

Mechanisms of BRCA1 Tumor Suppression

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ABSTRACT

The p220 BRCA1 tumor suppressor protein has been implicated in multiple biochemical and biologic functions since its molecular cloning 18 years ago. Here, we discuss those functions most relevant for its tumor-suppressing activities with an emphasis on new findings. In particular, this review focuses on what is known of the activities of those BRCA1-binding partners that have tumor suppressor functions, on the reversion of mutant *BRCA1* alleles concomitant with therapy resistance, on insights gained from studies of BRCA1 structure–function relationships, recent findings from animal models, and the potential role of BRCA1 in some nonhereditary tumors. From this information, a more detailed and refined picture of BRCA1 tumor suppression is beginning to emerge. Although key mysteries remain—such as why BRCA1 tumor suppression is focused on carcinomas of the breast and ovary—the pace of discovery is increasing.

Significance: BRCA1 functions as a clinically important classical tumor suppressor in hereditary breast and ovarian cancer; here, we review progress in understanding how BRCA1 operates to suppress tumor formation. *Cancer Discov*; 2(8); 679–84. ©2012 AACR.

INTRODUCTION

BRCA1 gene products are responsible for tissue-specific, clinically important tumor suppression. They are also vital participants in cellular responses to DNA damage. The *BRCA1* full-length gene product, p220, is a chromatin-interacting protein and operates as an E3 ubiquitin ligase when complexed with a heterodimeric partner, BARD1. How p220 delivers its tumor suppression function is the subject of this review.

Recent advances have led to a more refined picture of those functions of the protein most relevant for tumor suppression. Any such model should take into account a number of elements. First, it should reflect the presence of other tumor-suppressing proteins present in BRCA1-containing protein complexes and what is known about them. Second, it should encompass the recently discovered role of somatic *BRCA1* reversion mutations that emerge in response to therapy. Third, it must consider the known structure–function relationships of BRCA1. Fourth, it must be consistent with observations of BRCA1 dysfunction in relevant animal models. Finally, it should be compatible with what is known about sporadic tumors that are characterized by defective BRCA1 function.

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TUMOR SUPPRESSORS IN BRCA1 COMPLEXES

The best studied *BRCA1* gene product, p220, operates, at least in part, as a member of multiple, distinct protein complexes. Many BRCA1-interacting proteins have been reported (1–3); it is unlikely a coincidence that a number of the proteins in these complexes are themselves tumor suppressors, including BRIP1, ABRAXAS, PALB2, RAD50, NBS1, and BRCA2 (2, 4–12). PALB2 binds to the BRCA1 coiled coil domain (Fig. 1) and forms a bridge to BRCA2 (13, 14), whereas ABRAXAS (12) and BRIP1 (8) bind to the BRCA1 BRCT regions (Fig. 1), and RAD50/NBS1 have been reported to bind to BRCA1 residues 341–748 (9). Although these proteins carry out a variety of activities, one function that they share is a role in DNA repair, strongly implicating this process as an activity central to tumor suppression driven by them. The tumors that arise at increased frequency in those individuals born heterozygous for any of these genes tend to arise in the breast, ovary, pancreas, or prostate.

By comparing the characteristics of tumors that arise in the context of defects in each of these BRCA1-complex members, insight into gene-specific functions can be gained. For example, heterozygosity for most of the genes listed above usually results in tumors of the breast that are estrogen receptor (ER)-positive, whereas approximately 80% of the tumors arising in *BRCA1* heterozygotes are ER-negative (15, 16). These observations suggest that, in addition to a core DNA repair function shared by the other members of the above noted complexes, BRCA1 possesses functions that, when lost, alter the phenotype of a resulting tumor. A number of hypotheses could explain this observation. One suggests that BRCA1 is essential for ER expression (17) or function (18); however,

