BRCA1, BRCA2, and Rad51 Operate in a Common DNA Damage Response Pathway

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Abstract

The two major hereditary breast cancer susceptibility genes, BRCA1 and BRCA2, are associated with early-onset breast and/or ovarian cancer and encode products that each interact with the product of the eukaryotic RecA homologue, hRad51. We have recently found that BRCA1 and BRCA2 coexist in a common biochemical complex. The two proteins also colocalize in subnuclear foci in somatic cells as well as on the axial elements of developing synaptonemal complexes in meiotic cells. Thus, BRCA1 and BRCA2 participate in a common DNA damage response pathway associated with the activation of homologous recombination and double-strand break repair. Dysfunction of this pathway may be a general phenomenon in the majority of cases of hereditary breast and/or ovarian cancer. The BRCA1/BRCA2 complex may function in postreplication repair processes activated during the DNA synthesis stage of the cell cycle.

Introduction

The molecular basis of familial breast/ovarian cancer predisposition has recently been brought into sharper focus with the cloning of two genes that predispose an individual to these diseases when carried in the heterozygous mutant form in the germ line (1, 2). Termed BRCA1 and BRCA2, mutations in one or the other of the two genes can account for a significant proportion of cases of familial breast/ovarian cancer. The patterns of gene mutation, the genetics of inheritance, and the loss of the wild-type allele in tumors arising in familial cases strongly imply that BRCA1 and BRCA2 are tumor suppressor genes (3–5). How these genes can protect against cancer and how the loss of function of these genes can predispose to cancer are major questions in this field. Progress in answering them has the potential to influence therapeutic approaches to cases of hereditary breast/ovarian cancer and may provide insight into sporadic cases of these diseases.

The BRCA1 open reading frame predicts a polypeptide of 1863 amino acids (1). Analyses of disease-predisposing germ-line mutations in the BRCA1 gene indicate that truncation mutations are the major means of inactivating BRCA1. The most subtle disease-predisposing truncation mutation, Tyr1853→Term, deletes only the last 11 amino acids from the BRCA1 open reading frame (6). This implies that the extreme COOH terminus of BRCA1 contributes to its tumor suppressor function. In addition, missense mutations can also inactivate BRCA1. Although probable disease-predisposing missense mutations in BRCA1 have been detected throughout the open reading frame, there is some concentration of this type of mutation in two structural motifs: (a) an NH2-terminal zinc-binding RING domain; and (b) a COOH-terminal domain present as tandem repeats in BRCA1 termed BRCT3 (7–9). A missense mutation in the RING domain preferentially targets one of two zinc-binding cysteine residues (Cys63→Gly and Cys64→Gly; Ref. (5)). These data implicate the RING and the BRCT domains of BRCA1 in the performance of its tumor suppressor function.

Recently, Wu et al. (10) identified a novel gene whose product binds the BRCA1 RING domain in vivo and in vitro. Termed BARD1, the gene product is a polypeptide of M, ~100,000 bearing some structural similarity to BRCA1 in that it also possesses an NH2-terminal RING domain and two tandem repeated COOH-terminal BRCT domains. The ability of BRCA1 to bind BARD1 is abolished in the two known missense mutant forms of BRCA1 that lose BRCA1 RING domain function (Cys63→Gly and Cys64→Gly). Thus, BARD1-BRCA1 interaction may be important for BRCA1-mediated tumor suppression.

Various functions have been ascribed to the two BRCA1 BRCT domains present as tandem repeats near the COOH terminus. This segment of the BRCA1 polypeptide is generally acidic, which has led to the suggestion that it may be a transactivation domain. Consistent with this, the COOH terminus (including the BRCT region) can transactivate a GAL reporter gene when fused to the DNA binding module of GAL4 (11, 12). This function was suppressed when the wild-type BRCA1 COOH terminus was replaced with various clinically described missense mutants of this segment of BRCA1. These data suggest a role for BRCA1 in transcription control. In apparent support of such a role, BRCA1 was found to physically associate with components of the RNA polymerase II general transcription apparatus (also termed the holoenzyme; Ref. (13). Taken together, these data are also consistent with a role for BRCA1 in DNA repair because various DNA repair elements are seen in association with the RNA polymerase II holoenzyme (14).

BRCA1 mRNA is ubiquitously expressed in both human and mouse tissue. A relationship between BRCA1 and proliferation is seen in the elevated levels of BRCA1 in rapidly proliferating tissues of developing mouse embryos and in the induction of the BRCA1 mRNA at the G1-S phase border in cultures of human cell lines (15–17). Analysis of mice bearing homozygous targeted mutations of BRCA1 in the germ line revealed a further, paradoxical relationship between BRCA1 and proliferation. In particular, BRCA1−/− embryos died around the time of gastrulation, with an apparent proliferation deficit (18, 19). One study found that the mRNA levels of the cell cycle inhibitor p21 were greatly elevated in such embryos before cell death (18). The paradoxical phenotype of BRCA1−/− mice may be understandable in the light of more recent data that point to a role for BRCA1 in the maintenance of genome integrity (20, 21).

