The Role of BRCA1 and BRCA2 in Anticancer Drug Therapy

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1 Introduction

The genome is under constant assault from both endogenous and exogenous sources such as reactive oxygen species and ionizing radiation capable of inducing a wide array of mutagenic changes [1]. To maintain genomic integrity cells have evolved elegant mechanisms to recognize DNA damage, arrest the cell cycle, and activate specific repair pathways. One of the most cytotoxic lesions that a cell must contend with is a double-strand break (DSB) because even a single unrepaired DSB is capable of inducing cell death [2]. To repair a DSB, cells have at least four mechanisms at their disposal: homologous recombination (HR), single-strand annealing (SSA), nonhomologous end-joining (NHEJ), and microhomology-mediated end joining (MMEJ) (Fig. 1) [3]. HR relies on the sister chromatid as a template to fill in damaged or missing DNA, restoring the chromosome to its original condition. In cells with competent DNA repair mechanisms, HR is the preferred pathway of repair

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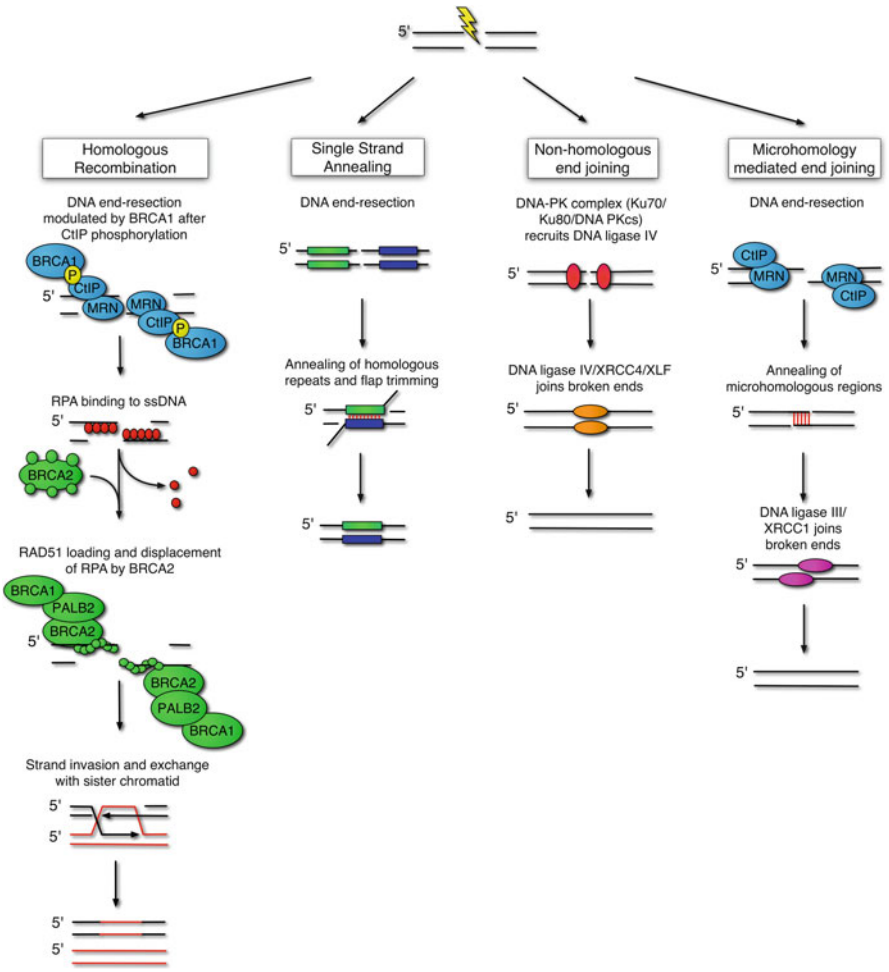

