BRCA1 at the crossroad of multiple cellular pathways: approaches for therapeutic interventions

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Abstract
Approximately 10% of the cases of breast cancer and invasive ovarian cancer are hereditary, occurring predominantly in women with germ-line mutations in the BRCA1 or BRCA2 genes. Low expression of these genes in sporadic tumors extends their significance to sporadic breast and ovarian cancers as well. For over a decade since its identification, extensive research has been directed toward understanding the function of the breast and ovarian tumor suppressor gene BRCA1. The long-term goal has been to identify the biochemical pathways reliant on BRCA1 that can be exploited for developing targeted therapies and benefit mutation carriers. To date, no one specific role has been identified, but rather it is clear that BRCA1 has significant roles in multiple fundamental cellular processes, including control of gene expression, chromatin remodeling, DNA repair, cell cycle checkpoint control, and ubiquitination, and overall is important for maintenance of genomic stability. Major findings and potential BRCA1-dependent therapies will be discussed.

Introduction
A decade ago, familial breast and ovarian cancer syndrome was linked to germ-line mutations in two genes (BRCA1 and BRCA2). The BRCA1 gene was mapped to chromosome 17q21, and BRCA2 was mapped to chromosome 13q12.3 (1, 2). Both sites often exhibited loss of heterozygosity in familial and sporadic tumors. In cancer-linked families, the loss of heterozygosity at chromosome 17q21 or 13q12 is associated with retention of the disease predisposing mutant allele, implying that BRCA1 and BRCA2 are tumor suppressor genes (3, 4).

In the western world, one of nine women is at risk of developing breast cancer during her lifetime. Of all cases, ~5% to 10% are the result of hereditary predisposition. The BRCA1 and BRCA2 genes show autosomal dominant transmission. Carriers of germ-line mutations in either BRCA1 or BRCA2 genes have up to ~80% increased lifetime risk of developing breast and ovarian cancers and elevated risk of developing other types of cancer, such as prostate and pancreas (5, 6). Mutations in the BRCA1 gene account for ~50% of familial breast cancer cases and for ~80% of all familial breast and ovarian cancer cases. Mutations in BRCA2 account for ~30% of familial breast cancer cases and are also linked to male breast cancer (5).

In contrast to other tumor suppressor genes, somatic mutations in BRCA1 and BRCA2 are extremely rare. Epigenetic changes in the BRCA1 gene in the form of promoter hypermethylation and loss of expression were reported in a subset of sporadic tumors (7). Only one of many reports describes methylation of the BRCA2 gene (8). The significance of BRCA1 epigenetic silencing is functionally equivalent to carrying a germ-line mutation. Both events lead to altered gene expression and a dysfunctional protein.

BRCA1 encodes a large protein of 1,863 amino acid with only a few familiar sequence motifs. A RING finger domain was identified at the NH2 terminus of the protein (amino acids 1-112; Fig. 1A). Recently, RING finger domain proteins were recognized as E3 ligase enzymes that participate in ubiquitination (9). The BRCA1 E3 ligase activity will be discussed later in this review.

The BRCT domain found at the COOH terminus of the protein between residues 1,646 and 1,863 is another sequence and a structural motif in BRCA1 (10). The two repeats that comprise this motif are often found in proteins involved in maintenance of genomic stability and in DNA repair proteins, such as p53-binding protein, MDC1, Rad9, etc. (10). The BRCT motifs were recently identified as phosphopeptide recognition modules and are shown to bind phosphorylated protein partners involved mainly in the DNA damage response (11, 12).

Based on its associations and physical interactions with other proteins, BRCA1 has been implicated in a wide array of cellular functions, including cell cycle regulation, DNA damage response, maintenance of genomic stability, transcription regulation, replication, and recombination as well as...
controls transcription, DNA repair, and cell cycle checkpoint activation. BRCA1 ubiquitylation may be the underlying mechanisms by which BRCA1 are intertwined to control genomic stability. Chromatin remodeling and/or functions of BRCA1 and a proposed model of how these multiple functions are intertwined to control genomic stability. Chromatin remodeling and/or ubiquitylation may be the underlying mechanisms by which BRCA1 controls transcription, DNA repair, and cell cycle checkpoint activation.

