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BRCA1 mutations in cancer: coordinating deficiencies in homologous recombination with tumorigenesis

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Abstract

Cancers that arise from *BRCA1* germline mutations are deficient for homologous recombination (HR) DNA repair and are sensitive to DNA damaging agents such as platinum and PARP inhibitors (PARPi). In vertebrate organisms, knockout of critical HR genes including *BRCA1* and *BRCA2* is lethal because HR is required for genome replication. Thus, cancers must develop strategies to cope with loss of HR activity. Furthermore, as established tumors respond to chemotherapy selection pressure, additional genetic adaptations transition cancers to an HR-proficient state. In this review, we discuss biological mechanisms that influence the ability of *BRCA1*-mutant cancers to perform HR. Furthermore, we consider how the HR status fluctuates throughout the cancer life course, from tumor initiation to the development of therapy refractory disease.

Keywords

BRCA1; BRCA2; RNF168; homologous recombination; breast cancer; ovarian cancer; DNA damage; DNA repair; 53BP1; RNF8

Introduction

BRCA1/2 gene mutations account for the majority of hereditary forms of breast and ovarian cancer (1). It is broadly accepted that both BRCA1 and BRCA2 proteins suppress the formation of tumors by facilitating homologous recombination (HR) DNA repair, which in turn, ensures genomic stability (2–4). Breaks frequently arise during DNA replication, and HR is required for DNA replication fork restart (5). Because HR is critical for genome duplication, it is obligatory for the viability of vertebrate organisms. Indeed, genetic disruption of critical HR factors, including *Brca1, Brca2* and *Rad51*, can induce early embryonic lethality in mice (6–12). In striking contrast, *BRCA1/2* mutant cancers are highly proliferative and malignant, despite being defective for HR. This apparent paradox raises several questions; including, why are cancers, but not normal cells, able to thrive without HR? Alternatively, do *BRCA1/2* mutant cancers retain residual HR activity? And, what are the biological mechanisms that provide HR in the absence of BRCA proteins?

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Conflicts disclosure

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The HR status of tumors is highly consequential for cancer patients. In particular, PARP inhibitors (PARPi) and platinum generate DNA damage during replication, which is usually repaired by the HR machinery (13–16). Patients with *BRCA1/2* mutation-containing tumors gain most benefit from PARPi therapy. In a portion of patients, PARPi provides long-term anti-tumor activity. However, in other cases, PARPi has minimal activity, despite the presence of *BRCA1/2* mutations and presumed HR-deficiency. Moreover, the majority of tumors are initially responsive, but then develop resistance (17–24). Often, cancers that are HR-deficient go on to acquire molecular adaptations that transition them to an HR-proficient state (25,26). The paradigm that *BRCA1* mutations are equal to HR deficiency may be over simplistic, and *BRCA1* mutation-containing cancers likely adapt strategies to maintain some level of HR. In this review, we focus on the relationships between *BRCA1* mutations, HR and cancer. We explore the concept that HR is not an all-or-nothing occurrence but a spectrum, and that where a tumor stands on this spectrum may have therapeutic relevance.

BRCA1 domains govern function

The human *BRCA1* gene comprises over 81 kilobases of DNA on chromosome 17. The largest transcript encodes an 1863 amino acid (aa) protein with an approximate mass of 220 kDa, referred to as BRCA1 full-length or p220. Within the full-length peptide, there are conserved RING, coiled-coil (CC), and BRCT domains, as well as a central unstructured region encoded by exon 11 that accounts for over half of the total protein (Figure 1A) (27,28). The molecular activity of BRCA1 within HR stems from protein interactions and the formation of distinct multi-protein complexes. BRCA1-BARD1 interact via their respective N-terminal RING domains. The BRCA1 BRCT repeats engender a phosphopeptide binding region that facilitates protein interactions, including CtIP, ABRAXAS, and BACH1. In contrast, the BRCA1 CC domain is only known to form a single direct protein interaction with the CC domain of PALB2 (27). Although the function of exon 11 is not clear, due to its large size, mutations in this region account for nearly 30% of pathogenic *BRCA1* mutations (29).