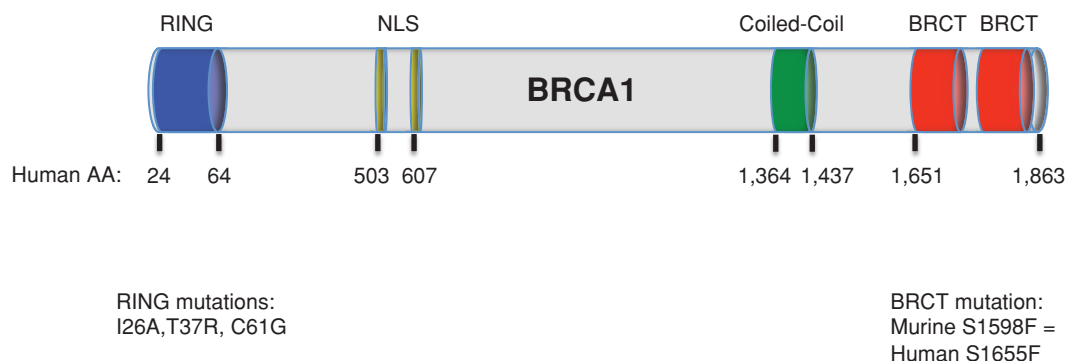


Figure 1. The domain structure of BRCA1 is shown, with the RING domain in blue, the tandem nuclear localization signals in yellow, the coiled coil domain in green, and the 2 BRCT domains in red. Amino acid (AA) locations of mutations referred to in the review are as shown.

BRCA1-related breast cancers tend to be of the basal subtype, which have a fundamentally different pattern of gene expression compared with ER-positive tumors, including those related to BRCA1 complex members, for example, BRCA2. The wholesale changes in the pattern of gene expression comparing basal to ER-positive breast cancers suggest that BRCA1-related tumors arise in a different precursor cell from ER-positive tumors, rather than arising in the same cell of origin which then suffers loss of the ER expression or function. Furthermore, approximately 20% of breast cancers arising in *BRCA1* heterozygotes are ER-positive, and these tumors lose the wild-type (wt) copy of *BRCA1* at the same frequency as ER-negative *BRCA1*-related breast cancers, arguing against the possibility that wt BRCA1 is required for ER expression (19). One intriguing article provides evidence suggesting that *BRCA1* loss perturbs the normal differentiation of mammary epithelial cells, which could, in turn, lead to a shift in the phenotype of the population of cells at risk for tumorigenesis after *BRCA1* loss (20). This shift could also alter the phenotype of a resulting cancer. Compatible with this hypothesis is the observation that *BRCA1* heterozygous individuals seem to have accumulated altered populations of breast precursor cells (21, 22). Conceivably, BRCA1 conducts a specific function in breast differentiation. On the other hand, it is possible that the DNA repair defect in breast stem or progenitor cells, resulting from either *BRCA1* heterozygosity or *BRCA1* loss, is more severe than the repair defects arising from lesions in BRCA1 complex-containing proteins. This more severe defect might be better tolerated in certain cell types, leading to the cell of origin shifts that are seen in *BRCA1*-related tumors. In this regard, recent evidence connects DNA damage and ensuing differentiation-associated checkpoint activity to the control of hematopoietic stem cell behavior (23).

REVERSION MUTATIONS

Another recent observation relevant to BRCA1 tumor suppression is the phenomenon of genetic reversion. Two groups have made pioneering observations regarding the ability of *BRCA1*- and *BRCA2*-mutated ovarian tumors to revert to a DNA repair competent state in response to treatments that target BRCA1/2-related DNA repair deficiencies (24–26). One class of genetic reversion events causes deletion of a