Figure 1. BRCA1 is implicated in multiple cellular functions based on its proteins interactions. **A**, proteins known to interact or associate with BRCA1. Red, interactions with proteins involved in ubiquitylation; blue, proteins involved in transcription; green, proteins involved in cell cycle checkpoint control; light blue, proteins involved in DNA repair; gray, proteins involved in chromatin modifications. **B**, the multiple cellular functions of BRCA1 and a proposed model of how these multiple functions are intertwined to control genomic stability. Chromatin remodeling and/or ubiquitylation may be the underlying mechanisms by which BRCA1 controls transcription, DNA repair, and cell cycle checkpoint activation.

as higher chromatin hierarchical control (Fig. 1A and B; ref. 13). Nevertheless, it is unclear which loss of function contributes to cancer initiation and progression. In this review, we will discuss the major findings on the different functions of the BRCA1 protein and their significance regarding developing different treatment options for patients who are BRCA1 mutation carriers or for sporadic breast cancer patients with loss of BRCA1 expression in their tumors.

**Mouse Models**

Several mouse models for BRCA1-associated breast cancer were generated by creating different null mutations in the Brca1 gene. Unlike humans, mice hemizygous for Brca1 do not develop tumors at a higher incidence than wild-type mice. Homozygous targeted disruption of Brca1 caused embryonic lethality between E5.5 and E13.5 depending on the site of disruption, suggesting that Brca1 plays an important role in embryonic development (14). Interestingly, embryonic defects in the Brca1 gene activated the p53 pathway and resulted in increased expression of p53 and its downstream target, cell cycle inhibitor p21\textsuperscript{Waf/cip}.

Therefore, coinactivation of Brca1 with p53 or p21\textsuperscript{Waf/cip} partially alleviates the embryonic lethality of Brca1 nullizygous embryos (15, 16). Complete rescue was observed only when targeted deletion of exon 11 hypomorphic Brca1 allele was crossed with mice heterozygous for p53 (16). In this latter mouse model, p53 heterozygosity not only rescued the embryonic lethality but also enhanced tumor development. To overcome the embryonic lethality and gain insight into mammary gland development, mouse models with mammary gland–specific disruption of Brca1 were created (17). Analysis of mutant mammary glands revealed abnormal ductal and alveolar development and an increase in apoptosis of epithelial cells. Eventually, most tumors developed alterations in the p53 gene (16, 17). Clinically, many BRCA1 tumors harbor a mutant p53 gene (18, 19). This tendency suggests that loss of p53 is a programmed hit in the pathway of BRCA1 tumor development and that p53 hypermutability is a mechanism to increase genomic instability in BRCA1 tumors. These animal models represent a potentially useful tool for preclinical studies that wish to determine the specificity and efficacy of different therapeutic compounds or to test novel chemoprevention agents. However, outcome of experiments needs to be cautiously interpreted because heterozygosity of Brca1 in mice, unlike in humans, is insufficient for tumor development. Recently, two reports described the utilization of these mouse models for evaluation of tamoxifen treatment and oophorectomy on mammary tumor development. Apparently, oophorectomy was proven quite useful in reduction of tumor growth, similar to the effect seen in patients who undergo this procedure (reviewed in ref. 20). However, Brca1 deficiency enhanced the agonist characteristic of tamoxifen in the mammary gland as well as in the derived cell lines. Thus, the tamoxifen experiment in mice did not recapitulate data from a case-control study where tamoxifen was a useful preventive agent for reducing the risk of contralateral breast cancer in BRCA1/BRCA2 carriers (20–22).

