancers arising in carriers of *BRCA1* or *BRCA2* mutations[66,67,107]. The extent of this type of DNA repair defect in other types of cancer is not known, as specific assays are needed to detect this phenotype. Our approach has been to test the functional integrity of the HR pathway directly in *ex vivo* human tumour samples by examining the formation of nuclear foci of RAD51 and BRCA1 induced by ionizing radiation[67] (such nuclear foci indicate the recruitment of DDR proteins to a site of DNA damage). Others have suggested that a 'BRCA-like' phenotype exists if tumour cells show the characteristic large-region gains and losses that are also seen in BRCA-deficient tumours (FIG. 4). We have initiated a more comprehensive assessment of the frequency and pathological associations of HR pathway defects both in primary breast tumours and in established breast cancer cell lines. We have found that there is a significant prevalence of HR defects in sporadic breast cancer (S.N.P., unpublished observations). In addition, we have observed the same phenotype in breast cancer cell lines, none of which has known *BRCA1* or *BRCA2* mutations or changes in BRCA1 and BRCA2 expression. The sporadic nature of these tumours with BRCA-like features would make the explanation less likely to be an unknown genetic alteration and more likely to be an epigenetic acquired event in tumorigenesis. However, the implications are profound — the number of patients who might be suitable for therapeutic strategies to target defects in HR would be substantially expanded.

Conclusions and perspectives

BRCA1 and BRCA2 function in the DDR during S and G2 phase by mediating HR to maintain replication fidelity. The loss of BRCA1 or BRCA2 function in normal cells results in growth defects, which are required, in combination with the subsequent loss of other DDR mediators, for tumour development. The genetic instability resulting from these growth defects and the loss of DDR mediators leads to multiple genetic gains and losses, but understanding which are the crucial secondary targets and which are nonspecific changes is one of the key research challenges in understanding the aetiology of cancers associated with BRCA loss. The observations that defects in HR can be acquired rather than inherited support the view that genetic instability provides a selective advantage to breast cancer cells. Both mechanisms result in genetic instability, which is perhaps triggered by oxidative metabolism producing replication stress. Therefore, the number of BRCA1–BRCA2–HR pathway-defective breast cancers may be 4 to 5 times greater than originally thought, making the number of patients who are amenable to DNA repair targeting strategies much higher than previously estimated.

Acknowledgments

The authors are supported by grants from the US National Cancer Institute and the Susan G. Komen For The Cure foundation.

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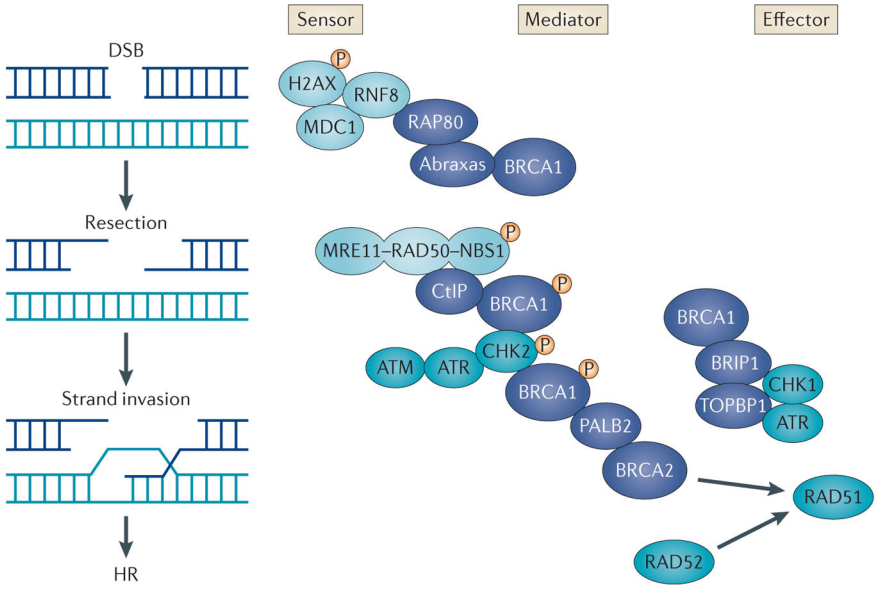