frameshift mutation or other compensatory changes that result in the correction of the original frameshift, producing an allele that contains a new mutation, but now encodes a DNA repair-proficient BRCA protein. A second class of mutations involves direct reversion to wt of the original mutation. These events appear to account for up to 46% of platinum resistance in hereditary ovarian cancer with the most common event being reversion of a mutated allele to wt within the mutated haplotype (27). Another related phenomenon has recently been reported in abstract form. Working with a xenograft of a sporadic triple negative breast cancer that did not express *BRCA1*, likely because of the presence of *BRCA1* promoter methylation, one group selected PARP inhibitor-resistant subclones. PARP inhibitors are thought to target BRCA1-deficient cells by creating DNA lesions that require BRCA1-directed homologous recombination to repair (28). These clones had translocated the coding sequence of *BRCA1* to an active promoter elsewhere on the same chromosome arm, restoring *BRCA1* expression (29). In all of these examples, BRCA1 function was restored, but there was no evidence that a fundamental property of the tumor other than therapy resistance had changed. Moreover, ovarian tumors that became platinum-resistant on this basis continued to spread. Therefore, the BRCA1-related activities required for tumor suppression need to be missing at some critical period during tumor development, but they no longer need to be missing after that critical point. In this setting, continued absence of BRCA1-related tumor suppression activity, at least in ovarian cancer, is not required for the maintenance of tumorigenicity. Even if BRCA1 tumor suppression activities are reexpressed, it is conceivable that tumor cells have developed functions that nullify them. For example, BRCA1 has been reported to operate in transcriptional control, and recent findings show that it participates in the transcriptional silencing of centric and pericentric heterochromatin, and that the latter contributes to tumor suppression (ref. 30; see below). One might imagine that the prolonged absence of these activities results in stable epigenetic changes that override elements of restored BRCA1 tumor-suppressing function.

The presence of reversion mutations is also completely compatible with models of tumor suppression that bring the role of BRCA1 in DNA repair to the fore. This is because the absence of DNA repair activity for a window of time during

tumorigenesis can result in genome instability that facilitates the development of permanent oncogenic changes in the genome. Subsequent restoration of DNA repair activity would have no effect on these changes.

FUNCTIONAL DOMAIN STUDIES IN ANIMAL MODELS

Two recent papers underscore some of the complications involved in assigning tumor suppressor functions to specific biochemical features of the polypeptide. Two domains of BRCA1 that are frequently mutated in families with hereditary breast and ovarian cancer are the N-terminal RING domain and the C-terminal BRCT repeats (Fig. 1). Both are suspected of contributing to BRCA1 tumor suppression function.

The RING domain is known to possess E3 ubiquitin ligase activity when heterodimerized with BARD1, another RING- and BRCT domain-containing protein. Relevant physiologic targets of this E3 activity are not yet clear. Interpretation of the phenotypes of BRCA1 RING domain mutations is complicated by the fact that at least some mutations of this domain prevent heterodimerization with BARD1, and the protein stability of both BRCA1 and BARD1 is significantly reduced without heterodimerization (31, 32). As a result, the precise biochemical functions of the RING domain necessary for tumor suppression have been the subject of debate.

The C-terminus of the protein contains tandem BRCT domains, which are a second structural element of BRCA1 sometimes mutated in hereditary breast and ovarian cancer. These are phosphopeptide-binding domains (33, 34), mediating interactions with certain key BRCA1 partner proteins including CtIP, ABRAXAS, and BRIP1. These domains are required for BRCA1 concentration in subnuclear repair foci that are present after DNA damage (12, 35, 36).

Shakya and colleagues examined the ability of *Brca1* alleles mutated in either the RING domain or the BRCT motifs to carry out tumor suppression in 3 separate mouse models dependent on *Brca1* tumor suppression (37). These investigators focused on the ability of an experimental RING domain mutation, I26A, to carry out functions important for genome integrity maintenance as well as tumor suppression. They found that cells homozygous for this mutation, which is thought to block ubiquitin ligase function but not to prevent *Brca1/Bard1* heterodimerization, are apparently normal with respect to proliferation, chromosome stability, senescence induction, centrosomal content, spindle formation, resistance to DNA-damaging agents, and the formation of characteristic polyubiquitin-containing foci at sites of damage. Furthermore, this allele functions identically to wt *Brca1* in its ability to suppress BRCA1-related tumor formation in 3 separate mouse models. In contrast, a mutation in the BRCT domain, S1598F, was clearly defective in genomic integrity control and was unable to function as a tumor suppressor in the same 3 models of BRCA1-dependent tumorigenesis. The authors conclude that E3 ligase activity is not necessary for tumor suppression, unlike the phosphopeptide binding activity of the BRCT domain (37).