**BRCA1 in DNA Damage Response and Cell Cycle Control**

Cell cycle checkpoints are surveillance mechanisms that halt cell cycle progression until DNA is intact to ensure genomic integrity passage between generations. BRCA1 expression is cell cycle regulated and its mRNA and protein are expressed mostly during S and G2-M phases (23). BRCA1 protein undergoes hyperphosphorylation during late G1 and S phases and in response to DNA damage (23). There are multiple phosphorylation sites of BRCA1 in DNA Damage Response and Cell Cycle Control (23). BRCA1 is phosphorylated mainly by the DNA damage sensors, the protein kinases mutated in the ataxia telangiectasia syndrome (ATM), ATM and rad3-related (ATR), and checkpoint 2 (Chk2), implicating it in the DNA damage
response (24–27). ATM and ATR phosphorylate BRCA1 at multiple serine residues within the SCD domain in vitro and in vitro following exposure to ionizing radiation, UV light, hydroxyurea, aphidocholin, and other genotoxic agents (24–27). There is some overlap between ATM and ATR phosphorylation sites, suggesting some redundancy in their function. However, there are also unique phosphorylation sites for each kinase, supporting a partially distinct signaling cascade that is initiated by each kinase depending on the stimuli. It has been suggested that the different phosphorylation sites (like in p53) direct the protein to participate in different multiprotein complexes and in different activities (28). To date, there are limited data as to how each phosphorylation event affects the function of BRCA1 in the DNA damage response and in the downstream signaling pathways. Phosphorylation on Ser1387 is essential for S-phase checkpoint activation following ionizing radiation and phosphorylation on Ser1423 is essential for G2-M checkpoint activation following ionizing radiation (29, 30). In addition, phosphorylation of Ser1453 and Ser1524 following UV damage was shown to regulate caspase-3 activation leading to apoptosis (31).

Chk2 kinase is a downstream target of ATM and ATR, and it phosphorylates BRCA1 in an ATM-dependent manner on a unique site outside of the SCD cluster (Ser1096; ref. 27). There is no evidence that Chk1, a downstream target of ATM/ATR and a fourth conserved kinase in the DNA damage pathway, phosphorylates BRCA1. On the contrary, we reported that BRCA1 is critical for Chk1 kinase activation and this activation is essential for the G2-M checkpoint activation following DNA damage (32). Our findings are supported by yeast data in which functional homologues of BRCA1, Rad9 of budding yeast, and Crb2/Rp9p of fission yeast act upstream of Chk1 and Cds1 (Chk2) and activate them in response to DNA damage (33, 34). Furthermore, BRCA1 physically interacts with Chk1 and this interaction was mapped to the BRCT domain of BRCA1, a motif recently identified as a phosphoprotein module (32, 35). Taken together, BRCA1 is an integral part of the DNA damage signaling cascade: downstream of ATM and ATR kinases and both downstream and upstream of the checkpoint protein kinases, Chk1 and Chk2, suggesting that there is a positive feedback loop to increase the magnitude of DNA damage response. In addition, BRCA1 regulates the expression and cellular localization of additional G2-M cell cycle checkpoint proteins, including Cdc25C and 14-3-3 protein (32), thus preventing unscheduled transition into mitosis at multiple levels of regulation. Many cancer cells depend on their G2-M checkpoint for survival, especially following exposure to DNA damage, because their G1-S checkpoint is defective due to oncogenic transformation. Therefore, it is reasonable to focus on abrogation of the G2-M checkpoint to induce apoptosis of cancer cells in response to DNA-damaging chemotherapeutic drugs. BRCA1 or its downstream effector, Chk1, may be useful molecular targets for sensitizing resistant sporadic tumors to chemotherapeutic drugs (Fig. 2).

BRCA1 and DNA Repair

Whether BRCA1 is involved in sensing and signaling DNA damage to DNA repair proteins or whether it plays a more proximal role in the repair process has not been resolved.

BRCA1 colocalizes in nuclear foci with Rad51, a human orthologue of the bacterial RecA, thereby implicating BRCA1 in homologous recombination (HR) during meiosis and repair of DNA double-strand breaks (DSB; ref. 36). These DSB arise following exposure to ionizing radiation or free radicals and following DNA damage during replication at the site of the replication fork. It is believed that DNA DSBs are the main cause of genomic instability and chromosomal rearrangements that are hallmarks of cancer. BRCA1 and BRCA2 are in complex with Rad51, but only BRCA2 binds directly to Rad51 via its BCR repeats and modulates its activity, thus playing a direct role in DNA repair (23).