Fig. 1 Schema describing DNA repair pathways following a double-strand break (DSB). Homologous recombination is the preferred pathway during S and G2 phases of the cell cycle and is considered an error-free pathway. NHEJ, MMEJ, and SSA, on the other hand, are thought to be error-prone pathways because they introduce deletions at broken-ends and may promiscuously ligate nonadjacent ends creating gross chromosomal aberrations. *XRCC1/4* X-ray repair complementing defective repair in Chinese hamster cells 1/4. *DNA-PK* DNA dependent protein kinase catalytic subunit. *MRN* Mre11-Rad50-Nbs1

during the S and G2 phase of the cell cycle when the sister chromatid is available [4]. SSA, a variant of HR that is thought to play a minor role in the repair DSBs, utilizes homologous repeats surrounding a DSB to anneal the broken ends resulting in the deletion of the intervening sequence. In contrast, NHEJ and MMEJ both operate throughout the cell cycle and directly ligate two ends of a DSB; however, MMEJ

always introduces small deletions at broken ends to produce a region of microhomology to facilitate ligation [5]. The important point to note is that HR is considered an error-free pathway whereas SSA, NHEJ, and MMEJ are error-prone because they can create gross chromosomal aberrations if ligation occurs incorrectly—potentially leading to neoplastic transformation [1].

BRCA1 and BRCA2 are tumor suppressors essential for the faithful repair of DSBs by HR [6]. However, BRCA1 also participates in other cellular functions important in maintaining genomic integrity including the assembly of the mitotic spindle [7], centrosome duplication [8], cell-cycle control [9–14], chromatin remodeling at sites of DSBs [15, 16], and DNA decantenation [17]. In contrast, the role of BRCA2 is primarily to regulate RAD51 filament formation, which is a critical step in catalyzing strand invasion and homologous recombination (Fig. 1).

Cells lacking BRCA1 or BRCA2 are unable to repair DSBs by HR and must resort to more error-prone pathways such as MMEJ and SSA. These cells display gross chromosomal rearrangements such as large deletions, translocations, and fusions during successive rounds of cell division [18]. While the vast majority of these lesions result in cell death, the genetic instability caused by loss of competent HR leads to a dramatically increased number of genetic alterations, which provide a rich background for Darwinian forces to act at the level of the tumor microenvironment, promoting the emergence of multiple clones, some of which have the capability to divide autonomously and metastasize [19]. The importance of BRCA genes in maintaining genomic integrity is underscored by patients who harbor germline mutations in *BRCA1* or *BRCA2* and have a markedly increased predisposition to develop, among others, breast and ovarian cancers [20].

Since the discovery of *BRCA1* and *BRCA2* more than 15 years ago [21, 22], understanding their function has been of primary importance and much progress has been made. In this review, we summarize the role BRCA1 and BRCA2 play in homologous recombination and how this knowledge can be utilized to target tumors deficient in this cellular pathway in hereditary as well as sporadic cancers.

2 Structure and Function of BRCA1

BRCA1 is composed of 1,863 amino acids and contains three functionally important domains (Fig. 2). At its amino terminal is a RING-finger domain with E3 ubiquitin ligase activity (Box 1). It is normally found in association with its heterodimeric protein partner BARD1 (which is itself a RING E3 ubiquitin ligase). This interaction stabilizes the complex, preventing its degradation [23] and enhances its E3 ligase function [24]. In addition, the ubiquitin ligase activity of BRCA1 is activated upon two post-modificational processes: auto-ubiquitination [25] and SUMOylation [26, 27]. It is not yet clear how the ubiquitin ligase activity of BRCA1 is increased; however, two possible scenarios can be envisaged. One is that ubiquitination or SUMOylation directly alters the conformation of the RING-finger domain increasing enzymatic activity. A second possibility could be that posttranslational