Interpretation of these experiments is complicated by lack of detailed information regarding the absolute nature of the

biochemical defect associated with the I26A mutation. By at least some measures, I26A is a hypomorph, perhaps retaining some residual E3 ligase activity. For example, Joukov and colleagues (38) described a role for BRCA1 in the formation of mitotic spindle. Ubiquitin ligase activity is thought to be important for this function. The addition of intact, I26A BRCA1 to *Xenopus* oocyte extracts rigorously depleted of wt BRCA1 led to partial, as opposed to full rescue of the spindle phenotypes observed in BRCA1-depleted extracts, suggesting some residual activity of this mutant (38). Adding to the difficulty associated with an *in vivo* assessment of residual ubiquitin ligase activity in any particular mutant BRCA1 protein is the molecular complexity of the ubiquitin ligase reaction and our ignorance of the relevant substrates for BRCA1 ubiquitin ligase activity. Without knowledge of the relevant E2 enzymes and E3 substrates, it is difficult to assign a pathophysiologic, nonfunctional phenotype to a particular BRCA1 RING mutation.

Jonkers and colleagues have investigated the effect of a different RING mutation, C61G, upon tumor suppression and therapy responsiveness (39). This RING mutant, one of the more common, pathogenic BRCA1 missense mutations in human cancer, destabilizes the BRCA1/BARD1 heterodimer in humans (31, 32) and grossly impairs ubiquitin ligase activity. They found that mice homozygous for this mutation, in a mutated p53 background, developed tumors even more rapidly than animals carrying a *Brca1*-null allele, confirming in a mouse model what is clear in patients: C61G is disease-related. Comparing tumors from C61G and null alleles, genomic disarray was indistinguishable among these 2 tumor species, as judged by comparative genomic hybridization (CGH), suggesting the existence of comparable defects in the maintenance of genomic integrity. Of interest, Western blots of several tumors suggested that murine *Brca1* C61G is about as stable as the murine wt protein; the degree of heterodimerization with murine *Bard1* is unknown at present. Orthotopic transplantation of *Brca1*-null or C61G tumors killed recipient mice equally quickly; however, treatment with the PARP inhibitor, olaparib, extended the life of mice bearing *Brca1*-null tumors considerably longer than mice with C61G tumors, suggesting that C61G retains some DNA repair activity and is, therefore, a hypomorphic mutation. Notably, C61G tumors exhibited an intermediate response between very sensitive *BRCA1*-null and *BRCA1*-wt tumors, which were unresponsive to olaparib. In addition, *Brca1*-null tumors never developed cisplatin resistance, a treatment that creates DNA interstrand crosslinks requiring *Brca1* function to repair. In contrast, C61G tumors developed therapeutic resistance approximately 50% of the time, and the resistance did not appear to be mediated by increased C61G expression or reversion, again suggesting that it is a hypomorph (39).

Certain DNA repair-related results support this hypothesis. For example, C61G homozygous cells revealed an intermediate number of RAD51 foci after gamma irradiation compared with wt (many foci) and null BRCA1 cells (none). Furthermore, C61G cells exhibited fewer pH2AX foci (H2AX is a variant histone which becomes phosphorylated at and near the sites of DNA damage; ref. 40) after olaparib treatment compared with null cells (39), possibly because of an increased ability to repair the DNA damage resulting from

this form of therapy. In summary, this work shows that while murine C61G seems to be completely deficient in tumor suppression, it still maintains some degree of residual Brca1-related DNA repair function.