In eukaryotes, there are two primary mechanisms of DNA DSB repair: HR and nonhomologous end-joining (NHEJ). HR is used in cells during the S and G2 phases of the cell cycle when sister chromatids are available as templates. NHEJ is a process of ligating DSB ends together without a homologous template and therefore is considered an error-prone mechanism. Studies reported by Moynahan et al. (37) and Snouwaert et al. (38) provided direct evidence linking BRCA1 to HR by showing a significant impairment of homologous repair in Brca1-deficient mouse embryonic stem cells. This impairment can be corrected by reexpression of wild-type BRCA1 (37, 38). Snouwaert et al. also reported an increase in the frequency of NHEJ in Brca1-deficient cells (38). Indeed, Brca1-deficient mouse models and BRCA1-deficient human tumors and cells exhibit centrosome amplification, gross chromosomal aberrations, and loss of genomic stability (39, 40).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Proposed strategies for therapeutic interventions tailored to specifically target BRCA1 tumors: PARP1 inhibitors inhibit repair of SSB and DSB in BRCA1 tumors, which become more sensitive to DNA-damaging agents. Lack of proper cell cycle checkpoint control renders BRCA1 tumors hypersensitive to platinum-based and DNA DSB-based chemotherapy. HDAC inhibitors restore gene expression that compensate for loss of BRCA1 and reduce the proliferative potential of tumors.
In addition, BRCA1 has been identified in a partially purified complex named BASC for BRCA1-associated surveillance complex (41). This mega-complex contains multiple proteins involved in DNA repair and cell cycle checkpoint control. Among the proteins in the complex are ATM, Mre11/Rad50/NBS1 (M/R/N) complex, Bloom syndrome protein, structural maintenance of chromosome proteins, and proteins directly involved in repair of DNA lesions, such as RFC and the complexes hMSH2-hMSH6 and hMLH1-hPMS2 that play a role in nucleotide excision repair. The BASC mega-complex may represent a combination of several complexes, as proteins from one DNA repair complex can shuffle and interact with proteins in other DNA repair complexes (28). BRCA1 interaction with the Mre11/Rad50/NBS1 complex was confirmed by direct binding. BRCA1 regulates the DNA single-strand resection activity of Mre11 (42). The Mre11/Rad50/NBS1 complex participates in HR, NHEJ, and checkpoint control. BRCA1 rapidly colocalizes to sites of DNA damage marked with phosphorylated histone H2AX (γ-H2AX; ref. 43). Additional repair proteins, such as Mre11/Rad50/NBS1 complex and Rad51, also colocalize with BRCA1 at these foci (43). Whether BRCA1 plays a role in DNA repair at the damaged sites or whether it plays a role in chromatin modifications, such as acetylation or ubiquitination (discussed below), around the DNA damage is not yet established nor is the exact function of γ-H2AX.

The involvement of BRCA1 and BRCA2 in DNA repair, and especially in repair of DSB, intrigued many researchers and clinicians to ask whether BRCA1 tumors and patients would respond favorably to treatments that generate DSB, such as ionizing radiation. Such treatment kills BRCA-deficient tumor cells more efficiently than sporadic tumor cells by loading them with damage that cannot be repaired and ultimately causes cell death (44). One caveat for this approach is that treatments are nonselective, and the accumulated DNA damage in the normal adjacent cells may pose a specific hazard for women with BRCA mutations because they carry only one normal allele. Ultimately, accumulation of nonrepaired DNA may induce malignant transformation of the adjacent cells. However, in a large clinical study, no significant differences in incidence of local reactions to radiation were reported in BRCA1/BRCA2 carriers versus noncarrier patients. Recurrence ratios following radiation treatments were shown to be similar in mutation carriers and in women without mutations (45).