BRCA1-BARD1 and HR

BRCA1 has several well-defined functions that contribute to the overall proficiency of HR. The BRCA1-BARD1 heterodimer is an E3 ubiquitin ligase, but the importance of this activity in HR is unclear (30–34). BRCA1-BARD1 E3 ligase substrates have been reported to include histone H2A and CtIP (33,34). The $BRCA1^{I26A}$ mutation is not found in cancer patients but disrupts E3 ligase activity, and there are conflicting reports regarding the degree to which this mutation is deleterious, with multiple studies showing little or no impact on HR or DNA damage sensitivity (31,35–37). In contrast, the physical association of BRCA1-BARD1 is crucial for the stability of each protein. The $BRCA1^{C61G}$ missense mutation is found in cancer patients and prevents BARD1 heterodimerization. Consequently, there is decreased expression of both proteins, loss of HR and DNA damage sensitivity (38–43). BRCA1-BARD1 have also been shown to directly interact with, and enhance the recombinase activity of, RAD51 (44).

Another critical function of BARD1 is to localize BRCA1 to sites of DNA damage. The BARD1-BRCT domain binds to poly-ADP ribose (PAR) and targets the heterodimer to double stranded DNA breaks (DSBs) early on in the response to DNA damage (45). However, at later time points, the BARD1-BRCT domain interacts with Lysine 9-dimethylated histone H3 (H3K9me2), which is mediated primarily by HP1γ, and is required for focal accumulation (46). Moreover, a screen identified the BARD1-ANK domain as efficiently binding H4 not methylated at lysine 20 (H4K20me0). BARD1$^{\Delta Ank}$ failed to be recruited to chromatin and resulted in cellular sensitivity to PARPi (37). The BRCA1-BRCT domain also contributes to recruitment to DSBs through an ABRAXAS-RAP80 complex (47–49). The interplay between these various mechanisms of BRCA1-BARD1 recruitment to chromatin and DSB sites is unclear.

BRCA1 and DNA end resection

The degradation of double stranded (ds)DNA to single stranded (ss)DNA overhangs by nucleases is termed DNA end resection and is required to initiate HR. Perhaps the most recognized function of BRCA1 in HR is in counteracting the 53BP1-RIF1-shieldin complex (50,51). A plethora of studies have emerged over recent years that have identified proteins that comprise the 53BP1-RIF1-shieldin-complex, which functions to inhibit DNA end resection. However, the precise biological mechanism by which BRCA1 counters this complex and activates resection is less clear. BRCA1 may physically displace 53BP1 (31,52), or recruit phosphatases that dephosphorylate 53BP1 and result in loss of RIF1 binding (53). Additionally, the exon 11 region and BRCT domain of BRCA1 have independently been shown to prevent RIF1 accumulation at DSBs (54,55). Moreover, the BRCA1-BRCT domain promotes CtIP localization to DSB foci, which in turn stimulates MRE11 nuclease activity. Key studies have shown that the BRCA1-CtIP interaction, although not crucial for end resection to occur, increases the efficiency and speed of resection (56–59).

BRCA1-PALB2 promotes RAD51 loading

Seminal studies published in 2009 uncovered a principal function of BRCA1 within HR. Here, BRCA1 was shown to directly bind to PALB2 via each proteins respective CC domains, resulting in the formation of a macro complex consisting of BRCA1-PALB2-BRCA2-RAD51 (60–62). The latter promotes the loading of RAD51 onto resected ssDNA, generating RAD51 filaments that are primed for strand invasion. Cell lines engineered to express *PALB2* or *BRCA1* mutations that disrupt the CC domains are defective for RAD51 foci formation (60–62). However, there remains debate regarding whether this is a critical interaction and function of BRCA1, or something that can be readily bypassed. Specifically, because 53BP1 knockout (KO) can rescue HR via the restoration of DNA end resection in *BRCA1* mutant cells, the significance of the BRCA1-PALB2 interaction is contentious. Recent studies have addressed this question, demonstrating that BRCA1 hypomorphs that retain the CC domain are required for efficient 53BP1 KO-mediated restoration of RAD51 foci (55,63,64). Other studies have shown that cell lines expected to be BRCA1 null had efficient RAD51 foci in the setting of 53BP1 deficiency (65–67). Therefore, in the context of 53BP1 KO, BRCA1-independent PALB2-BRCA2-RAD51 loading appears to be highly

variable, and potentially cell line-dependent. Moreover, BRCA1-independent PALB2 loading has been demonstrated to occur via mechanisms that depend on ATR and RNF168 activity (68–71). In summary, the BRCA1 protein has a multi-faceted and complex role within HR, with prominent actions in initiating DNA end resection and promoting RAD51 loading.