Figure 1. Molecular mechanisms of the DNA damage response

In response to DNA double-strand breaks (DSBs) or replication fork collapse (not shown), sensors (light blue) detect the damage, and signalling mediators recruit or activate effectors that repair the damage and activate cell cycle checkpoints. BRCA1-containing macro-complexes (dark blue) are crucial mediators of the DNA damage response. The BRCA1–abraxas–RAP80 complex associates with ubiquitylated histones near the sites of DNA damage; this is dependent on phosphorylation of histone H2AX (γH2AX), mediator of DNA damage checkpoint protein 1 (MDC1) and RING finger protein 8 (RNF8). The BRCA1–CtBP-interacting protein (CtIP) complex associates with the MRN complex (which is comprised of MRE11, RAD50 and Nijmegen breakage syndrome protein 1 (NBS1)), which senses DSBs and is responsible for DSB resection. The BRCA1–partner and localizer of BRCA2 (PALB2)–BRCA2 complex is important in mediating RAD51-dependent homologous recombination (HR). CHK2-dependent phosphorylation of S988 in BRCA1 appears to be required for the BRCA1–PALB2–BRCA2 effector complex, which is important in RAD51-mediated HR. The BRCA1–BRCA1-interacting protein C-terminal helicase 1 (BRIP1)–DNA topoisomerase 2-binding protein 1 (TOPBP1) complex is associated with DNA repair during replication and may help mediate ataxia telangiectasia and Rad3-related (ATR)–CHK1 signalling, but its precise function is unknown. DNA damage is also recognized by ataxia-telangiectasia mutated (ATM) and ATR kinases, which phosphorylate BRCA1, BRCA1-associated proteins and p53 and mediate signalling to form macro-complexes and activate cell cycle checkpoints.

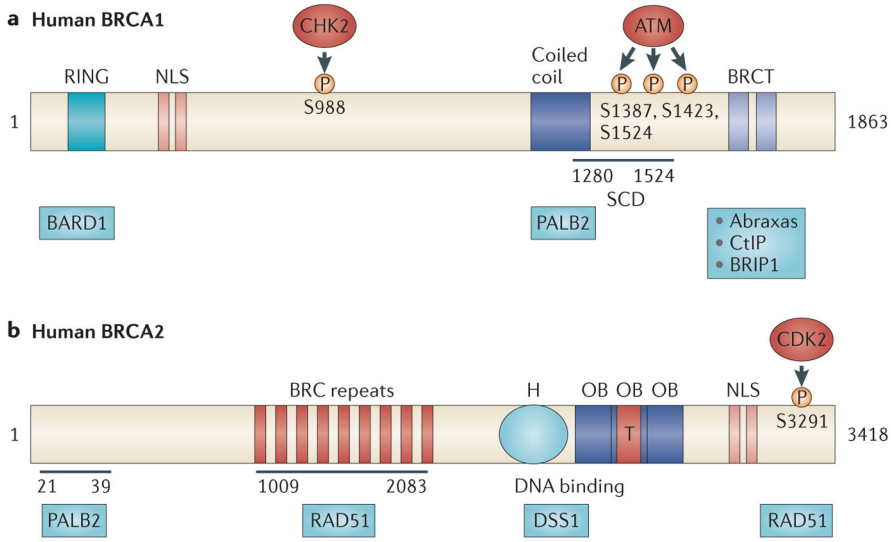

Figure 2. BRCA1 and BRCA2 functional domains

a | The BRCA1 amino terminus contains a RING domain that associates with BRCA1-associated RING domain protein 1 (BARD1) and a nuclear localization sequence (NLS). The central region of BRCA1 contains a CHK2 phosphorylation site on S988 (REF. 25). The carboxyl terminus of BRCA1 contains: a coiled-coil domain that associates with partner and localizer of BRCA2 (PALB2); a SQ/TQ cluster domain (SCD) that contains approximately ten potential ataxia-telangiectasia mutated (ATM) phosphorylation sites and spans amino acid residues 1280–1524; and a BRCT domain that binds ATM-phosphorylated abraxas, CtBP-interacting protein (CtIP) and BRCA1-interacting protein C-terminal helicase 1 (BRIP1). The BRCA1–abraxas complex is associated with BRCA1 recruitment to sites of DNA damage[19,20,108,109]. The BRCA1–BRIP1 complex, which also contains DNA topoisomerase 2-binding protein 1 (TOPBP1), is associated with DNA repair during replication[110]. The BRCA1–CtIP complex promotes ataxia-telangiectasia and Rad3-related (ATR) activation and homologous recombination (HR) by associating with the MRN complex (which is comprised of MRE11, RAD50 and Nijmegen breakage syndrome protein 1 (NBS1)) and facilitating DNA double-strand break resection[22]. The central region of BRCA1, which contains the SCD, is phosphorylated by ATM. This phosphorylation is important for BRCA1-mediated G2/M and S-phase checkpoint activation, as expression of a BRCA1 mutant that lacks three of the phosphorylation sites (S1387, S1423 and S1524) fails to rescue defective checkpoint activation and ionizing radiation hypersensitivity in a BRCA1-deficient cell line[111,112]. **b** | The N terminus of BRCA2 binds PALB2 at amino acids 21–39 (REF. 68). BRCA2 contains eight BRC repeats between amino acid residues 1009 and 2083 that bind RAD51. The BRCA2 DNA-binding domain contains a helical domain (H), three oligonucleotide binding (OB) folds and a tower domain (T), which may facilitate BRCA2 binding to both single-stranded DNA and double-stranded DNA[46]. This region also associates with deleted in split-hand/split-foot syndrome (DSS1)[42,44,45]. The C terminus of BRCA2 contains an NLS and a cyclin-dependent kinase (CDK) phosphorylation site at S3291 that also binds RAD51 (REF. 53).