A novel treatment idea for BRCA1/BRCA2 tumors takes advantage of the fact that single-strand breaks (SSB) near the replication fork are converted into a DSB, cause replication fork collapse, and trigger HR. Thus, BRCA1/BRCA2-deficient cells that are defective in homologous repair are more sensitive to SSBs than normal cells. Based on this notion, two groups have shown recently that BRCA1/BRCA2-deficient cells are extremely sensitive to inhibitors of the enzyme poly(ADP-ribose) polymerase 1 (PARP1) that is essential for SSB repair (46, 47). PARP1 is a conserved nuclear enzyme, predominantly involved in the recognition of DNA SSBs generated during base excision repair. PARP1 is responsible for activating DNA repair proteins by covalently attaching them with ADP-ribose moieties. Even more attractive is the notion that inhibition of the enzyme PARP1 is relatively nontoxic in itself but renders the cells hypersensitive when exposed to DNA-damaging agents or when DNA cannot be repaired because of a genetic defect in a repair gene. BRCA1/BRCA2-deficient cells were up to 1,000 times more sensitive to PARP1 inhibitors than wild-type cells, resulting in very high levels of apoptotic cell death (46, 47). Furthermore, the normal surrounding tissues around the tumor that retain the wild-type allele of BRCA1/BRCA2 are not susceptible to PARP1 inhibitors. Thus, this approach targets a specific cell population based on their lack of activity (Fig. 2).

**BRCA1 in Transcription and Chromatin Remodeling**

There is a growing list of BRCA1 transcriptionally regulated target genes that are involved in DNA damage response and repair. GADD45, a DNA damage-responsive gene and regulator of the G2-M checkpoint, is a transcriptional target gene of BRCA1. Conflicting results as to whether it is activated or repressed by BRCA1 seem dependent on the interaction between BRCA1 and the transcriptional repressor CtIP (48, 49). BRCA1 regulation of p53 and p53-regulated genes, such as the tumor suppressor gene p21<sup>waf1/cip1</sup> and GADD45 (48, 50), suggests both a convergence of the BRCA1 and p53 pathways and overlapping roles in checkpoint control and maintenance of genomic stability.

BRCA1 represses the transcription of estrogen receptor-α (ER-α) and its downstream estrogen responsive genes (51). BRCA1 regulation of ER offers a possible explanation to why BRCA1 mutations cause tissue-specific susceptibility in breast and ovary. Overexpression of cyclin D, which often occurs in sporadic breast cancer, antagonizes BRCA1 repression and relieves ER-α expression (52). Most BRCA1 carriers are ER negative and express low levels of cyclin D1 and additional ER targets, such as progesterone receptor and PS2. It was therefore assumed that the majority of BRCA1 tumors are estrogen insensitive and will not respond to hormonal therapy (53, 54). Theoretically then, tamoxifen should not reduce the incidence of BRCA1 tumors. The protective effect of tamoxifen for healthy women at high risk of breast cancer was evaluated by the National Surgical Adjuvant Breast and Bowel Project P1. Overall, tamoxifen provided a protective effect and reduced the risk of invasive breast cancer by about half for women at risk. No protective effect was observed for ER-negative women, including BRCA1 carriers, although only 8 BRCA1 carriers and 11 BRCA2 carriers were identified in the study (55). The small number of cases requires caution interpreting the data. In a later large case-control study, the use of tamoxifen reduced the risk of contralateral breast cancer in BRCA1 and BRCA2 carriers by about one half (20). Although the mechanism by which
tamoxifen reduces risk in ER-negative tumors is not understood, the authors believe that it is reasonable to offer tamoxifen to young women with BRCA1 or BRCA2 mutations and intact ovaries for the prevention of contralateral breast cancer.

Initially, it was reported that BRCA1 binds to DNA structures as Holliday junctions and cruciforms, whereas a subsequent study reported that BRCA1 binds to specific DNA sequences (56). Nevertheless, it is believed that BRCA1 modulates transcriptional control mainly via its interactions with many different transcription factors, such as p53, c-Myc, ChIP, ER, and ZBRK1, as well as with the RNA helicase A, a subunit of RNA polymerase holoenzyme (Fig. 1A; ref. 13). BRCA1 may link the basal transcription machinery with the different site-specific transcription factors. Taken together, BRCA1 up-regulates tumor suppressors and growth-inhibitory genes and represses cell proliferation genes, serving as a transcriptional coactivator and a corepressor depending on the specific target gene.