BRCA1 and replication fork protection

Proteins that are involved in HR are often also required to protect DNA fibers from nuclease mediated degradation in response to DNA replication fork stalling agents such as hydroxyurea (HU). The fork protection (FP) function of BRCA1 and BRCA2 appears to be distinct from their HR activities, with several separation-of-function mutations documented (72–74). For example, Brca1 CC domain mutations are highly disruptive to HR, but do not impact FP (55). Similarly, in mouse cells, Brca1-ΔRING was defective for HR but proficient for FP (70). In contrast, the Brca1-Δ11 protein is defective for both FP and HR (55,70,75). Some studies have shown FP disruptive mutations have minor effects on cell viability, others indicate more substantial effects on viability and response to therapy (74–77). Interestingly, proteins that are defective for fork protection, such as Brca1-ΔRING and Brca1-Δ11, retain the ability to provide residual HR, and can play a role in PARPi resistance upon selection pressure (29,78), although the precise interplay between partial HR and FP has not been addressed.

A requirement for HR in genome replication

HR is required to restart replication forks that pause after encountering spontaneously arising breaks and problematic DNA sequences or structures. While yeast and bacteria can replicate their genomes in the absence of essential HR genes such as *RecA*/*RAD51* and *RAD52*, the same is not true for vertebrate cells (5,79–82). *BRCA1*, *BRCA2* and *RAD51* genetic KO all result in early embryonic lethality in mice (6–12). *BRCA2* deficiency as well as mutations that specifically disrupt the HR activity of the protein were shown to trigger replication stress that is transmitted to the next cell cycle through DNA under replication. The latter caused chromosome segregation and the presence of 53BP1 nuclear bodies in G1 phase human breast epithelial cells, followed by senescence or apoptosis-associated loss of viability (76). Studies using DT40 chicken cells showed that genetic inactivation of *RAD51* caused cells to accumulate in G2/M before dying. Chromosome analyses presented multiple breaks spontaneously occurring in proliferating cells (83). Surprisingly, DT40 cells with *BRCA1* KO cell can proliferate, although at a slower speed, possibly due to RAD51 overexpression (84). In contrast, the human haploid HAP1 cell line is highly dependent on BRCA1 for viability, and a KO-knockin (KI) approach was able to identify potential pathogenic variants based on their ability to rescue cell proliferation (85).

BRCA1 and *TP53* mutations

Given the importance of HR for DNA replication, BRCA1 defective cells and mice are inviable. Thus, cells that undergo transformation require epi/genetic events that permit for loss of BRCA1 to become advantageous and subject to positive selection. The most well-

defined mechanism is the presence of *TP53* mutations, which are invariably detected in *BRCA1* mutant cancers (86,87). Loss of the p53-p21 signaling axis prevents DNA damage from triggering cell death (12,88). The ability of p53 and p21 KO to rescue viability in the presence of *Brca1* mutations has been illustrated using mouse genetics. Nonetheless, the efficiency of rescue is variable, and dependent on the specific *Brca1* mutation. For example, p53 KO rescues the embryonic viability of mice that are homozygous for hypomorphic *Brca1* alleles (89–91). In contrast, p53 KO had little effect, beyond an extra day of embryonic life, on homozygous *Brca1* null allele embryos (12,88,92). The latter suggests that *TP53* mutations alone may be insufficient for some cancers to maintain viability; and that BRCA1 hypomorphic protein products, in conjunction with *TP53* mutations, sustain viability. Significantly, while *TP53* mutations abrogate DNA damage checkpoints, they do not explain how *BRCA1* mutant cancers replicate their genome in the absence of HR.

BRCA1 hypomorphic proteins

The transformation process is thought to initiate when epithelial cells acquire *TP53* mutations, followed by loss of heterozygosity (LOH) at the *BRCA1* locus (93). When the wild-type copy of *BRCA1* is lost, the mutation-containing allele remains behind, which is unable to support HR and consequently lacks tumor suppressor activity. *BRCA1* mutant cancers have extensive genomic instability; thus, a reasonable prediction might be that the remaining mutation-containing locus might also eventually be lost in a portion of cells. However, *BRCA1* mutations are frequently detectable in established cancers, with few cases in which the locus is entirely absent (94–98). Similarly, established *BRCA1* mutant cancer cell lines invariably retain the mutation-containing allele, regardless of passage number or culture conditions (29,99). Thus, in the absence of wild-type *BRCA1*, mutant alleles may be subject to positive selection. Indeed, several copies of the mutation-containing allele are often observed in tumors (discussed in more detail below).