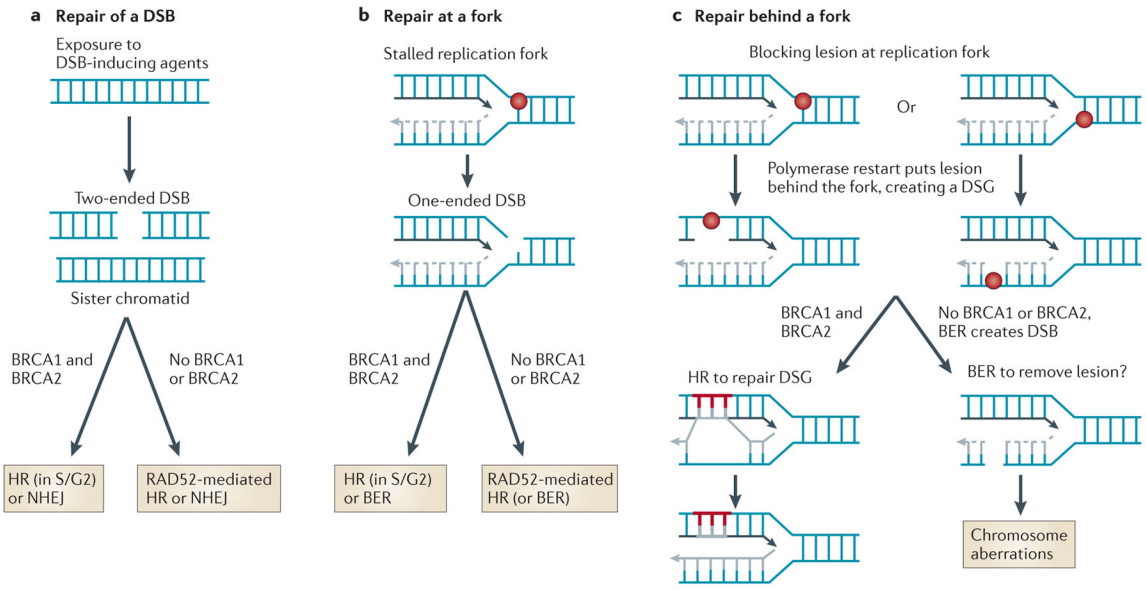

Figure 3. Homologous recombination at different types of DNA damage

Exogenous agents produce DNA double-strand breaks (DSBs) with two ends (**a**), whereas during replication, blocking lesions on the template strand can produce either one-ended DSBs (**b**) or daughter-strand gaps (DSGs) (**c**), both of which are preferentially repaired in the S and G2 phases of the cell cycle by the BRCA1–BRCA2-mediated homologous recombination (HR) pathway. During replication, template strand lesions may be repaired either behind the fork or at the fork. Gaps associated with lesions on the parental strand behind the fork cannot be removed by base excision repair (BER) because an undamaged template is needed for repair; therefore, HR is the only available pathway for the repair of DSGs. Cells lacking functional BRCA1 or BRCA2 exhibit an abundance of chromatid breaks, which indicates an attempt to repair DSGs in the absence of a functional BRCA-mediated HR pathway. DSBs that remain unrepaired behind a replication fork can also produce chromatid breaks or aberrant junctions or exchanges. Hence, BRCA1 and BRCA2 have crucial roles in the repair of replication-associated lesions at or behind the replication fork. NHEJ, non-homologous end-joining.