Results

Evidence of a role for BRCA1 in genome integrity maintenance came from our work in analyzing BRCA1 immunolocalization within the nucleus of cells in the S phase-G2 phase of the cell cycle. BRCA1 immunostaining revealed characteristic nuclear foci (dots) in every human cell line examined (22). We found that these foci also contain hRad51 (20), the human homologue of S. cerevisiae Rad51 recombinase, which is itself a close structural and functional homologue of the bacterial RecA protein (Fig. 1, J). All Rad51/RecA species examined to date play a role in homologous recombination processing of DNA in vivo and in vitro (23–25). In S. cerevisiae,
Fig. I. I, colocalization of BRCA1 with Rad51 in S-phase/G2-phase nuclear foci. MCF7 cells were synchronized in S phase by serum starvation, followed by release into medium containing high serum. After fixation in paraformaldehyde and permeabilization with Triton buffer, cells were immunostained and visualized by confocal microscopy. Antibody stains were as follows: A, mouse anti-BRCA1 mAb MS13 (FITC, green); and B, affinity-purified rabbit anti-Rad51 polyclonal antiserum (rhodamine, red). C, the composite picture demonstrates colocalization of BRCA1 and Rad51. Where green and red signals overlap, a yellow color is seen. Reproduced from Scully et al., Cell, 88: 265–275, 1997.

II, localization of BRCA1 to the developing synaptonemal complex. Spreads of primary human spermatocytes were prepared, fixed in paraformaldehyde, and permeabilized with Triton buffer. Spreads were stained with SCP3, a rabbit polyclonal antiserum (shown here in white pseudocolor) that recognizes a structural component of the synaptonemal complex, and with mouse anti-BRCA1 (rhodamine, red). The image depicts the contents of a single spermatocyte, staged at late zygotene. The red BRCA1 stain is seen to overlie predominantly unsynapsed regions (axial elements) of the developing synaptonemal complex. Reproduced from Scully et al., Cell, 88: 265–275, 1997.

III, recruitment of BRCA1 to PCNA-positive regions after acute hydroxyurea treatment of S-phase cells. MCF7 cells were synchronized in S phase as described in the legend to part I. Parallel cultures were harvested untreated (A–C) or harvested after a 1-h treatment with HU at a final concentration of 1 mM (D–F). Cells were fixed and permeabilized in methanol/acetone buffer and then immunostained with mouse anti-BRCA1 mAb MS13 (A and D; FITC, green) and with human anti-PCNA antiserum AK (B and E; rhodamine, red). C, composite picture of A and B. Note that in untreated cells, BRCA1 and PCNA appear to localize to distinct nuclear structures. F, composite picture of D and E. Where green and red signals overlap, a yellow color is seen. Note that in HU-treated cells, BRCA1 and PCNA now colocalize extensively. Reproduced from Scully et al., Cell, 90: 425–435, 1997.

Rad51 mutants reveal profound defects in two processes closely linked to homologous recombination: (a) double-stranded break repair; and (b) meiotic recombination (26). Interestingly, mice bearing homozygous mutations of Rad51 in the germ line are approximate phenocopies of BRCA1−/− mice, revealing an early deficit in proliferation associated with early embryonic lethality (27). Consistent with the colocalization of BRCA1 with Rad51 in S-phase/G2-phase cells, an endogenous complex containing the two
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A phosphorylation of BRCA1 in S phase after DNA damage. S-phase MCF7 cells were exposed to the treatments shown for 1 h before harvesting. HU treatment was added at a final concentration of 1 mM. Mitomycin C was added at a final concentration of 20 μg/ml. Cell extracts were then immunoblotted for BRCA1 using mAb MS 110. B, S-phase specificity of the BRCA1 damage response. MCF7 cells were released from serum starvations into G1 for 7 h. Cells were harvested 1 h after treatment with DNA-damaging agents (while still in G1), as shown, and immunoblotted for BRCA1. Extracts of S-phase MCF7 and HU-treated S-phase cells were analyzed in parallel to show the relative migration of the G1, G2/damage, S-phase, and S-phase/damage forms of BRCA1. Note that HU, mitomycin C, and low-dose UV treatment (10 J/m²) each failed to shift the G1 form of BRCA1 under conditions in which the S-phase form had undergone damage-induced phosphorylation (compare with A). Reproduced from Scully et al., Cell, 90: 425-435, 1997.

proteins was detected in extracts of human cells. In addition, Rad51 and BRCA1 colocalized on the axial element of developing synaptonemal complexes in primary human spermatocytes, strongly suggesting a role for BRCA1 in meiotic as well as mitotic recombination (Ref. (20); Fig. 1, II).