BRCA1 missense mutations produce full-length proteins that can have hypomorphic activity. For example, mice with *Brca1*^{C61G} mutant mammary cancers rapidly developed PARPi and cisplatin resistance due to increased expression of the Brca1^{C61G} protein (100). Moreover, mice containing some *Brca1* hypomorphic mutations can survive embryogenesis. *Brca1*$^{CC/CC}$ mice were born with Fanconi anemia-like defects, and Brca1 CC in-frame mutant proteins were capable of displacing RIF1-shieldin, thereby promoting DNA end resection, but were defective for RAD51 loading (55). In contrast, frameshift mutations that block the production of proteins are unable to contribute to HR. Nevertheless, there are several examples of *BRCA1* frameshift mutations that are predicted to be unable to generate protein, but in fact produce unanticipated BRCA1 isoforms.

BRCA1 mutations that arise in the germline of patients can be broadly grouped by mutation location (Figure 1B). *BRCA1* 5’ located mutations, including 185delAG, can generate proteins that lack the RING, but contain all other domains, through initiating translation from start sites that are downstream of the mutation-induced stop codon (78,101). Meanwhile, *BRCA1* exon 11 frameshift mutations remain capable of expressing the BRCA1-Δ11q alternative splice isoform, which is without exon 11, but contains all conserved functional domains (29,102). BRCA1-Δ11q can provide residual growth in the

presence of PARPi or cisplatin, but remains defective relative to the full-length protein. BRCA1 BRCT domain mutations are highly destabilizing and result in proteasomal degradation (103,104). However, gene re-arrangements can generate proteins that lack the entire BRCT domain, including the destabilizing mutation (105).

Proteins with folding issues may also be stabilized by HSP90 (106). BRCA1- ΔRING, -ΔBRCT, and -Δ11q proteins can be readily detected under PARPi selection pressure and are capable of partially supporting RAD51 loading due to retention of the CC domain (63) (Figure 1B). However, lower levels of BRCA1 mutant protein expression are sometimes detected in the absence of PARPi selection pressure and likely support residual HR activity.

Typical methods used to quantify HR activity include the DR-GFP reporter, RAD51 foci formation, and PARPi colony assays. However, while these methods are effective at uncovering HR defects, they may lack the sensitivity required to detect residual HR activity. Our laboratory has adopted a modified colony assay that is capable of uncovering low levels of HR activity afforded by BRCA1 mutant proteins. The MDA-MB-436 breast cancer cell line has undetectable BRCA1 protein expression and is used to generate stable BRCA1 cDNA addbacks with a lenti-virus expression system. We seed cells at increasing densities, and incubate with a low concentration of PARPi that prevents the negative control cDNA-expressing cells from forming colonies, but has no effect on cells that express the BRCA1 wild-type positive control. Hypomorphic proteins enable colonies to form when cells are seeded at higher densities, whereas negative control cells do not form any colonies. Using this approach, the ability of BRCA1 mutant proteins to provide residual HR can be visualized and quantified.

BRCA1 locus modifications

The paradigm for *BRCA* mutant cancer development is centered on the “two-hit” hypothesis, where one allele is lost, and the remaining has a mutation that results in loss-of-function (107). However, recent insights suggest further complexity. In an analysis of patients with pathogenic germline mutations, depending on the cohort, 16-27% of *BRCA1/2* tumors analyzed fit the classic hypothesis where one allele was lost and the mutant allele remained. However, 23-42% lost the wild-type allele but had 2 copies of the mutant allele, and 16-36% had 3 copies of the mutant allele. Another 4-36% of tumors retained both the wild-type and mutant gene copy (98).

Retention of the wild-type allele has obvious implications for cancer development and therapeutic outcomes. Ovarian cancers that did not demonstrate LOH were associated with worse survival (98), likely from poorer response to platinum or PARPi therapies. Cancers that do not demonstrate LOH have additional driver mutations, such as *KRAS* or *EGFR*, and are better characterized by tumor lineage as opposed to *BRCA* mutation status (108). Possible explanations for retention of wild-type *BRCA1* could be that HR re-wiring adaptations are not in place to support survival in the event of LOH. Alternatively, cancer promoting alterations occur prior to *BRCA1* LOH and make the latter unnecessary.

