Deciphering the BRCA1 Tumor Suppressor Network

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Abstract

The BRCA1 tumor suppressor protein is a central constituent of several distinct macromolecular protein complexes that execute homology directed DNA damage repair and cell cycle checkpoints. Recent years have borne witness to an exciting phase of discovery at the basic molecular level for how this network of DNA repair proteins acts to maintain genomic stability and suppress cancer. The clinical dividends of this investment are now being realized with the approval of first in class, BRCA targeted therapies for ovarian cancer and identification of molecular events that determine responsiveness to these agents. Further delineation of the basic science underlying BRCA network function holds promise to maximally exploit genome instability for therapy of hereditary and sporadic cancers.

The breast cancer early onset genes, BRCA1 and BRCA2 were discovered by positional cloning approaches in kindreds with a high prevalence of breast and ovarian cancer. The initial lack of clarity presented from the domain structure of the proteins was remedied by a series of discoveries that revealed the proteins biochemically interact in large nuclear foci in response to DNA damage, and are required to execute homology directed DNA repair and cell cycle checkpoints (1). These observations strongly suggested that a common function in genome integrity maintenance is necessary to suppress cancer. Subsequent advances in protein purification and mass spectrometry methodologies have led to the expansion of this concept by the discovery that at least 13 different tumor suppressor proteins interact with BRCA1 and BRCA2. Despite these striking similarities, a linear model of BRCA dependent DNA repair and tumor suppression is challenged by multiple other observations. Namely, only about 5% of each protein exists in association with the BRCA1-BRCA2 complex, and breast cancers occurring in individuals with germline BRCA1 or BRCA2 mutations typically display different histopathologies and gene expression profiles. Coupled with the realization that chemo-resistance mechanisms in BRCA1 and BRCA2 mutant cancers also differ, these features have inspired a network model relating BRCA molecular function in DNA repair to tumor suppression. We highlight recent insights into the BRCA tumor suppressor network and stress the connections between basic molecular knowledge of these proteins and their roles in genome integrity, tumor suppression, and response to therapy.

Overview of BRCA1 Structure and Cancer Susceptibility

The BRCA1 protein has several definable structural domains that suggest it uses modular and potentially cooperative interactions to execute its DNA damage response functions (Fig 1). The amino terminal RING (Really Interesting Gene) domain enables E3 ubiquitin ligase activity through its interaction with E2 enzymes. Clinical mutations to the RING domain are associated with cancer susceptibility. However, knockin of a rationally designed BRCA1 RING mutant I26A that disrupts interaction with E2 enzymes did not abrogate genome stability or confer cancer predisposition in mice (2). Conversely, a known clinical mutant C61G that also disrupts E3 activity did cause genome instability and cancer in mice (3). Complicating interpretation of this phenotype as proof that BRCA1 E3 activity suppresses cancer, is the confounding issue that C61G mutation disrupts RING architecture and association with BARD1, a stoichiometric binding partner of BRCA1 (4). The C61G mutation may impart cancer susceptibility by loss of E3 ligase activity, diminished BARD1 interaction, or a combination of these deleterious events.

Approximately 60% of the BRCA1 protein is comprised of the centrally located exon 11 encoded region. This poorly conserved region lacks definitive domain elements or interacting partners, yet is required for full HR and checkpoint function, as well as tumor suppression (5, 6). Nonetheless, exon 11 mutations allow an in frame splice between exons 10 to 12 and the production of a partially active protein that localizes to DNA damage sites. Downstream of exon 11, BRCA1 harbors a coiled coil region near its carboxy terminus that interacts with PALB2, which biochemically bridges BRCA1 to BRCA2 in a tumor suppressor supercomplex (Fig 1). PALB2 mutations confer high penetrance breast
cancer phenotypes (7), similar to BRCA1 and BRCA2. C-terminal to this region are the BRCA1 C-terminal Repeats (BRCT domains), which bind to phospho-peptides. Mutually exclusive interactions with 3 bona fide BRCT interacting proteins allow segregation into at least 3-different protein complexes. The distinct BRCA1-containing complexes, which will be discussed in detail in the following sections, are thought to work together in response to double stranded breaks (DSBs) (8). The BRCT domain of BRCA1 contributes to most of its functional interaction with different protein complexes, where BRIP1, Abraxas, CtIP all contain consensus BRCT-interacting motif, SXXF, which is phosphorylated on serine to mediate the interaction (9, 10).