Clinically relevant mutations in the BRCT domain at the COOH terminus of BRCA1 disrupt its structure and reduce its transcriptional activity (57). Interestingly, these mutations also disrupt the binding of BRCA1 to the histone deacetylase (HDAC) complex (58). The ability of BRCA1 to bind chromatin-modifying enzymes, including HDAC, histone acetylase, Rb-associated proteins, and chromatin remodeling factors SWI/SNF (58–60), offers the plausible mechanism that BRCA1 regulates transcription via chromatin modifications and these two activities are essential for BRCA1 tumor suppression activity. Two working models could be offered: BRCA1 may recruit HDAC complexes to specific promoters to modulate local chromatin structure, thereby making specific sites less accessible for transcription factors and repressing transcription of a particular gene. Conversely, BRCA1 may activate transcription by displacing HDAC complexes from specific promoters. The two opposing roles of BRCA1 in chromatin condensation and activation are also supported by its interaction with the acetylation factor CBP/p300 (59, 60). Equally important is the relationship between chromatin modifications and DNA repair. As mentioned above, BRCA1 is recruited to nuclear foci of phosphorylated γ-H2AX sites that flag DSB immediately after they occur (43). Thus, the multiple functions of BRCA1 in DNA repair, DNA damage response, and transcriptional control may be due to a common underlying mechanism of chromatin remodeling. To date, however, there is no direct evidence that specific genes are regulated by BRCA1-dependent chromatin modifications on their local chromatin structure either in steady state or in response to any stimulus. Identification of such genes will provide essential information on the mechanisms by which BRCA1 suppresses tumor development. Histone acetylation is tightly regulated by two classes of enzymes: histone deacetyltransferases and HDACs. It is believed that deregulated histone acetyltransferase and HDAC activity plays a role in the development of a range of cancers (61). Consequently, inhibitors of these enzymes may serve as potential anticancer agents. Several HDAC inhibitors have been described, whereas only a few inhibitors of histone acetylation transferases have been disclosed (62). The attractive antitumor model of HDAC inhibitors is based on an increase in histone acetylation that leads to the transcriptional activation of few genes (<2% of the expressed genes), the expression of which causes inhibition of tumor growth. Among the frequently induced genes are the cell cycle inhibitors p21^wan/cip, p16^ink4a, and p27^kip. At the same time, an equal or greater number of genes is repressed, including cyclin D1 (63). Interestingly, some of these genes are downstream targets of BRCA1 or interfere with BRCA1 function (50, 64) Thus, HDAC inhibitors represent potentially successful treatment/prevention options for BRCA1 tumors by compensating for lack of BRCA1 expression and possibly restoring growth control in BRCA1-deficient tumor cells (Fig. 2).

**BRCA1, Stress Response, and Apoptosis**

Several studies have implicated BRCA1 in the regulation of apoptosis following cellular stress and DNA damage. Ectopic overexpression of wild-type BRCA1 induced c-Jun NH2-terminal kinase/stress-activated protein kinase as well as Fas/Fas ligand-dependent apoptosis (48, 66). Conversely, lack of both BRCA1 expression and ectopic expression of dominant-negative truncated BRCA1 diminished the apoptotic effect (65). Chemotherapeutic drugs commonly used in treatment of breast cancer act by producing DNA damage that ultimately leads to programmed cell death of tumor cells. Therefore, it is a high priority to identify the drugs that are most effective for BRCA1 mutation carriers. Several studies showed that apoptotic response of breast cancer cells, following treatment with antimicrotubule drugs, such as taxanes (paclitaxel and docetaxel) and vinorelbine, is dependent on the expression of functional BRCA1 (44). This suggests that women with BRCA1 mutations would not be good candidates for the antimicrotubule drugs type of chemotherapy.