Cancer cell lines and PDX tumors often harbor several copies of the *BRCA1* mutation-containing allele (105). Extra copies permit for transcription from multiple alleles and may enhance mutant protein production. Moreover, the presence of a second allele has been shown to facilitate *Alu*-mediated rearrangements in the *BRCA1* locus that enables the production of hypomorphic proteins (105). In summary, the presence and retention of single or multiple *BRCA1* mutant alleles enables truncated protein isoforms to be expressed, which support residual HR and the viability of cancers.

RNF168-53BP1 and basal viability

While PARPi treatments induce high levels of expression of BRCA1 hypomorphs, during cancer initiation and prior to chemotherapy selection pressure, truncated BRCA1 protein expression may be low or absent, and alternative re-wiring mechanisms are required to support HR. Deletion of 53BP1 can restore resection and HR in *Brca1* mutant mice and cancers (50,51). In the setting of *Brca1* null alleles, 53bp1 KO was insufficient to provide robust HR and PARPi resistance, or genome stability and protection from tumor development. Nonetheless, 53bp1 KO did restore the ability of Brca1 null embryonic cells to divide and produced pups at close to the expected Mendelian rate (63,64). Thus, in addition to therapy resistance, modulation of the 53BP1 pathway may be pertinent to *BRCA1* mutant tumor initiation (Figure 2).

Genetic disruption of proteins that are either up or downstream of 53BP1 can also restore end resection and HR. The RIF1-shieldin complex acts downstream of 53BP1, and KO of components have been shown to restore HR and promote PARPi resistance in a manner similar to 53BP1 (109). TRIP13 amplification also contributes to loss of shieldin complex activity (110). The RNF8-RNF168 pathway functions upstream and is required to recruit 53BP1 to DSB sites. In response to DNA damage, RNF8 binds to phosphorylated MDC1 and promotes the recruitment of RNF168, both are E3 ubiquitin ligases that conjugate Lys 63-linked ubiquitin chains onto histone H2A. Additionally, RNF168 monoubiquitinates H2A at K13/15, which serves as a recruitment module for 53BP1 (111). Thus, depletion of RNF168 and RNF8 have both been shown to decrease 53BP1 recruitment and consequently promote DNA end resection in the absence of BRCA1 (70,112,113). Moreover, the TIRR protein interferes with 53BP1 recruitment and can influence HR activity (114).

In the setting of BRCA1 and 53BP1 deficiency, RNF8 and RNF168 ubiquitin activity contributes to BRCA1-independent RAD51 loading, likely through a direct interaction between RNF168 and PALB2 (69–71,113). Therefore, in BRCA1 and 53BP1 deficient cells, RNF168 may play a key role in regulating HR. In BRCA1 null cancers, RNF168 protein expression is finely regulated. Here, low levels of RNF168 protein expression were detected, and consequently reduced 53BP1 recruitment activated resection and provided residual HR. However, RNF168 remained essential and was required for RAD51 loading in the absence of BRCA1. Indeed, a further decrease in RNF168 expression abolished HR and was detrimental to cellular viability. Of note, *BRCA1* mutant cells that expressed high levels of hypomorphic proteins retained the ability to promote residual HR without modulation of RNF168 expression, and were not dependent on reduced RNF168 for viability (113). Hence, residual HR may be supported by a variety of means, which are potentially determined by

whether mutant alleles are capable of expressing high enough levels of hypomorphic proteins.

RAD18 is another H2A-ubiquitin binding protein and is recruited to DSBs in an RNF168-dependent manner. In studies where RNF168 was ectopically overexpressed in BRCA1 null cancers, DNA end resection was blocked via 53BP1 recruitment. Moreover, DNA repair events at stalled replication forks were redirected toward a break-induced replication (BIR)-like mechanism through a RAD18-SLF1 signaling axis (115). Significantly, mutational signatures associated with tandem duplications arise from BIR-like events can be readily viewed in *BRCA1* mutant cancer genomes (116,117). Thus, RNF168 is a master regulator of multiple aspects of DNA repair, particularly in the setting of BRCA1-deficiency.

BRCA1 mutations and therapeutics

Tumor HR status is a critical determinant of PARPi and platinum therapy response. In the standing paradigm, the *BRCA1* wild-type genotype confers therapy resistance, and mutations sensitivity (13,14). In the clinical setting, *BRCA1/2* mutant cancers demonstrate overall better progression free survival (PFS) than wild-type cancers from PARPi (17–24). However, PARPi's have been particularly successful in ovarian cancers, where efficacy is observed in both *BRCA* wild-type and mutant cancers, and there is a wide spectrum of responses (118). *BRCA* wild-type ovarian cancers are thought to have defects in other HR genes that account for sensitivity, including *PALB2*, *RAD51D*, and multiple *FANC* genes (119,120).