Connecting BRCA Network Biochemistry to the DNA Damage Response

BARD1 has a similar domain structure as BRCA1, with an amino-terminal RING domain and C-terminal BRCT repeats. Unlike BRCA1, the BARD1 RING domain does not interact with E2 enzymes and its BRCT repeats do not bind phosphopeptides. The stoichiometric interaction between BRCA1-BARD1 instead provides stability to both proteins, enhanced DNA damage site recognition, and increases BRCA1 E3 ubiquitin ligase activity in vitro. The BRCA1-BARD1 RING domains ubiquitylate H2A in vitro and in vivo at K127-129 (11), though the relationship of this activity in DNA repair is unknown. The crystal structure of the most similar E3 ubiquitin ligase heterodimer, Ring1B-Bmi1 bound to a nucleosome suggests a possible mode for how BRCA1-BARD1 could directly interact with nucleosomes. A high level of conservation shared between the Ring1B nucleosome binding loop and the corresponding BRCA1 region predicts that BRCA1 targets to K127-129 by binding to a nucleosomal H2A-H2B acidic patch using its own basic residues within the nucleosome binding loop (12). It will thus be interesting to understand the basis for how BRCA1 ubiquitinates different lysines on H2A than RING1B-Bmi1, and importantly how K127-9 ubiquitination contributes to BRCA1 function.

The BARD1 BRCTs were reported to interact with poly(ADP) ribose (13), and more recently to specifically recognize histone H3 dimethylated at lysine 9 (H3K9me2) through its interaction with HP1 proteins. This interaction was reported to anchor BRCA1-BARD1 at DNA damage sites (14). Genetically, the BRCA1-BARD1 interaction is unique among the BRCA1 network in that it appears to be necessary for the majority of BRCA1 in vivo function. BARD1 deficiency fully recapitulates BRCA1 nullizygosity, with BARD1 knockouts displaying embryonic lethality, genomic instability, and cancer susceptibility (15, 16).

Abraxas resides in a five-member complex (RAP80, Abraxas, MERIT40, BRCC45, BRCC36) that preferentially binds to lysine-63 linked ubiquitin through the RAP80 tandem ubiquitin interaction motifs (UIMs). The finding that BRCA1-RAP80 interaction was required for foci formation first implicated nondegradative ubiquitin as a DNA damage recognition platform during DSB signaling (17-19). DSB ubiquitination occurs as a result of a γH2AX initiated signaling cascade that brings RNF168 to perform large-scale DSB chromatin ubiquitination at H2AK13 and K15 (20). Several other DNA damage response proteins rely on RNF168 E3 activity for DSB localization, including 53BP1, which is a specific reader of the H2AK15-Ub mark (21). In addition to ubiquitin, SUMOylation also contributes to BRCA1 DSB recruitment. RAP80 contains SUMO interacting motifs (SIM) domains amino terminal to its tandem UIM repeats, and shows ~80 fold higher affinity for hybrid SUMO-ubiquitin chains than K63-linked ubiquitin alone, suggesting a mixed SUMO-ubiquitin targeting signal (22). The RAP80 complex specifically de-ubiquitinates the K63-Ub chains through the actions of its associated Zn²⁺ dependent deubiquitinating enzyme (DUB), BRCC36. Unresolved questions remain as to whether DUB activity serves to terminate DNA damage association by removing the K63-Ub recognition signal for RAP80, or alternatively, in a ubiquitin editing capacity, whereby it removes K63-Ub, thus allowing accumulation of either mono-ubiquitin or other ubiquitin topologies that have been reported at DSBs (23). The data that loss of any member of the RAP80 complex eliminates observable BRCA1 foci formation at DSBs raises the question of whether the RAP80 complex accounts for BRCA1 function in HR. Interestingly, loss of
RAP80 leads to over-resection and increased sister chromatid exchanges in response to DSB inducing agents, indicating that RAP80 complex is required to fine-tune the HR efficiency by controlling resection level (24, 25).

