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Emerging Roles of BRCA1 in Transcriptional Regulation and DNA Repair

YUMAY CHEN,[1] WEN-HWA LEE,[1] AND HELEN K. CHEW[2,3]*

[1]*Department of Molecular Medicine/Institute of Biotechnology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas*
[2]*Division of Medical Oncology, Department of Internal Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas*
[3]*Medical Research Division, South Texas Veterans Health Care System, San Antonio, Texas*

BRCA1 was the first breast cancer susceptibility gene to be identified and cloned. In individuals from high-risk families, mutations in *BRCA1* increase the lifetime risk of developing breast cancer eight to tenfold, compared to the general population. How the BRCA1 protein product normally functions to suppress tumor formation and how defects in the gene can ultimately lead to breast cancer have been the focus of intense scrutiny by the scientific and medical communities. BRCA1 has intrinsic transactivation activity and is able to activate the p21 promoter. In addition, *BRCA1* is linked to a number of genes involved in transcriptional regulation, including CtIP, c-Myc, the RNA holoenzyme complex, and the histone deacetylase complex. Moreover, BRCA1 is essential for cellular response to DNA damage repair. Inactivation of *Brca1* in mouse embryonic stem and fibroblast cells results in increased cell sensitivity to DNA-damaging agents. In human cells, BRCA1 binds to both Rad50 and Rad51 and colocalizes with these proteins at repair foci. Part of BRCA1's response to DNA damage may in fact be corroborated through transcriptional regulation. The expression of GADD45, a DNA damage-responsive gene, is increased immediately after induction of BRCA1. Recently, BRCA1 was shown to repress estradiol (E2)-responsive ER-α-mediated transcriptional activity, potentially linking the multiple functions of BRCA1 to specific tissue targets. These recent developments in BRCA1 function are an encouraging step toward understanding the role of *BRCA1* in breast cancer formation. J. Cell. Physiol. 181:385–392, 1999.

Breast cancer is the most common cancer affecting women in the United States. In 1998, 178,000 American women were projected to be diagnosed with breast cancer, while 44,000 were expected to die of the disease. It is second only to lung cancer as the leading cause of cancer-related deaths. Although multiple factors influence a woman's lifetime risk of developing breast cancer, family history has long been recognized as one of the most powerful predictors. Approximately 5–10% of all breast cancers are hereditary, secondary to the autosomal dominant inheritance of a germline mutation in a breast cancer susceptibility gene. Two such genes, *BRCA1* and *BRCA2*, have been recently identified and cloned (Hall et al., 1990; Hall and Lane, 1997; Wooster et al., 1994, 1995) and an intense effort has been undertaken to characterize the function of their protein products.

Through linkage analysis of multiple families affected by early-onset breast and ovarian cancer, the first breast cancer susceptibility gene, *BRCA1*, was mapped to chromosome 17q21 in 1990 and cloned 4 years later (Hall et al., 1990; Miki et al., 1994). At the same time, *BRCA2* was mapped to chromosome 13q and rapidly cloned (Wooster et al., 1994, 1995). Mutations in *BRCA1* are responsible for nearly all of the hereditary breast and ovarian cancer families and up to 40–50% of families with hereditary breast cancer only (Easton et al., 1993). *BRCA2* mutations are linked to a similar percentage of inherited breast cancers. However, in contrast to *BRCA1*, they also predispose to male breast cancer. Together, defects in these two genes account for the majority of inherited breast cancers.

Mutations in other known tumor susceptibility genes, such as the retinoblastoma gene *RB*, *p53*, and the adenomatous polyposis gene *APC*, are found in both familial as well as sporadic tumors. In contrast, mutations in *BRCA1* and *BRCA2* are rarely detected in the more common sporadic cases of breast cancer (Futreal et al., 1994b), which account for 90–95% of all breast cancers. The reason for this remains unclear.

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*Correspondence to: Helen K. Chew, Division of Medical Oncology, Department of Internal Medicine, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284. E-mail: chewh@uthscsa.edu

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However, an understanding of the roles that *BRCA1* and *BRCA2* play in suppressing breast tumorigenesis may be applied to benefit all breast cancer patients.

In this review, we focus on our current understanding of the evolving roles of the *BRCA1* gene product, with particular emphasis on transcriptional regulation and DNA repair.