Whether a particular group of *BRCA* mutations confer worse or better response to PARPi or impact the duration of effectiveness is an important question, but one that has not yet been fully resolved (121). Analyses of cohorts of ovarian cancer patients showed that *BRCA1* exon 11 mutation carriers may have worse overall response to platinum and overall survival (OS) compared to other mutation types (29,122). This may be due to expression of the BRCA1-Δ11q splice variant that can promote therapy resistance. However, there are many examples of patients with similar mutations, including those in *BRCA1* exon 11, which have a complete, partial, as well as no response to PARPi. Thus, it does not appear that specific *BRCA1* mutations can be used to predict therapy efficacy or the duration of PARPi response. A range of factors likely determine response and resistance, much of which, in the clinical setting, are unknown. The collection of paired PARPi pre- and post-resistant tumor samples from patients is currently limited and future efforts along this front will be crucial.

Insights into PARPi resistance mechanisms have largely come from studies using *BRCA1* mutant human cancer cell lines and mouse models, and are too numerous to review here (26). It should be noted they include non-HR related events; however, we will focus on mechanisms that restore HR. With respect to the role of *BRCA1* mutations, we and others have observed increased expression of a range of BRCA1 truncated proteins in multiple human cancer cell lines (29,78,100,101,105,106,123). Importantly, RNAi or CRISPR/cas9 targeting of truncated BRCA1 proteins reversed PARPi resistance. However, ectopic overexpression of truncated BRCA1 proteins only provided partial or low levels of HR and PARPi resistance. Although BRCA1 isoforms were not as efficient as wild-type, they did

enable significantly more colonies to grow in the presence of PARPi compared to cells that were null for BRCA1 expression (29,78,105). Thus, BRCA1 hypomorphs likely promote resistance in combination with additional events that together provide robust PARPi resistance. In several instances, we have observed reductions in 53BP1 protein levels in conjunction with BRCA1 hypomorph expression in cells that acquired PARPi resistance (63,106). In another example, BRCA1 hypomorphic protein expression was critical for *PARP1* mutation-induced PARPi resistance (124).

BRCA1 mutant proteins might not be expressed at high enough levels or may have insufficient HR activity to induce resistance. Here, secondary mutations that restore the reading frame and expression of the full-length protein can promote resistance (26). Full-length BRCA1 proteins are more efficient at promoting PARPi resistance, and a preference for reversion mutations might be expected. However, reversion mutations are only detected in a portion of PARPi resistant cancers (125), and determinants of the acquisition of reversion mutations versus other mechanisms of resistance are unclear. Conceivably, reversion mutations may be more readily generated than alternative translation, alternative splicing, and protein folding re-wiring events that are required for BRCA1 hypomorph protein expression. Of note, specific mutations do not appear to determine whether reversions occur or hypomorphs are expressed, as there are examples of both mechanisms in RING, exon 11 and BRCT mutation-containing cancers. Moreover, intra-tumoral heterogeneity could play a role in PARPi resistance. Additional studies of patient tumors are required to determine whether multiple mechanisms are engaged within the same tumor.

Implications and future directions

Despite progress, there is still much unknown, and hereditary *BRCA* mutations continue to significantly contribute to breast and ovarian cancer mortality. The process of HR is fundamental to the study of BRCA cancer. HR re-wiring mechanisms are emerging, but there are likely many additional means by which cells adapt to survive in the absence of wild-type *BRCA1*. Further complexities, such as the relationship between replication fork protection and HR will be important to decipher, particularly in determining their relative impact on cell viability and therapeutic response. Accumulating DNA damage also triggers luminal to basal/mesenchymal trans-differentiation events that permit for cells to tolerate BRCA1 loss (126). An understanding of these events and pathways could reveal additional therapeutic vulnerabilities.

During the development of *BRCA1* mutant cancer, HR deficiency is advantageous, but in established tumors is an Achilles heel, providing a therapeutic window for targeting cancer cells over non-transformed cells. Consequently, HR-deficiency is often transient, and determined by therapy selection pressure (Figure 2). Retaining a state of HR-deficiency would prolong PARPi sensitivity and patient survival. While efforts are underway to develop therapeutics that target HR defective cells, the selective targeting of cancers that have acquired HR-proficiency may provide a complementary approach to PARPi. Moreover, loss of HR activity confers dependencies on alternative DNA repair pathways. Microhomology-mediated end joining (MMEJ) has been shown to be one such pathway (127,128), and DNA polymerase theta inhibitors are under development (129,130). Continued research into the

basic biology of DNA repair pathways is of critical importance for the discovery of new and effective therapeutic targets. Finally, routine collection of tumor material from patients who have progressed on PARPi therapy will be of enormous benefit in understanding resistance and designing new treatments.