In contrast to embryonic lethality of BRCA1 knockout mice, RAP80, Abraxas or MERIT40 knockout mice are viable and do not exhibit apparent developmental defects (26-29). Indeed, RAP80 was recently shown to target BRCA1 to chromatin regions that are approximately 1kb removed from break sites and to affect checkpoint responses and not DSB repair (30). Despite this relatively mild DNA repair phenotype, germline mutations exist in RAP80 and Abraxas in familial breast cancer, and numerous somatic mutations are observed in all members of the complex (28, 31, 32). Moreover, RAP80 and Abraxas mice are tumor prone, developing lymphomas in about 20% of mice at 1 year of age (26-28). These observations are consistent with the concept that complete loss of BRCA function in the DNA damage response (DDR) is not necessary for cancer susceptibility.

BRIP1 (also known as BACH1/FANCJ) was first cloned and identified as a putative DNA helicase protein interacting with BRCT domain of BRCA1 (33). Phosphorylation of S990 on BRIP1 allows its interaction with the BRCA1 BRCT repeats (34). BRIP1 deficient cells show reduced HR efficiency in the DR-GFP assay (35). Interestingly, BRIP1 contributes to DNA crosslink repair pathway independent of its interaction with BRCA1 (36). BRIP1 deficiency causes severe DNA crosslink sensitivity and is biallelically mutated in Fanconi Anemia. However, loss of interaction with BRCA1 does not confer MMC sensitivity (36, 37). Instead this is thought to affect a balance between HR and translesion synthesis at crosslinks. BRIP1 has bona fide in vitro helicase activity and resolves G-quadruplex structures with 5'-3' polarity. Concordant with this in vitro specificity, both the C. elegans and human cells deficient in BRIP1 show loss of repetitive G-rich DNA and telomere abnormalities (38, 39). However, if BRIP1 helicase activity contributes to maintenance of GC rich regions through its association with BRCA1 remains an unresolved question.

CtIP (also known as RBBP8) interacts with the Mre11/Rad50/NBS1 (MRN) complex and stimulates its nuclease activity, in turn mediating end resection in S/G2 phases of the cell cycle that initiate homologous recombination (40). The interaction between CtIP and BRCA1 depends on CDK phosphorylation on S327. Knockin of human phospho-deficient mutant CtIP S327A (equivalent to S326 in mouse), revealed no impact on resection in chicken and mouse cells, and was not essential for resection mediated repair, tumor suppression or viability (41-43). Although BRCA1-CtIP interaction was not required for end resection, it enhanced the speed of this process (44). Therefore, BRCA1 might interact with CtIP to ensure optimal end resection timing, a subtlety that may not be easily detected by other assays. Similarly, PALB2 knockin mice have been generated that disrupt interaction with BRCA1. In contrast to either BRCA1 or PalB2 null mice, these PalB2 mutants are viable, indicating that while the interaction between BRCA1 and PALB2 contributes to HR, either protein is largely functional in the absence of this interaction (45). This again signifies that interaction with BRCA1 is not equivalent to function and together with data showing disruption of BRCA1-BRIP1 also did not recapitulate null phenotypes, suggests caution when interpreting the importance of BRCA1 protein-protein interactions. Guilt by association is clearly an oversimplification.