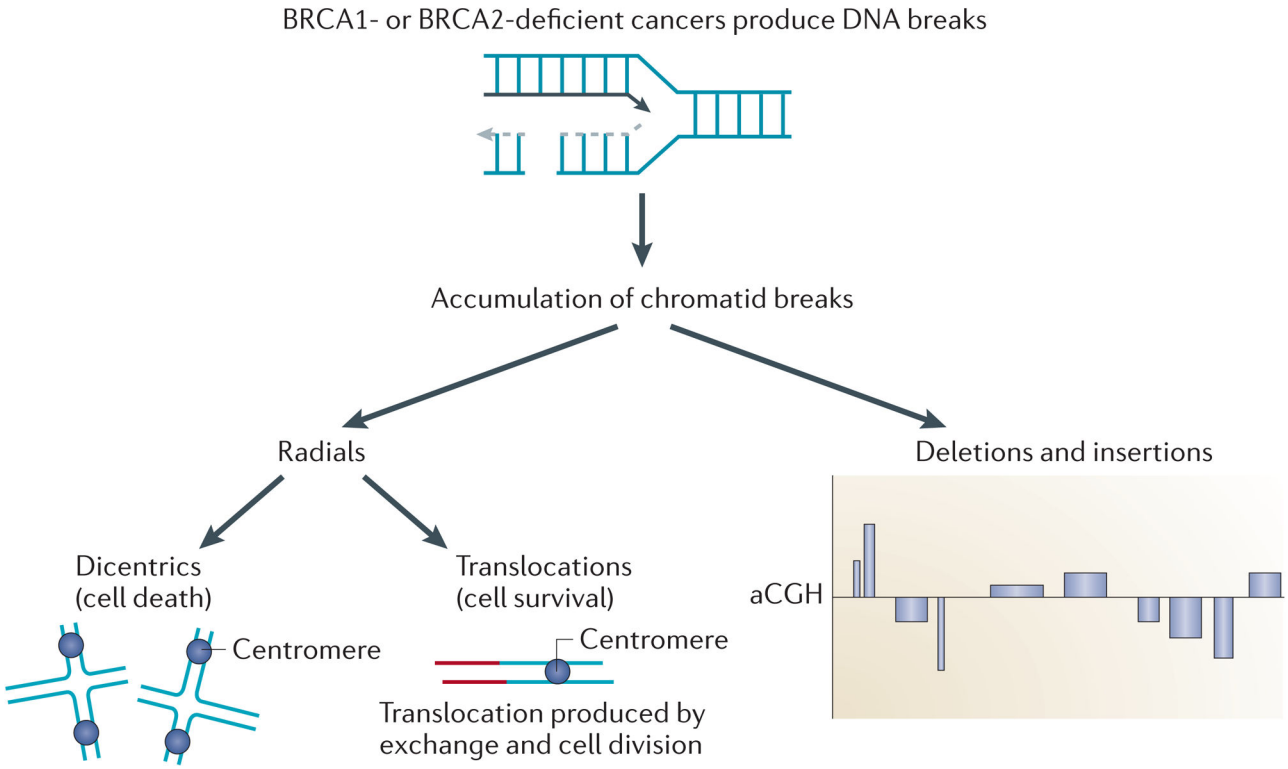

Figure 4. BRCA-deficient cells accumulate chromatid breaks and chromatid exchanges

In the absence of BRCA1 or BRCA2 function, chromatid breaks accumulate, resulting in aberrant chromatid exchanges or other processes involving illegitimate end-joining. If two chromatid breaks are joined to produce a chromosome structure containing two centromeres, a dicentric quadri-radial chromosome is formed, which leads to cell death at mitosis. If an exchange is made with a chromatid fragment without a centromere, processing and cell division can produce a viable cell with a translocation. All of the hallmarks of BRCA-deficient cancers can be explained by the production of chromatid breaks and illegitimate end-joining. Without exchange events between different chromosomes, interstitial deletions, terminal deletions and insertions of chromosome fragments can originate from the chromatid break. In the absence of homologous recombination, the resulting phenotypes can be seen either by spectral karyotyping or by array-comparative genomic hybridization (aCGH), which detects large losses and gains across the genome.