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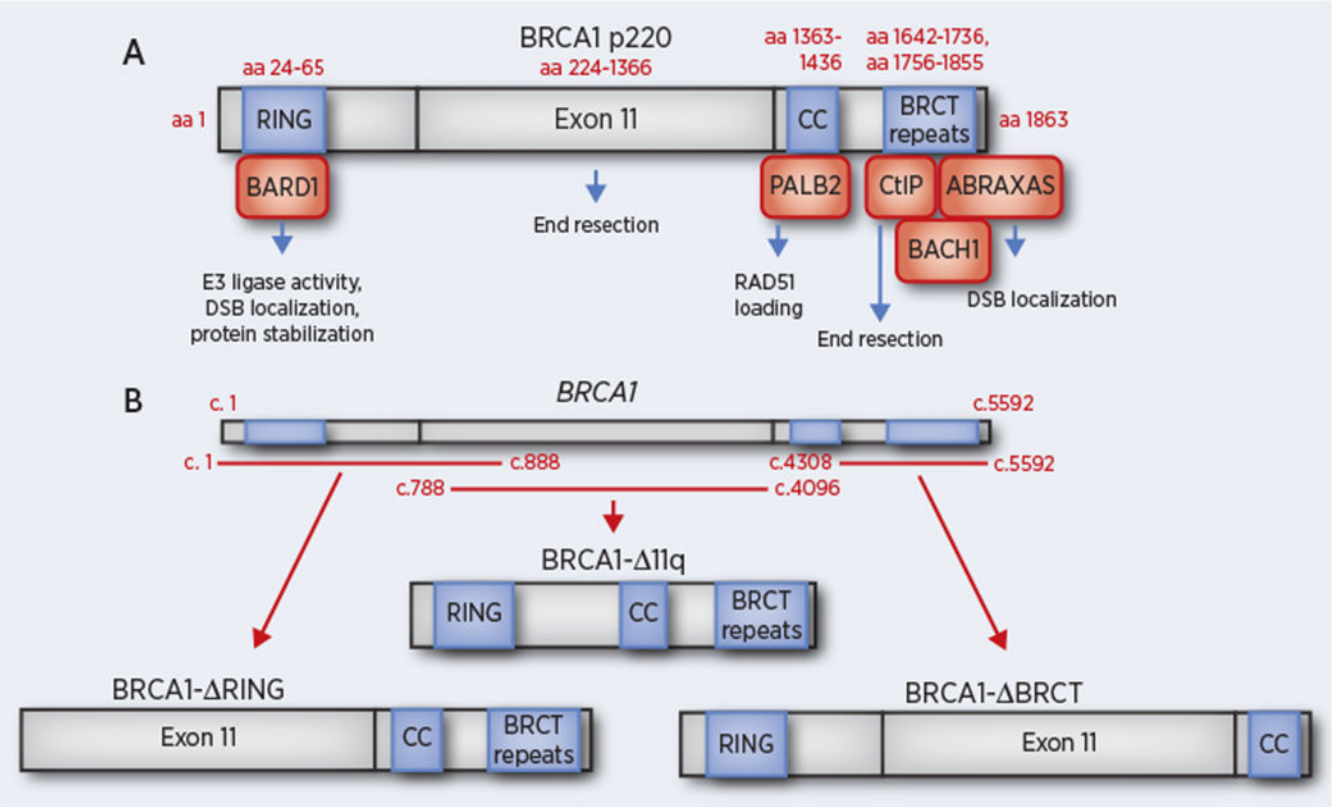

Figure 1.

Schematic representation of BRCA1 full-length and hypomorphic proteins that have been detected in cancers. (**A**) The full-length (p220) BRCA1 protein is depicted along with known functional domains and corresponding protein interactions. Amino acid (aa) residues corresponding to predicted domain start and end sites are indicated as well as potential functional activities of each domain. (**B**) *BRCA1* cDNA is shown (above) with corresponding base pairs indicated. Redlines indicate regions where frameshift mutations may be present but remain capable of expressing either the BRCA1-ΔRING, BRCA1-Δ11q, or BRCA1-ΔBRCT proteins.