Transcription Influences DNA Damage Recognition by BRCA1 complexes

Questions remain as to where in the genome does the BRCA1 network execute its DNA damage response functions. Recent reports reveal strong connections between BRCA1 genome integrity maintenance and transcription. BRCA1 forms a complex with mRNA-splicing machinery in DNA damage dependent manner to regulate pre-mRNA splicing of genes involved in DNA damage signaling (46). BRCA1 has also been shown to participate in transcription-associated damage control, where it functions at stalled or defective transcription sites to assist transcription restart and resolve RNA-DNA hybrids (R-loop) that are known to promote DSB formation (47). This link to transcription is buttressed by the finding that BRCA1, PALB2, and other HR proteins have been found to co-reside at transcriptionally active
regions throughout the genome (48-50). Moreover, active transcription was directly implicated in BRCA1 targeting to DNA damage sites, revealing RNA and transcription associated chromatin modifications in BRCA1 DNA damage recognition and repair. In support of this assertion, disruption of BRCA1 interaction with senataxin (SETX) led to R-loop driven DNA damage (51). BRCA2 also prevents R-loop accumulation to prevent transcriptional stress (52), implicating R-loop stability as a common mechanism for both proteins to maintain genome stability. This data also raises the intriguing possibility that the transcriptional profile of a cell could influence genome instability in the context of BRCA deficiency. Perhaps this could play a role in tissue specificity of its tumor suppression.

Multifactorial Responses of the BRCA Network to Replication Stress

PALB2 bridges the interaction between BRCA1 and BRCA2. The BRCA1-BRCA2-PALB2 complex promotes RAD51 nucleofilament formation, thus initiating homology directed repair (53). It has more recently become apparent that BRCA1 and BRCA2 also participate in replication fork protection and restart. BRCA2 was found to prevent degradation of nascent strands at stalled replication forks by MRE11 and the C-terminal site of BRCA2 is critical for replication fork protection (54, 55). BRCA1 also contributes to fork protection in a similar manner to BRCA2, suggesting that BRCA1-PALB2-BRCA2 maintains genome stability by contributing to both HR and to replication fork maintenance (56).

These concerted activities are interesting in light of the hypothesis that genomic instability in BRCA deficient cells is thought to arise primarily in S-phase as a consequence of replication stress. By adapting the bacterial replication terminator Tus/Ter complex to induce site-specific replication fork stalling in mouse cells, it was shown that both the tandem BRCT repeats and exon 11 regions of BRCA1 are required for HR at stalled replication forks in mammalian cells (57). BRCA1 also regulates multiple aspects of replication to promote DNA inter-strand crosslink (ICL) repair independent of RAD51 nucleofilament formation. Studies using Xenopus egg extracts revealed that BRCA1, possibly through its interaction with RAP80, is required to unload the replicative CMG (Cdc45, MCM, GINs) helicase complex upon bidirectional replication forks colliding with an ICL (58), thus affording access to crosslink recognition proteins. This data is in agreement with genetic studies implicating BRCA1 in early stages of ICL repair that occur prior to HR.

Consistent with replication stress being the limiting aspect of BRCA1 genome integrity control, primary mammary epithelial cells from patients with heterozygous BRCA1 or PalB2 status experience higher replication stress than cells with two wildtype copies of either gene (59, 60). Conversely, other BRCA1 functions in DSB repair and checkpoint activation were proficient in BRCA1*+/* cells (60). This observation supports the hypothesis that BRCA haploinsufficiency in resolving replication stress might contribute to high risk of cancer in mutation carriers. Genetically engineered mouse models of pancreatic cancer and ovarian cancer are consistent with this hypothesis, as BRCA1 or BRCA2 heterozygosity accelerated tumor formation in both instances (61, 62).