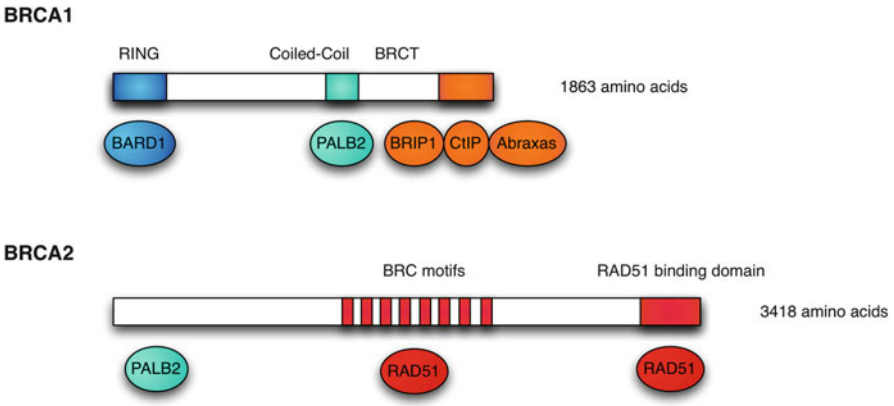

Fig. 2 Functional domains and interacting partners of human BRCA1 and BRCA2 proteins. Only domains (*listed above*) and protein partners (*drawn below*) that were discussed in this review are described. Proteins are color-coded with its corresponding interacting domain

modifications increase affinity for the E2 conjugating enzyme UbcH5a, accelerating ubiquitin transfer. BRCA1 has been shown to ubiquitinate various proteins including histones (H2A, H2AX, and H2B) [25, 28], CtIP [29], γ-tubulin [8], nucleophosmin [30], RNA polymerase II [31, 32], and ERα [33]. How ubiquitination of these target proteins modifies their function is unclear; however, germ-line mutations derived from patients with breast cancer that abolish RING finger ligase activity are observed to result in checkpoint deregulation and sensitivity to ionizing radiation [34, 35]. Strikingly these effects are independent of homologous recombination [36]. To reconcile this apparent paradox, Zhu et al. propose that BRCA1 acts in vivo to regulate expression of satellite DNA that is normally silenced by ubiquitination of H2A and that overexpression of satellite transcripts is linked to genomic instability [28]. However, the function of satellite transcripts and how its aberrant expression leads to tumor development are currently unknown.

At the carboxyl end of BRCA1 are tandem BRCT domains which contain a phosphate binding core providing an interface for phosphorylated proteins [37, 38]. Phosphorylation, mediated primarily by the kinases ATM and CHEK2, is an important spatiotemporal regulator of proteins involved in check-point control and DNA repair. The tandem BRCT domain of BRCA1 helps localize it to nuclear foci by binding to different phosphorylated intermediates including Abraxas [10, 14, 39], CtIP [40], and BRIP1 [13]. These protein complexes form three distinct entities during HR and each has important functions at sites of DNA breakage. For instance BRCA1 in association with Abraxas and RAP80 has been shown to regulate the G2-M checkpoint. When BRCA1 is bound to CtIP coupled with MRN, however, it regulates DNA end-resection diverting the pathway away from MMEJ towards HR (Fig. 2) [41].

BRCA1 also plays a more central role in HR. BRCA1 contains a coiled-coil domain present near the carboxyl terminal which binds PALB2 (Partner and Localizer of BRCA2) [42, 43]. PABL2 physically bridges BRCA1 to BRCA2. This complex in turn mediates the final enzymatic step of RAD51 assembly, and strand exchange between homologous chromosomes (described below).

Box 1

Ubiquitination is a posttranslational modification in which ubiquitin, a small peptide molecule of 76 amino acids, covalently tags larger proteins. This process requires the sequential coupling of three enzymatic reactions: an E1 activating enzyme, an E2 conjugating enzyme and an E3 ligase. The unique combination of a diverse array of E2 and E3 enzymes allows specific proteins to be targeted for ubiquitination. In a similar process, SUMOylation involves the tagging of larger proteins by a small ubiquitin-like modifier (SUMO) using a different but parallel enzymatic cascade consisting of E1, E2 and E3 enzymes. Modification of a protein by ubiquitin or SUMO can alter its conformation or modify its surface to allow or prohibit protein interactions.