STRUCTURE OF THE *BRCA1* GENE

BRCA1 contains 24 exons, which encode an 1863 amino acid protein (Miki et al., 1994). Exons 1 and 4 are noncoding, and exon 11 is unusually large, accounting for one half of the entire coding region of the gene. From sequence analysis, the amino-terminus of *BRCA1* contains a zinc RING finger domain of the C3HC4 structure. The biological function of this zinc finger domain remains unclear, although such structures may be involved in protein-protein interactions (Bienstock et al., 1996). In addition, an acidic domain at the carboxy-terminal region was shown by yeast one-hybrid assay to possess transactivation activity (Chapman and Verma, 1996; Chen et al., 1996a; Monteiro et al., 1996). Because of these structural motifs, BRCA1 is believed to function as a transcription factor or cofactor, which regulates the expression of specific genes.

Moreover, at the distal carboxy-terminus (amino acids 1640–1863), BRCA1 contains two BRCT (BRCA1 C-terminal) repeats. These repeats have been found in many proteins involved in DNA repair including Rad9, XRCC1, and three eukaryotic DNA-ligases (Bork et al., 1997; Callebaut and Mornon, 1997; Koonin et al., 1996). The minimal binding region of the p53 binding protein, P53BP1, also contains BRCT repeats (Iwabuchi et al., 1994), suggesting that such domains may be involved in protein-protein interactions, possibly in response to DNA damage. Recently, the structure of the XRCC1 BRCT domain was resolved by X-ray crystallography at 3.2 A resolution (Zhang et al., 1998b). Structural analysis revealed that the BRCT domain comprises a four-stranded parallel beta-sheet surrounded by three alpha-helices that forms an autonomously folded domain, which again suggests potential protein-protein interaction sites.

These potential functions, predicted from the primary amino acid sequence, and their role in tumor suppression will be discussed.

MUTATIONS IN THE *BRCA1* GENE

Mutations in *BRCA1* are linked to approximately 45% of hereditary breast cancer families and nearly all (79%) breast and ovarian cancer families (Easton et al., 1993). The majority of the mutations are either frameshift or nonsense mutations that result in a truncated protein product and provide few clues to the function of the BRCA1 protein. In addition, these mutations are scattered throughout this large gene, making mutation screening difficult. Few missense mutations have been found and most are located in the amino- or carboxy-termini, which result in disruption of the RING finger domain or BRCT repeats, respectively (BCIC, Breast Cancer Information Core). Tumor-derived mutations in these two domains support the observation that these structures are functionally important for BRCA1's tumor suppressor activity. The significance of some other mutations, however, is unknown and they may represent polymorphisms.

Somatic mutations of *BRCA1* are rarely found in sporadic breast tumors (Futreal et al., 1994a), nor are they found in established breast cancer cell lines. The establishment of HCC1937, a breast cancer cell line homozygous for a germ-line-inactivating *BRCA1* mutation, 5382insC, has provided an excellent tool with which to study the function of BRCA1 (Tomlinson et al., 1998). In HCC1937 cells, 5382insC results in the erroneous translation of protein sequence distal to codon 1755 and the termination of translation at codon 1829.

The genetic evidence linking *BRCA1* mutations to inherited breast cancer is striking. Deciphering how these different mutations affect the normal function of BRCA1 and predispose mutation carriers to breast cancer is an exciting challenge. Based on recent studies, BRCA1 appears to be involved in both transcriptional regulation and in the DNA repair pathway as discussed below.

BRCA1 IN TRANSCRIPTIONAL REGULATION

The acidic domain (amino acids 1142–1643) of BRCA1 fused to the Gal4-DNA-binding domain contains strong transactivation activity in yeast (Chapman and Verma, 1996; Chen et al., 1996a; Monteiro et al., 1996). The distal C-terminal region has minimal transactivation activity in the same assay system. Within this region, mutations found in familial tumors abrogate this transactivation activity, supporting speculation that the transactivation activity of BRCA1 is crucial for its tumor suppression. Recently, investigators have demonstrated that the C-terminus of BRCA1, fused to Gal4-DNA-binding domain, activates transcription in vitro (Haile and Parvin, 1999). Although BRCA1 appears to have transactivation activity, specific DNA-binding activity has not been demonstrated. At the amino-terminus of BRCA1, the zinc RING finger domain may permit interaction with DNA either directly or, more likely, indirectly through protein-protein interactions. The RING finger domain of BRCA1 contains one of the more frequent missense mutations found in familial breast cancer, notably the Cys61Gly mutation. BRCA1 may not recognize a specific DNA sequence, but may function as a coactivator or corepressor for sequence-specific binding proteins.