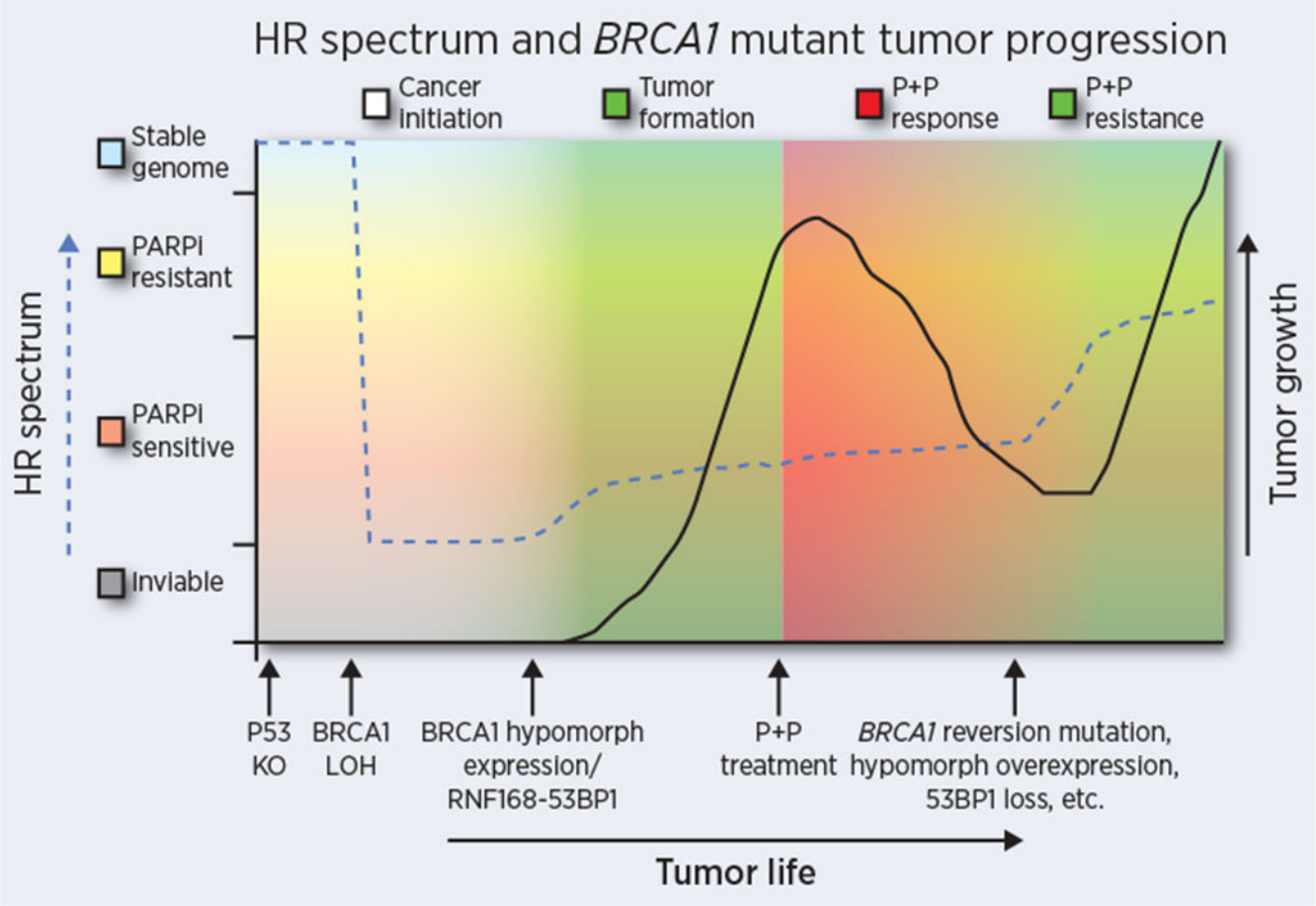

Figure 2.

The HR spectrum over the life course of a *BRCA1* mutant cancer. Partial HR activity is likely required for tumor initiation and growth, but may also play a role in therapy resistance, and could account for some patients demonstrating poor responses in the treatment naïve setting. Dashed line indicates HR activity and solid line indicates cancer growth over time. Arrows along the x axis indicate significant events over the course of cancer development and progression. Shading colors correspond to cancer development, cancer growth, PARPi and Platinum therapy (P+P) responsiveness and resistant growth.