3 Structure and Function of BRCA2

Although bearing similar names, BRCA2 is structurally and functionally distinct from BRCA1. It is a much larger (3,418 amino acids) protein containing eight BRC motifs, which enable binding to RAD51 [44, 45] and another distinct RAD51-binding domain at its terminal end [46, 47] (Fig. 1). Full-length human BRCA2 had never been purified to sufficient quantities due to its large molecular size. As such, its functions could only be derived from studying BRCA2 orthologues and smaller protein fragments. Recently, however, three teams using different approaches have managed to obtain purified full-length human BRCA2, providing an unprecedented in vitro analysis of its molecular functions [48–50]. All three papers were able to demonstrate that BRCA2 mediates loading of RAD51 onto ssDNA while displacing RPA (a protein that binds ssDNA preventing secondary DNA structures from forming). In addition, Jensen et al. and Thorslund et al. show that BRCA2 prevents RAD51 association to dsDNA, which would inhibit HR, and favors RAD51 association to ssDNA or dsDNA with ssDNA tails. Jensen et al. and Liu et al. demonstrate that BRCA2 inhibits RAD51 hydrolysis of ATP, which stabilizes the nucleoprotein filament. Much more is still to be learned about BRCA2. For example, there is direct evidence to demonstrate that, despite being evolutionary conserved domains, not all BRC motifs are required for competent HR to be elicited in the presence of DNA DSBs, suggesting that they may have alternate or modulatory roles in HR [51]. Understanding how its interacting partners—such as PALB2 and BRCA1—affect BRCA2 function is also unclear. With full-length BRCA2 at hand characterization of its complex molecular functions will be more readily answered.

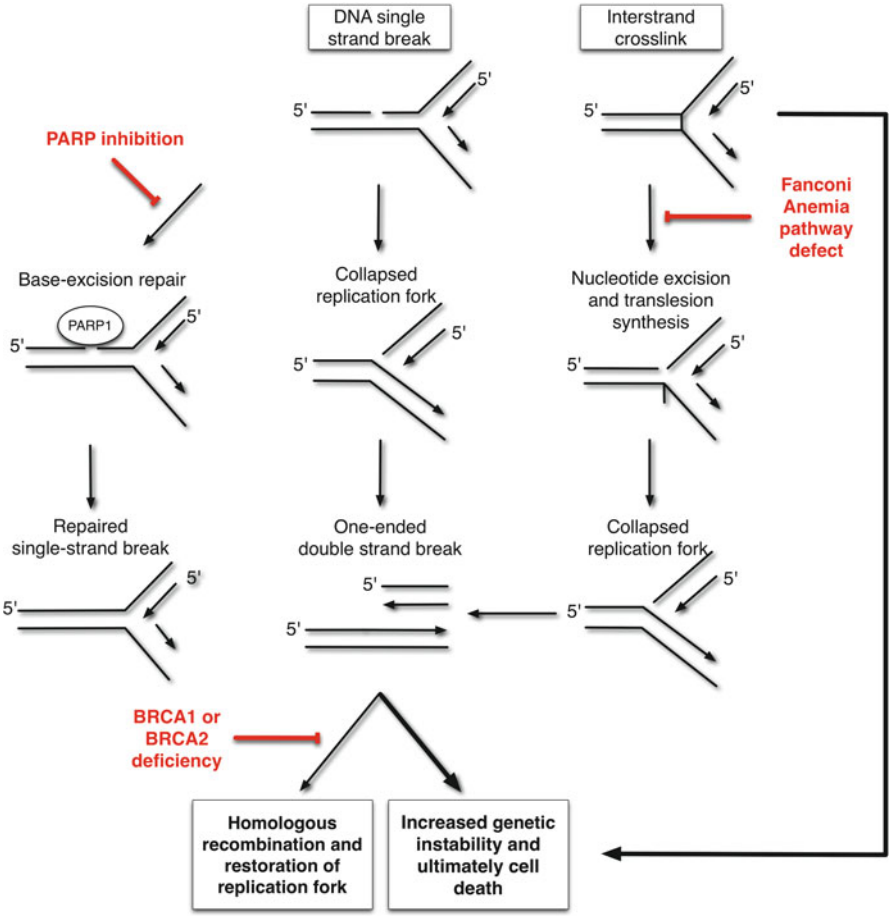

Fig. 3 Schema describing DNA repair pathways following a single-strand break and interstrand cross-link (ICL). A single-strand break is normally repaired by base-excision repair (BER). If PARP is inhibited, however, BER is defective and a double-strand break is induced during the S phase of the cell cycle requiring homologous recombination (HR) to mediate repair and regenerate the replication fork. Tumor cells unable to properly repair DNA damage by both BER and HR, resort to more error prone mechanisms such as NHEJ, MMEJ and SSA, which induces genomic instability and ultimately cell death. Interstrand cross-links require both intact Fanconi anemia (FA) and HR pathways to mediate its repair. A defect in any one of these pathways leads to chromosome breakage and cell death

4 Fanconi Anemia and Homologous Repair

An interstrand cross-link (ICL) is another highly cytotoxic lesion that prevents separation of complementary strands of DNA during replication. A specialized pathway is necessary to recognize and remove a cross-link but in so doing, a DSB is generated, requiring the HR machinery to complete the repair (Fig. 3) [52].