The existence of such mutations with defective tumor suppression but hypomorphic DNA repair function creates a new level of complexity for the development of therapeutics targeting BRCA1-deficient tumor cells, as the absence of certain BRCA1-dependent repair processes (e.g., repair of double-strand breaks by homologous recombination or DNA crosslink repair) is required for drug sensitivity (41, 42). Thus, it will be interesting to see how provocative data from mouse models, like those described above, translate to the clinic. Patients with the C61G mutation have been enrolled in ongoing clinical trials of cisplatin. At least one such patient treated with neoadjuvant cisplatin experienced complete elimination of detectable tumor (43).

NEWLY DISCOVERED FUNCTIONS OF BRCA1

Other recent observations have led to a new hypothesis regarding BRCA1 tumor suppression activity (30). BRCA1 knockout or depletion was observed to decrease the number and morphology of pericentromeric heterochromatic foci in a variety of mouse cells and, concomitantly, derepress transcription of satellite repeat sequences in mouse and similar sequences in human cells. Effective repression of this transcription required intact, BRCA1-directed ubiquitin ligase activity, as 2 RING mutants, T37R and I26A (cf. above), failed to function in this regard. Furthermore, effective repression of satellite RNA transcription required BRCA1-directed ubiquitination of H2A. In a particularly powerful demonstration of this finding, the authors showed that a synthetic protein consisting of H2A coupled to ubiquitin overcame the failure of transcriptional repression in the absence of BRCA1. Finally, synthetic overexpression of satellite RNA induced increased abnormal mitotic figures, centrosome amplification, H2AX foci, and mitotic spindle checkpoint defects, all phenotypes which have been associated with BRCA1 deficiency. The authors strengthened further their case for a role of BRCA1 repression of heterochromatic transcription in BRCA1 tumor suppression by showing that human *BRCA1*-mutated tumors often reveal increased transcription of satellite sequences and that BRCA1 T37R, which forms an effective heterodimer with BARD1, failed to carry out any heterochromatic functions of BRCA1 (30). In addition to these interesting and novel observations, other studies support a role for BRCA1 in the life of heterochromatin. For example, studies have shown that BRCA1 participates in maintaining proper XIST RNA localization on one X chromosome in somatic cells (44, 45), a process important for maintenance of the fully heterochromatic state of that chromosome (46, 47).

Because BRCA1 likely participates in tumor suppression, at least in part, through DNA repair/genome integrity functions and its role in pericentric heterochromatic repression, questions related to the interconnectedness of the 2 processes are pertinent. For example, do certain members of the various BRCA1-containing complexes active in DNA repair and tumor suppression contribute to heterochromatic

transcriptional suppression? Furthermore, do other mutants of BRCA1 known to lose tumor suppression also lose their ability to carry out heterochromatic transcriptional suppression functionality? In addition, does the presumed satellite promoter reactivation that occurs in BRCA1-depleted/mutant cells depend upon coemerging DNA damage?

ROLE AT THE REPLICATION FORK

Two recent articles support the role of BRCA1 activity at the replication fork as being central to its DNA repair function and likely to its tumor suppression activity as well. Pathania and colleagues (48) have showed a new role for BRCA1 in DNA repair, that is, the removal of bulky adducts such as UV photoproducts from stalled replication forks and the resolution of these abnormal structures. In this context, BRCA1 plays a role in executing checkpoint function, enabling damage excision and repair, and suppressing error prone translesional polymerase-mediated replication. This work highlights the possibility that bulky adducts may, in some circumstances, come under the purview of BRCA1-mediated repair and, thus, that this process may be related to tumor suppression. One intriguing hypothesis suggested by this observation is that certain estrogen metabolite DNA adducts might be repaired in this manner, perhaps a mechanism which links BRCA1 dysfunction to the subsequent development of cancers in estrogen-responsive tissues. Notable in this regard are results showing that breast cancer development in BRCA1-affected women is an estrogen-dependent process (49, 50).