In support of a role for BRCA1 in DNA repair or in processes connected with genome integrity maintenance, the BRCA1 S-phase dots were found to be labile in the face of acute DNA damage. The dispersion of BRCA1 from S-phase foci was accompanied by two temporally related phenomena: (a) after hydroxyurea treatment or low-dose UV treatment of cells, BRCA1 was found to have relocated onto subnuclear regions containing PCNA (a marker of replication areas; Fig. 1, III); and (b) BRCA1 underwent specific phosphorylation shortly after incurring DNA damage in S phase (Ref. (21); Fig. 2).

This phosphorylation was distinct from the known phosphorylation change that occurs in BRCA1 during the G1-S-phase transition (21, 28). In its relocation onto PCNA-positive replication sites, BRCA1 was accompanied by both Rad51 and BARD1 (a BRCA1-associated protein, see the "Introduction"). This colocalization phenomenon could be interpreted as a DNA repair response, especially in view of the persistent association of BRCA1 with Rad51 under such circumstances. More broadly, the specific phosphorylation of BRCA1 after DNA damage in S phase is likely to indicate that BRCA1 is the target of one or more DNA structure-dependent cell cycle checkpoints (21).

Certain features of BRCA2, including a similar clinical outcome of women bearing BRCA1 and BRCA2 germ-line mutations and a similar embryonic lethality phenotype of BRCA1 and BRCA2 knockout mice (29), suggest that it may cooperate with BRCA1 in a common biochemical pathway. One common element on such a pathway is Rad51, which can interact with both BRCA1 and BRCA2 (30). In view of these hints of a common function for BRCA1 and BRCA2, we asked whether BRCA1 and BRCA2 could interact in vivo.

Using a panel of newly developed, affinity-purified polyclonal antibodies to BRCA2, we found that BRCA1 and BRCA2 coexist in a common biochemical complex within naive cell extracts of several different cancer cell lines (Fig. 3). A BRCA2-interacting surface on BRCA1 was identified within residues 1314-1863 of BRCA1 (31).

Subcellular localization studies performed on multiple different human cancer cell lines using two-color immunofluorescence staining and confocal microscopy revealed colocalization of BRCA2 with BRCA1 and Rad51 in S-phase-G2-phase-correlated nuclear foci (Fig. 4). In meiotic cells, the three proteins colocalized on the axial element of the developing synaptonemal complex. After hydroxyurea or UV treatment of S-phase cells, BRCA2 was noted to colocalize with BRCA1 and Rad51 (31).
Fig. 4. Colocalization of BRCA1 and BRCA2 in discrete nuclear foci. DU145 cells were fixed and permeabilized as described in the Fig. 11 legend and stained with anti-BRCA1 mAb SD118 (FITC, green) and affinity-purified anti-BRCA2 antibody and anti-BRCA2C (rhodamine, red) and imaged by confocal microscopy. A, BRCA1 stain; B, BRCA2 stain; C, the composite BRCA1 and BRCA2 stains. Where the green and red signals overlap, a yellow pattern is observed, indicating the colocalization of BRCA1 and BRCA2. Reproduced from Chen et al., Mol. Cell, 2: 317–328, 1998.

Discussion

The work described above indicates that BRCA1, BRCA2, and Rad51 participate in a common DNA damage response pathway that is likely connected, at least in part, with homologous recombination. In principle, other DNA repair systems could also be involved. When disabled, this pathway appears to predispose women to early-onset breast and/or ovarian cancer. Thus, other genes participating in this pathway may also be found to be hereditary breast cancer genes. Furthermore, it is conceivable that this pathway is implicated in sporadic cases of breast cancer.

Because BRCA1 and BRCA2 colocalize and interact before and after DNA damage, our original speculations on the nature of BRCA1 function, based upon localization data and association with Rad51, can now be extended to BRCA2. In this regard, hydroxyurea or UV treatment of S-phase cells may generate persistent regions of parental ssDNA in close proximity to replication forks. These ssDNA regions or their derivatives (double-stranded DNA breaks) may be recombinogenic, which may account in part for the recruitment of Rad51 to BRCA1/BRCA2/BARD1 complexes to PCNA-containing sites in these circumstances. These complexes may therefore function in a process analogous to prokaryotic “daughter strand gap repair,” an error-free RecA-dependent homologous recombinational response to ssDNA lesions generated during attempted replication across a DNA adduct (reviewed in Ref. 32). Defects in such a process could explain some of the spontaneous anomalies in chromosome structure and sensitivity to DNA adducting agents noted recently in the cells of BRCA2 mutant mouse embryos (33). If this homologous recombinational process were saturable, then either a high load of added DNA to the replication machinery or a quantitative defect in the homologous recombinational pathway might translate into inefficient gap repair and hence into increased cancer risk.