Patients with defects in ICL repair develop a rare genetic condition known as Fanconi anemia (FA), characterized by aplastic anemia, multiple congenital defects, susceptibility to both hematologic and solid malignancies, and sensitivity to ICL agents such as platinum drugs and mitomycin C. It is a heterogeneous disease caused by defects (either by recessive or X-linked mutations) in 1 of 13 genes, three of which—BRIP1, PALB2, and BRCA2—are also proteins involved in HR, providing further evidence that these two pathways are closely interrelated. See chapter "Repair of DNA Interstrand Cross-links Produced by Cancer Chemotherapeutic Drugs" for a detailed review of ICL repair.

5 Targeting BRCA1 and BRCA2-Mutated Tumors

Nearly all ovarian carcinomas and most breast cancers derived from patients with germ-line *BRCA1* and *BRCA2* mutations have lost their remaining wild-type allele and thus the ability to repair DSBs by HR [53, 54]. These cancers instead rely on complementary pathways such as NHEJ, to maintain some degree of genomic stability. By contrast, "healthy" cells with only one functional BRCA gene still have an intact HR pathway, a biological difference that can be exploited. In cells with a defective HR pathway, agents that introduce ICLs, such as mitomycin C and platinum drugs, are not effectively repaired and induce cell death, while those still capable of repairing DSBs by HR are relatively spared. Early data from platinum-based regimens on carriers of BRCA1 mutations have suggested some efficacy in treating breast cancer in the neoadjuvant setting [55]; however, stronger data will be needed before its clinical use in treating BRCA-associated cancers can be routinely proposed [56]. Anthracyclines, which intercalate DNA causing DSBs, may also be effective [57, 58]; however, reports from cell and clinical data are conflicting [55, 59]. Based on in vitro data, taxanes (traditionally used in the treatment of sporadic breast and ovarian cancer) may be of lesser benefit in hereditary cancers, at least for those lacking BRCA1 [60, 61]. Nevertheless, available clinical data do not support this view.

A novel class of drugs called PARP (Poly ADP-ribose polymerase) inhibitors was developed to target cells deficient in HR pathways [62, 63]. By inhibiting PARP, base-excision repair (BER) is impaired leading to the accumulation of unrepaired single-strand breaks, which during S phase lead to stalling and/or collapse of replication forks, and eventually degenerate into DSBs (Fig. 3). While normal cells have the capacity to compensate for PARP inhibitor-mediated loss of BER via HR, cells without the means to repair the damage by HR have to resort to error prone mechanisms (i.e., SSA, NHEJ, and MMEJ) to repair the DNA DSBs. These observations have led to the development of synthetic lethal approaches to target BRCA1 and BRCA2 deficient cancers [62, 63]. An extended phase I study [64] and two phase 2 clinical trials in *BRCA1* and *BRCA2* carriers have shown promise in the treatment of both metastatic breast [65] and ovarian cancers [66]. Further clinical trials are underway to evaluate whether PARP inhibitors act synergistically in combination with other chemotherapeutic agents such as cisplatin.

A caveat to cisplatin and PARP therapy is the cancer's inevitable progression towards drug resistance. Studies have described the mechanism of drug resistance in *BRCA1*- and *BRCA2*-mutant tumors as intragenic deletions and secondary mutations induced by error-prone repair pathways such as NHEJ and SSA that restore an open-reading frame resulting in the expression of a functional BRCA1 or BRCA2 protein [51, 67–69]. It would seem that while loss of BRCA1 or BRCA2 is advantageous early in the progression of tumor development, the presence of BRCA1 or BRCA2 in its later stages may have little if any effect on tumor viability. In addition, it is thought that mutations are stochastic events and therefore the larger the tumor population the greater the likelihood that a revertant mutation will arise. Taken together, this would suggest that treatment with cisplatin or PARP inhibitors in the very early stages of cancer would have the greatest chance of eliminating disease, while treatment beyond a certain stage of development will likely end in relapse.