Fanconi Anemia provides a Window into Dysfunction of the BRCA Network

Fanconi anemia (FA) is a rare hereditary syndrome characterized by developmental defects, progressive bone marrow failure and cancer susceptibility that is associated with DNA crosslink repair deficiency and sensitivity to endogenous aldehydes (63). In contrast to heterozygous mutations that cause hereditary breast and ovarian cancer, FA requires biallelic mutations within a network of 17 genes dedicated to DNA crosslink repair. Mutations within several members of the BRCA1 network are causative for FA. BRCA2 (FANCD1) and PALB2 (FANCN) interact with the BRCA1 coiled coil domains, while BRIP1 (FANCJ) interacts with the BRCA1 BRCT repeats (Fig 1). More recently, two patients have been identified with missense mutations (R1699W; V1736A) within the first BRCA1 BRCT domain and an FA-like syndrome, establishing BRCA1 as a Fanconi gene (FANCS). Both patients had one allele with missense mutation in BRCT repeats and another deleterious mutation in either exons 10 or 11 (64, 65). Patient derived BRCA1/FANCS cells were obtained and...
found to have reduced BRCA1 BRCT interactions, diminished RAD51 foci formation and MMC and PARP inhibitor (PARPi) hypersensitivity, in contrast to a sibling control that had only one mutated BRCA1 allele. These findings thus mimic the clinical context of a biallelically mutated tumor and heterozygous patient. Indeed, BRCA mutant tumors are hypersensitive to platinum salts or PARPi, while increased systemic toxicity in noncancerous tissues is not seen in BRCA carriers.

Comparison of FA phenotypes derived from mutations to different genes within the BRCA network has also been illuminating (66). BRCA2/FANCD1 and PALB2/FANCN patients displayed bone marrow failure and severe FA phenotypes associated with solid tumors outside of the typical BRCA spectrum, including Wilms Tumor and Medulloblastoma. BRIP1 FA patients displayed bone marrow failure and leukemia. In contrast, neither of the BRCA1/FANCS patients developed spontaneous bone marrow failure and both maintained breast or ovarian cancer predilection, with early onset ovarian cancer at age 28 and breast cancer at age 23. The difference in patient phenotype is especially interesting in relation to BRIP1/FANCJ, as both BRCA1/FANCS patient mutations occurred in the BRCT region and disrupted the BRCA1-BRIP1 interaction. The BRCT BRCA1/FANCS mutations also abrogate interaction with CtIP and the RAP80 complex, possibly accounting for these differences. In addition, it is clear that BRIP1/FANCJ has many BRCA1 independent activities.

Determinants of Synthetic Lethality with Dysfunction of the BRCA Network

Poly (ADP-ribose) polymerases (PARP) are highly abundant proteins that are responsible for PARylation during the DDR. PARP1/2 can bind to DNA single-strand breaks (SSBs) and facilitate resolution of these lesions. Collision of active replication forks with SSBs in S-phase is thought to lead to one-sided DSBs that necessitate BRCA-RAD51 mediated HR for repair (Fig 3). Indeed, landmark studies showed that PARPi was synthetic lethal in the context of BRCA-deficiency, ushering in new era of targeted therapy for patients with BRCA mutant (67, 68). PARPi (olaparib, trade name Lynparza) was been rapidly translated to the clinic with successful phase I (69) and phase II (70-72) clinical trials leading to FDA approval in December of 2014 for advanced BRCA mutated ovarian cancer. However, resistance mechanisms for PARP inhibitor treatment have arisen in patients and in the laboratory setting in cells exposed to chronic PARPi, indicating a more thorough understanding of resistance mechanisms and alternative targets is needed.

Resistence Mechanisms to PARP Inhibition

HR proficiency is a key determinant of cellular sensitivity to PARPi. This reasons that restoring HR capacity would produce PARPi resistance. Indeed, genetic reversion of mutated BRCA1 or BRCA2 has been found in human tumors. Secondary mutations that restore reading frame lead to generation of partially functional BRCA proteins and render these cells HR proficient and resistant to PARPi or cisplatin (75, 76). A reported 46.2% of platinum-resistant recurrences had secondary mutation restoring BRCA1/2 (77), indicating genetic reversion is a common resistance mechanism.