In contrast, multiple studies have shown that BRCA1-deficient cells exhibit a radiosensitive phenotype following exposure to DNA-damaging agents that cause DSB like ionizing radiation, the radiomimetic drug bleomycin, and the topoisomerase inhibitors etoposide and camptothecin. Meanwhile, restored expression of functional BRCA1 increases apoptotic resistance to DSB-damaging agents (44, 66). This is most likely due to involvement of BRCA1 in G2-M checkpoint and HR repair of DSB that allow the cells to survive. The mechanisms by which BRCA1 induces apoptosis are not all understood. A correlation between BRCA1 and the antiapoptotic Bcl2 expression in breast tumors was reported (67). In addition, on UV radiation, BRCA1 phosphorylation on Ser1423 and Ser1524 regulates caspase-3 activity and apoptosis (31).

It was recently established that BRCA and Fanconi anemia (FA) proteins act in a common DNA damage/signaling pathway as they interact with common proteins...
the protein complex and activates its autoubiquitination (72, 74) that paradoxically stabilizes studies indicate that the BRCA1-BARD1 complex is capable of linkage through Lys6 (K6), instead of the conventional complex is polyubiquitinated (72, 74). A special ubiquitin BRCA1-BARD1 complex is more efficient when the activity. This is apparent as ubiquitination of H2A by the BRCA1, such as response to DNA damage and ability to mutations in the RING domain inactivate BRCA1 E3 ligase finger and a BRCT domain (Fig. 1). Cancer-predisposing finger of BARD1, a BRCA1-associated protein with a RING and is enhanced when it heterodimerizes with the RING drug cisplatin (66). Germ-line mutations in the BRCA genes also increase breast and ovarian tumor sensitivity to carboplatin (20, 69). A clinical trial is under way to assess the effectiveness and safety of carboplatin treatment of metastatic breast cancer in women who are BRCA1/BRCA2 carriers (70).

**BRCA1 and Ubiquitination**

Ubiquitination, a post-translational modification process of covalently attaching ubiquitin groups to lysine residues in proteins, most often targets those proteins for destruction by the proteasome. In recent years, it became apparent that ubiquitination serves a wider array of functions. Proteins modified by polyubiquitin chains are often destined for degradation, whereas monoubiquitination is often contemplated as a regulatory modification (71). Monoubiquitination of histones similar to other histone post-translational modifications may alter chromatin structure and affect transcriptional control, gene expression profiles, and DNA repair. BRCA1 E3 ligase activity is localized to the RING finger domain at the NH2 terminus of the protein (9, 72) and is enhanced when it heterodimerizes with the RING finger of BARD1, a BRCA1-associated protein with a RING finger and a BRCT domain (Fig. 1). Cancer-predisposing mutations in the RING domain inactivate BRCA1 E3 ligase activity and affect other tumor suppressor activities of BRCA1, such as response to DNA damage and ability to activate the G2-M checkpoint (73).

The enzymatic specificity and the substrates of BRCA1 E3 ligase activity and how they are related to its tumor suppression activity are still unknowns. In vitro and in vivo studies indicate that the BRCA1-BARD1 complex is capable of autoubiquitination (72, 74) that paradoxically stabilizes the protein complex and activates its in vitro E3 ligase activity. This is apparent as ubiquitination of H2A by the BRCA1-BARD1 complex is more efficient when the complex is polyubiquitinated (72, 74). A special ubiquitin linkage through Lys6 (K6), instead of the conventional linkage through Lys48 (K48), might be the key difference between degradation and stabilization of the polyubiquitinated BRCA1. The usage of K48 linkage that is commonly used when proteins are targeted for degradation has not yet been reported for BRCA1 but cannot be ruled out for different BRCA1 target proteins. Several reports show that BRCA1 E3 ligase is also capable of in vitro monoubiquitination of histones H2A and its subtype H2AX (73, 74). If proven by in vivo studies, this will add another level of chromatin structure regulation by BRCA1 in the context of transcriptional regulation and DNA repair.

Two recent reports suggest nucleoplasmin B23 (75) and γ-tubulin (76) as in vivo substrates of BRCA1 E3 ligase activity, although the specificity of the latter could not be shown by in vitro studies (76). Both proteins are present in centrosomes and apparently are not targeted for degradation by BRCA1-mediated modifications. Thus, the significance of their ubiquitination by BRCA1 to its tumor suppression function remains unknown. As BRCA1-null cells exhibit centrosome amplification (39), the authors suggest that ubiquitination of nucleoplasmin B23 and γ-tubulin plays a role in regulating centrosome number and maintenance of genomic stability by unknown mechanisms.