Evidence to support this speculation is accumulating. *Brca1* null mutant mouse embryos have elevated expression of the cyclin-dependent kinase (cdk) inhibitor p21 and decreased expression of mdm2 (Hakem et al., 1996, 1997). Furthermore, wild-type, but not mutant BRCA1, can transactivate the p21 promoter in human cells (Somasundaram et al., 1997). The observation that BRCA1 interacts with several transcription factors, including c-myc (Wang et al., 1998), CtIP (Li et al., 1999; Wong et al., 1998; Yu et al., 1998), and RNA helicase A (RHA; Anderson et al., 1998), argues that BRCA1 may regulate transcription as a cofactor. In support of this, a recent study demonstrates that BRCA1 represses ER-α-mediated transcriptional repression (Fan et al., 1999). These exciting data link BRCA1's repressor activity with tissue specificity. Some of these specific interactions are discussed below.

C-Myc and BRCA1

C-Myc belongs to a helix-loop-helix (HLH) transcription factor family. The transcriptional activation of c-Myc requires its interaction with another HLH-family protein. For example, heterodimerization of c-Myc and Max is required for c-Myc-mediated transcriptional activation. The amino-terminus of BRCA1 (amino acids 433–511) is sufficient to interact with the HLH domain of c-Myc and repress Myc-mediated transcription (Wang et al., 1998). Furthermore, BRCA1 can inhibit the phenotype of embryonic fibroblasts transformed by Ras and Myc. Whether mutations in BRCA1, which cannot interact with c-Myc, fail to inhibit the transformation phenotype remains to be tested.

CtIP and BRCA1

The BRCT repeats of BRCA1 are frequently found in proteins involved in DNA repair, DNA recombination, and cell cycle control. Using the yeast two-hybrid system, three groups have found that the BRCA1 BRCT repeats (residues 1602–1863) can interact with several cellular proteins including CtIP (Li et al., 1999; Wong et al., 1998; Yu et al., 1998). CtIP was first identified as a corepressor with the CtBP transcription factor (Schaeper et al., 1998). The PLDLS motif in CtIP mediates its interaction with CtBP. This motif is found in E1A and several *Drosophila* transcription factors, but not in BRCA1 (Nibu et al., 1998; Schaeper et al., 1998). CtIP apparently uses different regions to interact with CtBP and BRCA1, perhaps acting as an intermediate to allow CtBP to associate with BRCA1. BRCA1 specifically interacts with CtIP both in vitro and in vivo in human cell lines (Li et al., 1999). Germ-line mutations in the BRCT repeats of BRCA1, found in familial breast cancer families, abrogate BRCA1's interaction with CtIP.

Furthermore, BRCA1 can transactivate p21 expression in human cells and transactivation of the p21 promoter is regulated by the complex of CtBP, CtIP, and BRCA1 (Li et al., 1999). Coexpression of CtIP and CtBP with BRCA1 can inhibit BRCA1-dependent transactivation of the p21 promoter. However, expression of CtBP and CtIP alone has no effect. The repression of BRCA1-dependent transactivation of the p21 promoter by CtBP and CtIP depends on their association with BRCA1. In addition, treatment with DNA-damaging agents, including UV and gamma irradiation and Adriamycin, disrupts the interaction between BRCA1 and CtIP/CtBP. Consistent with this finding, p21 was upregulated in these treated cells. These results suggest that the CtIP/CtBP interaction may negatively regulate the transactivation activity of BRCA1 on the p21 promoter (Fig. 1).

Studies of BRCA1-mediated transactivation of p21 were carried out in human cell lines with intact wild-type BRCA1. Of great interest will be whether BRCA1-mediated transactivation activity and its purported regulation differ in the BRCA1-null cell line HCC 1937 when wild-type BRCA1 is exogenously expressed.

RHA and BRCA1

BRCA1 specifically co-purifies the RNA polymerase II holoenzyme (Scully et al., 1997a). This association is possibly mediated by RHA (Anderson et al., 1998). In yeast, RHA interacts with the BRCT repeats of BRCA1. Overexpression of truncated RHA retains binding to BRCA1 in human cells, but inhibited the transcriptional activation mediated by the BRCA1 C-terminal region. Whether the complex of BRCA1 and RHA indeed forms in vivo remains to be answered. However, this provides further evidence that BRCA1 is linked to the transcriptional machinery.