Competition between repair pathways also appears to be a key determinant of PARPi sensitivity (Figs 2 and 3). Loss of 53BP1 suppresses embryonic lethality, HR deficiency, and PARPi hypersensitivity in BRCA1 null mice (78, 79). Remarkably, double knockout mice are also not
cancer prone, firmly connecting loss of HR to BRCA1 cancer susceptibility (80). A putative mechanism underlying this observation is that 53BP1 blocks end resection in BRCA1 deficient cells and promotes toxic NHEJ in S-phase (Fig 3). In turn, loss of 53BP1 renders these cells proficient in HR in the absence of BRCA1 by permitting excessive single stranded DNA, the initial substrate of RAD51 dependent HR (78). Similarly, deficiency in several 53BP1 interacting partners RIF1, PTIP, REV7, and Artemis participate in blocking end resection. Loss of these four effector proteins also promotes PARPi resistance to varying extents in BRCA1 mutant cells (81-84). So how is this competition between the BRCA1 and 53BP1 networks regulated? Cell cycle seems to be a key determinant, with BRCA1 exclusion from foci in G1, whereas RIF1 and PTIP have limited association with 53BP1 damage foci in S/G2 when HR is favored. Acetylation also appears to play a role in this balance, in part through reducing 53BP1 affinity for H4 histone tails containing methylated K20 residues (48, 85).

Novel Targets for BRCA Network Dysfunctional Cancers

Given the numerous possibilities of acquired PARPi resistance, it would be prudent to develop alternative strategies that selectively kill BRCA mutant cells. An attractive target is the HR protein RAD52, which is synthetically lethal with combined deficiency in either BRCA1, PALB2, or BRCA2 (86, 87). RAD52 was proposed to execute alternative repair pathways that perform RAD51-mediated HR independent of BRCA1-PABL2-BRCA2 complex. Alternatively, RAD52 is also known to catalyze the process of single strand annealing (SSA) and may function as a salvage pathway in the absence of BRCA proteins (88). A second exciting potential target to selectively treat HR deficient cancers is DNA polymerase theta (Polθ), which mediates error-prone alternative NHEJ (Alt-NHEJ, also called micro-homology-mediated end joining, MMEJ) (89-92). Polθ promotes MMEJ and restricts RAD51-mediated recombination. Combined FANCD2 and Polθ nullizygosity produced embryonic lethality in mice, and loss of Polθ in BRCA1 or BRCA2 deficient cells demonstrated significantly reduced plating efficiency, indicating a synthetic lethal relationship with HR deficiency (90, 91). Both Polθ and RAD52 are upregulated in HR deficient cancers and are potentially druggable targets. While the clinical utility of such agents is to be ascertained, it will be interesting and feasible to determine whether RAD52 or Polθ inhibition would restore PARPi sensitivity in BRCA1 and 53BP1 double mutant cells. Should either of these modalities be toxic to double knockout cells, this would suggest potential use for resistance of BRCA1 mutant cancers to PARPi.

Elevated replication stress is frequently experienced in rapidly replicating cancer cells, particularly in those experiencing HR deficiency. Inhibition of replication stress activated kinases, ATR or Chk1 inhibition sensitizes ovarian cancer cells with defective HR to commonly used chemotherapy agents, and both ATR and Chk1 inhibition sensitize BRCA mutant cancer cells (93). In a possible example of proof of concept, Chk1 inhibition caused synthetic lethality with MRN mutation and showed a curative clinical response in a tumor harboring Rad50 mutation (94).