The concept of a common BRCA1/BRCA2 hereditary breast and ovarian cancer pathway suggests at least one hypothesis for understanding the tissue specificity of BRCA1/BRCA2-linked disease. A potentially “universal” carcinogen can give rise to tissue-specific disease because it is concentrated in certain specialized cell types (so-called “remote carcinogenesis,” reviewed in Ref. 32). Conceivably, the breast ductal epithelium accumulates such a carcinogen and therefore suffers an unusually high rate of DNA damage of a type that stresses postreplicative homologous recombination and possibly other forms of DNA repair, the activity of which depends upon the presence of a normal BRCA1/BRCA2 pathway. In this setting, the tissue-specificity of BRCA1/BRCA2-linked disease might reflect, in part, an inadequate DNA repair response to tissue-specific DNA adduction.

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References


Discussion

Dr. Bruce Ponder: Thank you very much for an elegant exposition of a complicated subject. Questions? Thank you for the generous acknowledgment to my laboratory. I should say that the work on BRCA2 is from my department but from Ashok Venkitaramen’s laboratory. I wouldn’t like to claim credit for that.

Dr. Phillip Sharp: Why is this specific to breast cancer? This is a DNA repair process. We would expect the same processes to be active in most cell types, taking care of a very general problem. Why do you see it in breast versus other tissues?

Dr. Livingston: The best I can do is speculate. As I’ve learned from my experiences here at the Cancer Institute, certain laboratories have shown that certain estrogen derivatives generated by conversion through enzymes such as COMT and the appropriate P-450 isofrom(s) acquire alkylyating activity. In effect, they become carcinogenic, at least in rats. So one speculates on the possibility that estrogen is a remote carcinogen in humans in those cells that concentrate it, i.e., cells that have the estrogen receptor. If the cells are BRCA1 +/−, they may be at special risk.

Dr. Letricia Ben: I have a question on BRCA1 and DNA damage and repair. All the data you presented are very beautiful, but on the other hand, this could be simply a correlation because of the colocalization with Rad51. Alternatively, BRCA1 might function in checkpoint control. I just wonder if you have any data or if other people have data to show that BRCA1 is actually involved in the repair process?

Dr. Livingston: At the moment, we have no direct evidence that it is.

Dr. Ponder: Could I add a comment to that? In BRCA2, there is in vitro evidence that you mentioned from Tony Kouzarides’ laboratory linking BRCA2 function to transcription. Recently, several families have been described in which there are mutations in exon 3, which is the putative transactivational domain. One of the mutations is an in-frame deletion. One presumes that the rest of the peptide is there, but we don’t know it. It might be unstable. The other is a missense mutation. So there is some suggestion that the loss of exon 3 function alone might be sufficient to cause the tumor phenotype; then the question is what price the DNA repair function?

Dr. Livingston: BRCA2 is an enormous protein, ~3200 amino acids. There should be a significant number of potential functions for a polypeptide that big. Therefore, the clinical phenotypes (or lack thereof) might be dependent on which BRCA2 functions remain after a given deletion. Depending on the mix of remaining activities, a given mutation may or may not be tumorigenic.

Dr. Ponder: You suggested that BRCA1 and BRCA2 were possibly in the same pathway. What would you expect the phenotype of a compound heterozygote to be?

Dr. Livingston: If there’s anything to the notion that a single heterozygote, a cell with single haploinsufficiency, experiences a mutator phenotype, then the double heterozygote might be in considerable trouble.

Dr. Ponder: There have been one or two reports. There was one report from my laboratory, which involved a Hungarian lady who was 49 years old or something who had both breast and ovarian cancer. Do you know whether the mouse crosses have been made yet? I don’t.

Dr. Livingston: I do not know for sure, but one of the things that Jean Feunteun and my laboratory are working on at the moment, thus far without anything to report, is whether or not the presence of heterozygosity in either BRCA1 or BRCA2 renders the relevant cells unable to perform certain homologous reactions.

Dr. Ponder: You left the tantalizing question regarding sporadic tumors dangling at the end. What about the sporadic tumors?

Dr. Livingston: LOH and a residual wild-type copy of BRCA1 and BRCA2 is a not uncommon phenomenon in sporadic breast cancer. In fact, there’s a recent report in the Lancet within the past couple of years demonstrating that LOH, both in 13q and 17q, is present in about 30% of the cases of sporadic breast cancer that were studied, if I remember correctly. Very large chunks of DNA are deleted in these cases, and it’s a stretch to argue that LOH covering megabases means hemizygosity at BRCA1 and/or BRCA2. However, if it does, then one might be able to make a connection between sporadic disease and the loss of BRCA1 and/or function.

Dr. Ponder: In sporadic breast cancer? What about sporadic ovarian cancer?

Dr. Livingston: Yes, in ovarian cancer.
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