Histone deacetylase complex and BRCA1

Using purified BRCT polypeptide as a probe to screen a human placenta cDNA expression library, the Rb-binding proteins, RbAp46 and RbAp48, were identified as BRCA1-interacting proteins by Far Western analysis (Yarden and Brody, 1999). Moreover, the BRCT repeats associate with the histone deacetylases HDAC1 and HDAC2. From binding assays, RbAp46 and RbAp48 show in vitro and in vivo interactions with BRCA1. These results demonstrate that BRCA1 interacts with components of the histone deacetylase complex and, therefore, may explain the involvement of BRCA1 in chromatin remodeling (Hu et al., 1999).

p53 and BRCA1

Two groups have shown that BRCA1 can activate the expression of p21 in a p53-dependent manner (Ouchi et al., 1998; Zhang et al., 1998a), implying that these two genes may work together in the DNA damage repair process and/or in cell growth arrest. p53 and BRCA1 interact in vitro and in vivo, and BRCA1 can activate p53-mediated transcription, a response reduced in the presence of C-terminal BRCA1 mutants (Ouchi et al., 1998). How these two genes interact in transcriptional regulation, as well as in cell cycle control and DNA damage response, will be of great interest.

ER-α-mediated transcription

As discussed, there is strong evidence to support a role for BRCA1 in transcriptional repression. However, how aberrations in such a broad function can result in predominantly breast and ovarian tumors has not been well understood.

Investigators recently demonstrated that wild-type BRCA1, in a dose-dependent manner, repressed E2-mediated transcriptional activation by the transcriptional activation function AF-2 of the estrogen receptor ER-α. In transient transfection assays of human breast cancer and prostate cancer cell lines, wild-type BRCA1 nearly abolished ER-α signaling in the presence of E2 and the estrogen response element (ERE). This was not seen with the empty vector. BRCA1 appears to inhibit the AF-2 function of ER-α when AF-2 was linked to yeast Gal4DBD. Moreover, BRCA1 did not appear to regulate the activity of other transcription factors including E2F1, Sp1, or c-Jun.

These results link the function of BRCA1 as a transcriptional regulator to tissue specificity. Breast and ovaries are highly responsive to estrogen. Wild-type BRCA1 may normally function as a repressor of E2-reponsive ER-α-mediated transcription. When BRCA1 is mutated or diminished, E2-responsive transcription proceeds unimpeded and may stimulate breast cells already initiated by other factors. Clinically significant *BRCA1* mutant constructs, corresponding to those mutations seen in familial breast cancer, were not evalu-