Table 1

BRCA1 and BRCA2 functions: their domains and binding partners

Function	Domain	Direct binding	Indirect binding	Refs
BRCA1				
Recruitment to DNA damage sites	BRCT	Abraxas	RAP80	19,20, 108,109
DNA end resection	BRCT and RING?	CtIP	MRN complex	13,14,22
G2/M checkpoint	BRCT	Abraxas	RAP80	20,21
	BRCT	CtIP	MRN complex	13
	SCD (S1423 and S1524 phosphorylation)	ATM	MRN complex	111
S-phase checkpoint	SCD (S1387 phosphorylation)	ATM	MRN complex	112
	BRCT	BRIP1	TOPBP1	40
Repair during DNA replication	BRCT	BRIP1	TOPBP1	110
HR	Coiled-coil and S988 phosphorylation	PALB2	BRCA2	16–18,25
BRCA2				
HR	BRC	RAD51		46,50,52
	DBD	DSS1		43–46
	N terminus	PALB2	BRCA1	16–18,25
	C terminus	RAD51	CDK2	53

ATM, ataxia-telangiectasia mutated; BRIP1, BRCA1-interacting protein C-terminal helicase 1; CDK2, cyclin-dependent kinase 2; CtIP, CtBP-interacting protein; DBD, DNA-binding domain; DSS1, deleted in split-hand/split-foot syndrome; HR, homologous recombination; MRN, MRE11, RAD50 and Nijmegen breakage syndrome protein 1 (NBS1); PALB2, partner and localizer of BRCA2; SCD, SQ/TQ cluster domain; TOPBP1, DNA topoisomerase 2-binding protein 1.

Table 2

Human cancers arising in *BRCA1* or *BRCA2* mutation carriers

Cancer type	*BRCA1* mutations	*BRCA2* mutations	Notes
Breast	70–80% lifetime risk	50–60% lifetime risk	Breast and ovarian cancer is the dominant cancer predisposition in *BRCA1* and *BRCA2* mutation carriers. *BRCA1* mutation carriers develop breast and ovarian cancer at a younger age than *BRCA2* mutation carriers[113]
Ovarian	50% lifetime risk	30% lifetime risk	Breast and ovarian cancer is the dominant cancer predisposition in *BRCA1* and *BRCA2* mutation carriers. LOH of the wild-type BRCA allele is always found
Prostate	Ashkenazi Jewish founder mutations are associated with increased risk	20-fold increased risk	<1% of *BRCA2* mutation carriers have prostate cancer. Prostate cancer is even rarer in *BRCA1* mutation carriers, except in members of the Ashkenazi Jewish population with *BRCA1* mutations
Pancreatic	Anecdotal evidence and case reports only	Tenfold increased risk	<1% of *BRCA2* mutation carriers have pancreatic cancer. No incidence has been clearly documented in *BRCA1* mutation carriers
Gastric	None reported	Limited reports	It is unclear whether stomach cancer is associated with *BRCA2* mutations
Others	None reported	Brain, medulloblastoma, pharyngeal, CLL and AML	Fanconi anaemia subtype D1 (caused by *BRCA2* mutations) is associated with cancer of the central nervous system
Fallopian tube	Observed, but rare	Rare	This cancer type is like ovarian cancer, but it is a rare cancer overall and is still uncommon in BRCA mutation carriers

AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; LOH, loss of heterozygosity.

Table 3

Characteristics of *BRCA1*- and *BRCA2*-mutation-associated breast cancers

Phenotype	*BRCA1*	*BRCA2*	Notes
ER expression	Negative in 80–90%	Positive in 60–65%	One of the major mysteries to be solved
PR expression	Predominantly negative	Positive in the majority of cases	Less complete data relative to ER expression
ERBB2 amplification	Usually absent	~15% have amplification	*ERBB2* amplification can occur in BRCA mutation carriers
Early onset	Highly prevalent between 30 and 50 years of age	Less prevalent between 40 and 70 years of age	
Lobular cancers	Less likely	As frequent as in sporadic breast cancer (~15%)	
High grade	Likely	Common	More common than sporadic cancers
Basal markers	Frequent	Less common	Tumours have cytokeratin profile of basal or myoepithelial markers
HR function	Defective	Defective	Some debate over the frequency of LOH for the wild-type allele
Prognosis relative to sporadic cancer at the same stage	No difference overall. Local recurrence in the breast is increased with conservative surgery and radiation therapy	No difference	

ER, oestrogen receptor; HR, homologous recombination; LOH, loss of heterozygosity; PR, progesterone receptor.