Concluding Thoughts

The past 5 years has brought forward a revolutionary increase in basic understanding of BRCA molecular function in the DNA damage response, the first clinically approved therapies to selectively treat BRCA mutant cancers, and a host of resistance mechanisms that connect restoration of HR to therapeutic response. Still, there are many questions remaining. As detailed above, most BRCA1 protein-protein interactions do not satisfactorily explain its roles in DNA repair or tumor suppression. This in part relates to a lack of knowledge regarding how BRCA1 functions in DNA repair. In stark contrast to seminal studies describing BRCA2 dependent RAD51 nucleofilament formation by displacement of RPA (95), meaningful in vitro assays to dissect BRCA1 repair function have not been reported. The development of such approaches would represent an important advance because it remains a matter of debate as to whether the primary BRCA1 function resides in its ability to promote DNA repair transactions, or act as a competitor to 53BP1 to counteract toxic NHEJ. Close links between basic discovery and clinical observations...
should help resolve these questions as well as pose new and unanticipated issues. They should also continue to enable translation of new and existing therapies and further refine our knowledge of the BRCA1 Tumor Suppressor Network.

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87. Lok, B. H., Carley, A. C., Tchang, B., and Powell, S. N. (2012) RAD52 inactivation is synthetically lethal with deficiencies in BRCA1 and PALB2 in addition to BRCA2 through RAD51-mediated homologous recombination. *Oncogene.* 32, 3552–3558


Figure Legends

Figure 1. Domain structure of BRCA1/BARD1 and key interacting partners in the BRCA network
Key interacting partners and mutations referred to in the text are listed. R1669W and V1736A were found in patients with BRCA1 biallelic mutations. K619A, C645R and V695L mutations within the BARD1 BRCT repeats fail to interact with PAR.

Figure 2. Alternative DNA repair pathways compensate for BRCA deficiency
Nonhomologous end joining (NHEJ) and homologous recombination (HR) are the primary DNA double-strand (DSB) repair pathways. End resection produces single stranded DNA overhangs that is required for HR. Alternative DSB repair pathways, single stranded annealing (SSA) and microhomology mediated end joining (MMEJ) also necessitate end resection and represent compensatory DNA repair mechanisms when BRCA dependent HR is impaired. Proteins that execute alternative DNA repair mechanisms represent potential targets to selectively kill BRCA mutated cells.

Figure 3. Determinants of PARP inhibitor response
PARP inhibition creates trapped PARP-DNA complexes that require BRCA HR activity during DNA replication. Resistance mechanisms occur due to events that restore HR in BRCA1 mutant cells. This entails secondary reversion mutations that lead to production of partially functional BRCA1 protein. Alternatively, restoration of HR and resistance can occur due to loss 53BP1 or several of its interacting partners.
Figure 1

BRCA1

RING
1863 AA

BRCT BRCT

BARD1

RING
200 300

NLS

Exon11

PALB2
BRCA2

S(p)XXF (phospho-protein binding)
Abaxas
BRIP1
CtIP

PAR

PLVLI (PvVxL) motif mediates binding to HP1

BARD1

RING
8 96

NLS

Exon11

PALB2
BRCA2

S(p)XXF (phospho-protein binding)
Abaxas
BRIP1
CtIP

PAR

PLVLI (PvVxL) motif mediates binding to HP1
Figure 2

DSB

NHEJ

Resected ends

RPA

Rad51

BRCA1/2

HR

MMEJ

Polθ

Synapse formation

Microhomology annealing and extension

RPA

SSA

Long resected ends

Rad52

Rad52 mediated annealing

D-loop

Rad52

Long resected ends
PARP-mediated SSB repair

DSB formation

BRCA1/2 mediated HR

Rad51

Repair breaks and cell survival

PARP inhibitor

Loss of 53bp1 and its effectors
Partially restored HR

Reversion of BRCA1/2 mutations
Restored HR activity

BRCA1/2 deficient cells
Error prone NHEJ
Cell death

Resistance Mechanisms

Figure 3