6 Targeting Sporadic Cancers Lacking Homologous Recombination

Do sporadic cancers harbor defects in HR and FA pathways, and if they do, would targeting them with cisplatin and PARP inhibitors be effective? A logical first step in answering this question would be to determine whether BRCA1 and BRCA2 are mutated in sporadic cases. About 20% of high grade serous ovarian carcinomas [70] and a similar percentage in triple-negative breast cancers (TNBC) [71] have germ line or somatic mutations in BRCA1/2; however, BRCA1/2 are also found to be down-regulated by other means such as epigenetic silencing [72–76] and transcriptional repression [77, 78]. In the latter example, the hypothesized role of *EMSY* amplification and BRCA2 suppression has been called into question as it appears that EMSY amplification in cancer cell lines is not associated with impaired HR function or increased sensitivity to cisplatin or PARP inhibition [79]. It has been previously suggested that the consequences of early BRCA1 deficiency dictate tumor lineage and phenotype [80] and that cell phenotype or "BRCAness" may be used as a surrogate marker for an underlying *BRCA1* mutation [81]. Cells with a BRCA2 deficiency, however, seem not to follow a particular lineage, which is reflected by a lack of an association for *BRCA2*-associated tumors to a histopathologic phenotype that distinguishes them from sporadic cancers.

BRCA1 deficient breast cancers are characteristically "triple-negative" meaning they lack estrogen and progesterone receptors and do not over-express HER2 [82]; a tendency that could be explained by a haploinsufficiency of BRCA1 leading to a failure of luminal-progenitor cells to differentiate [83] thus creating a comparatively larger pool of basal-like stem cells that have the potential to give rise to a triple-negative phenotype [84, 85]. The unique biology of BRCA1 may underlie the phenotype seen in sporadic TNBCs providing the rational for clinical trials targeting TNBC with cisplatin [86] and PARP inhibitors [87]. However, promising results in a phase 2 trial for the novel therapeutic drug iniparib [87], a previously ascribed PARP inhibitor,

failed to meet clinical outcomes in a subsequent phase 3 trial (http://en.sanofi-aventis.com/research_innovation/rd_key_figures/rd_key_figures.asp). Although the mechanism by which iniparib achieves its antitumor effects is unclear, its failure in the phase 3 trial may be due to the plasticity by which BRCA1 is down-regulated allowing tumor cells to more readily reactivate BRCA1 function leading to earlier resistance. Another possibility could be because TNBC is a convergent phenotype of a heterogeneous disease with only a small subgroup having an underlying BRCA1 defect. More reliable methods at predicting HR and FA function are being sought such as gene expression profiling [88] and radiation-induced RAD51 foci formation [89]; however, it is expected that next-generation sequencing technologies may ultimately prove to be the "gold standard" in the prediction of the ability to repair DNA. A genomic landscape not only characterizes all the mutations found within HR and FA related genes, but also describes the genetic signature of HR dysfunction. A comprehensive understanding of tumor biology however will rely on more than just genomic data. As a testament to the rapid advances made in sequencing technology and bioinformatics, a recent paper demonstrated the monumental task of analyzing 466 tumors across different platforms, integrating copy number variation, exomic, epigenomic, transcriptomic and proteomic data, providing a comprehensive understanding cancer drivers and drugable targets for the major breast cancer subtypes [90].

7 Conclusion

Studying the molecular pathways underlying hereditary breast and ovarian cancers has elucidated the processes that drive tumor progression, processes that are also common to sporadic cancers. Novel therapies are available to target cells defective in HR and FA pathways; however, determining which tumors have an underlying HR and FA defect is complex with no single method capable of providing a complete picture. As we begin to enter the genomic age, next-generation sequencing should allow full molecular characterization of cancer architecture and function including the tumor's ability to respond to DNA damage—setting the stage for personalized medicine.

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