Phosphorylation of BRCA1 either during S phase or following DNA damage may activate its own E3 ligase activity. A phosphorylation-enhanced ubiquitination model has been reported for MDM2, a RING finger protein with an E3 ligase activity that is enhanced following its phosphorylation by AKT (77).

Preliminary results from our laboratory suggest that BRCA1-BARD1 is an E3 ligase of several cell cycle proteins both in vivo and in vitro, capable of their polyubiquitination and their targeting for degradation via the proteasome. Such activity of BRCA1 may explain the mechanism by which it regulates cell cycle checkpoint control following DNA damage. An inhibitor of the proteasome may in principle abrogate the degradation of these proteins. Thus, combination of a proteasome inhibitor with radiation or chemotherapeutic drugs might potentiate resistant BRCA1-proficient breast cancer cells to therapy (Fig. 3).

BRCA1 interacts with a ubiquitin-hydrolase protein, BAP1 (78), and so ubiquitination-deubiquitination of the BRCA1-BARD1 complex or its substrates may be tightly regulated by BRCA1. In addition, BRCA1 colocalizes with the monoubiquitinated and activated FA protein, FANCD2, in nuclear foci during S phase and following DNA damage. This latter association seems to modulate HR DNA repair (68).

Some questions regarding the activity of the BRCA1-BARD1 E3 ligase in vivo remain unsolved. The most obvious issue is to identify specific cellular substrates that will lead to better understanding of BRCA1 functions in cells and in tumor suppression. It is plausible that, similar to chromatin modifications, ubiquitination of diverse substrates is the mechanism underlying BRCA1 multiple roles in the maintenance of genomic stability.

**Conclusions: BRCA1 as a Molecular Target**

Understanding and exploiting the genetic reasons why some tumors respond to treatment, whereas others do not is a central goal of current medical research. Many of the
conventional anticancer treatments kill cells by damaging DNA or disrupting cell division. Because these treatments are nonselective for cancer cells, patients often suffer from adverse effects. Efforts to specifically target cancer cells by taking advantage of their lack of DNA repair activity or by abrogating their G2-M checkpoint and sensitizing them to conventional treatments are under way.

Much has been learned on BRCA1 function in the past decade following its identification. However, the precise function of BRCA1 as a tumor suppressor is not fully understood. Accumulating evidence suggests that BRCA1 is involved in DNA damage response via multiple cellular and biochemical functions, such as DNA repair, transcription, chromatin modifications, cell cycle control, and ubiquitination. The multiple functions of BRCA1 suggest that it is using numerous pathways to ensure genomic stability. Thus, the potential for multiple intervention strategies for therapy and prevention of BRCA1 tumors may exist depending on particular secondary hits and tumor characteristics (Fig. 2). Identifying those events will enable development of specific therapeutic approaches. Even with the current knowledge of BRCA1 function, knowing the BRCA1 status of a tumor may be invaluable in deciding which type of chemotherapy to use. Numerous studies illustrate that cancer cells without a functional BRCA1 protein are resistant to antimicrotubule drug-based chemotherapy but are sensitive to DNA-damaging agents that cause DSb (bleomycin and ionizing radiation) and interstrand cross-linking agents as platinum-based drugs (cisplatin and carboplatin). More excitingly, Farmer et al. and Bryant et al. showed in animal models how it is possible to exploit the loss of HR function in BRCA1/BRCA2 tumors to specifically target the tumor tissue with minimal or no side effects to the surrounding tissues (46, 47). Therefore, it is important to continue elucidating BRCA1-dependent pathways to design specific therapies capable of targeting those specific tumors. In addition, there may be merit in targeting BRCA1 function in combination with radiotherapy or chemotherapy as a second line of therapy in sporadic breast cancer that has become resistant to these treatments to increase sensitivity of tumor and overcome their resistance.

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Molecular Cancer Therapeutics

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*Mol Cancer Ther* 2006;5:1396-1404.

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