Recently, Birkbak and colleagues explored the nature of the imprint that BRCA1 loss leaves on the tumor genome (51). Nonhomologous end joining and other processes that may be used in the absence of BRCA1-mediated homologous recombination can lead to the production of telomeric chromosomal fragments no longer connected to centromeres; these are often present in quadriradial chromosomes, which are abnormal chromosomal structures prevented by BRCA1. Such acentric fragments are frequently lost after cell division, leading to the prediction that BRCA1-deficient tumors will display a high level of allelic imbalance extending to telomeres. In fact, increased telomeric allelic imbalance was observed in *BRCA1*-mutated ovarian cancers, and in sporadic triple-negative breast cancers telomeric allelic imbalance correlated with low BRCA1 mRNA levels (51), a deficiency believed to be important for the development of these tumors (52). Breakpoints leading to these regions of telomeric allelic imbalance were found to be located at a disproportionately high rate in the vicinity of regions of copy number variation (CNV; ref. 51), which themselves are thought to arise from replication errors arising at genomic locations that cause fork stalling (53). Thus, this work again points to a defect in the recovery of stalled replication forks as being important to the biology of tumors deficient in BRCA1.

These telomeric imbalance results highlight the possibility that a fraction of sporadic breast cancer is a product of noninherited BRCA1 dysfunction. This observation suggests that a BRCA1-related failure of tumor suppression is shared between hereditary breast and ovarian cancers and a subset

of sporadic cancers of these organs where germ line *BRCA1* mutations are absent. This is a situation that is, in part, related to that manifest by other tumor suppressor genes involved in hereditary cancer syndromes that also contribute to nonhereditary tumor development, for example, *VHL*, *APC*, certain mismatch repair genes, and *RB*.

CONCLUSIONS

Eighteen years after the molecular cloning of *BRCA1*, the majority of the evidence strongly favors the maintenance of genomic integrity as a principal tumor suppressor activity of *BRCA1*. There is also growing evidence that correlates *BRCA1* function with proper mammary epithelial differentiation, and it too may prove to be a contributor to *BRCA1* tumor suppression. Whether this and its genome integrity maintaining function are linked remains to be seen.

Subtleties differentiate the tumor-suppressor activity of *BRCA1* from that of its partners in biochemical complexes. A detailed understanding of exactly which biochemical activities of *BRCA1* are required for tumor suppression still eludes us; new activities in the repression of transcription of heterochromatin and in bulky adduct repair add to the complexity of pinpointing relevant functions but offer useful, new insights. Because a detailed understanding of the relevant biochemical activities is still missing, predictions about which functional domains of *BRCA1* are required for tumor suppression are difficult, but the clinical importance of RING and BRCT domain mutations is indisputable, as inherited mutations in each of these domains lead to the hereditary breast and ovarian cancer syndrome.

Furthermore, significant, evidence-based insight into the remarkable tissue specificity of *BRCA1* tumor suppression is lacking. Whether the *BRCA1* heterozygous state is more important (i.e., in being haploinsufficient) to tumorigenesis than simply being one step closer to total loss-of-function remains unknown. Remarkably, we are still unsure of when loss of the wt copy of *BRCA1* occurs during the genesis of a tumor relative to other changes. Ultimately, progress in understanding the biochemical nature of the tumor-suppressing functions of *BRCA1* is likely to help physicians to distinguish clinically relevant mutations from harmless *BRCA1* polymorphisms and to aid in the clinical management of those individuals who have inherited a pathologic *BRCA1* mutation.

Disclosure of Potential Conflicts of Interest

D.M. Livingston has a commercial research grant from Novartis and is a consultant/advisory board member for Novartis and Nextech Venture. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: D.P. Silver, D.M. Livingston
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.P. Silver, D.M. Livingston
Writing, review, and/or revision of the manuscript: D.P. Silver, D.M. Livingston

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