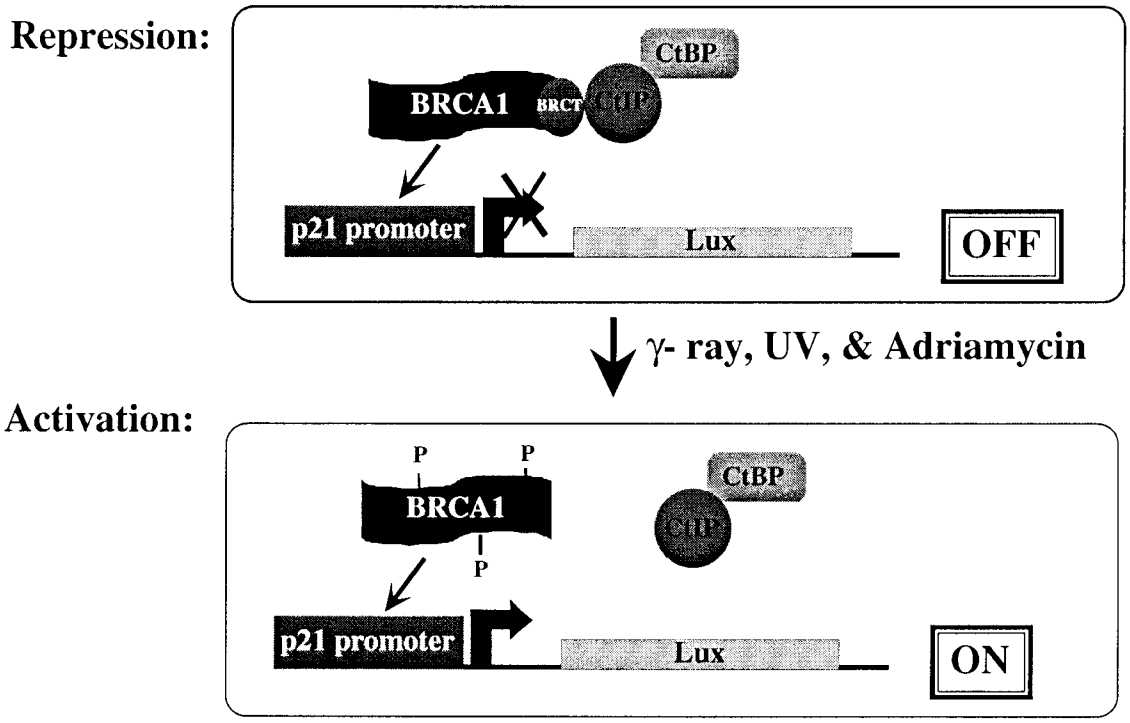

Fig. 1. The CtIP/CtBP complex interacts with BRCA1, through CtIP, to repress BRCA1-mediated p21 transactivation. This repression is abrogated upon DNA damage. BRCA1 itself is phosphorylated in response to damage and can transactivate the p21 promoter. Whether BRCA1 transactivates p21 directly or indirectly is unknown.

ated. Such results would have strengthened these important findings.

BRCA1 AND DNA REPAIR

BRCA1 is a 220-kDa nuclear phosphoprotein whose expression and phosphorylation are cell cycle regulated (Chen et al., 1996b). Kinase assays suggest that BRCA1 can be phosphorylated by cdk2, cyclin A, and cyclin D-dependent kinases. Phosphopeptide mapping suggests that BRCA1 is phosphorylated on at least 13 sites, most of which represent serine residues. The precise phosphorylation sites and the functional significance of this phosphorylation remain to be explored. Furthermore, BRCA1 is hyperphosphorylated in response to DNA damage (Scully et al., 1997b; Thomas et al., 1997).

Murine embryos carrying a Brca1 null mutation are hypersensitive to gamma irradiation. Brca1-deficient mouse embryonic stem (ES) cells are also hypersensitive to ionizing radiation and hydrogen peroxide (Gowen et al., 1998). These ES cells are unable to carry out transcription-coupled repair, a process in which damage is repaired more rapidly in transcriptionally active loci compared with the whole genome. The impaired transcription-coupled repair in Brca1−/− cells strongly suggests that BRCA1 plays an important role in DNA repair. The observation that BRCA1 dissociates from the CtIP/CtBP complex upon DNA damage, with resultant upregulation of the p21 promoter (Li et al., 1999), may link BRCA1's role as transcriptional regulator with DNA repair.

Furthermore, BRCA1 interacts with RAD51, the human homolog of the bacterial RecA protein (Scully et al., 1997c). The role of human RAD51 is not clear; however, yeast RAD51 participates in the DNA double-strand-break repair pathway and in mitotic and meiotic recombination events. By immunostaining of MCF7 cells, BRCA1 and RAD51 both exhibited an overlapping punctate-expression pattern in S-phase nuclei. However, this colocalization was transient and varied from cell to cell. Using the GST pull-down assay, the authors found that Rad51 interacts with the 3′ end of BRCA1 exon11, but direct binding has not been confirmed (Scully et al., 1997c). Finally, both BRCA1 and Rad51 colocalized on human synaptonemal complexes, suggesting a role for these two proteins in chromosome recombination and maintenance of genome integrity (Scully et al., 1997b). However, Rad51 foci formation in HCC1937, a breast cell line with a homozygous mutation of *BRCA1*, is not affected in response to DNA damage (Zhong et al., 1999). Thus, the significance of the interaction between Rad51 and BRCA1 in the DNA repair pathway remains to be clarified.

BRCA1 also interacts with Rad50 both in vitro and in vivo (Zhong et al., 1999). Rad50 forms a complex with Mre11 and p95 (NBS protein); this complex plays an important role in the DNA double-strand break repair pathway in yeast. BRCA1 is detected in discrete foci in the nucleus that colocalize with Rad50 after irradiation. BRCA1, Rad50, Mre11, or p95 foci, induced by irradiation, were dramatically reduced in HCC1937

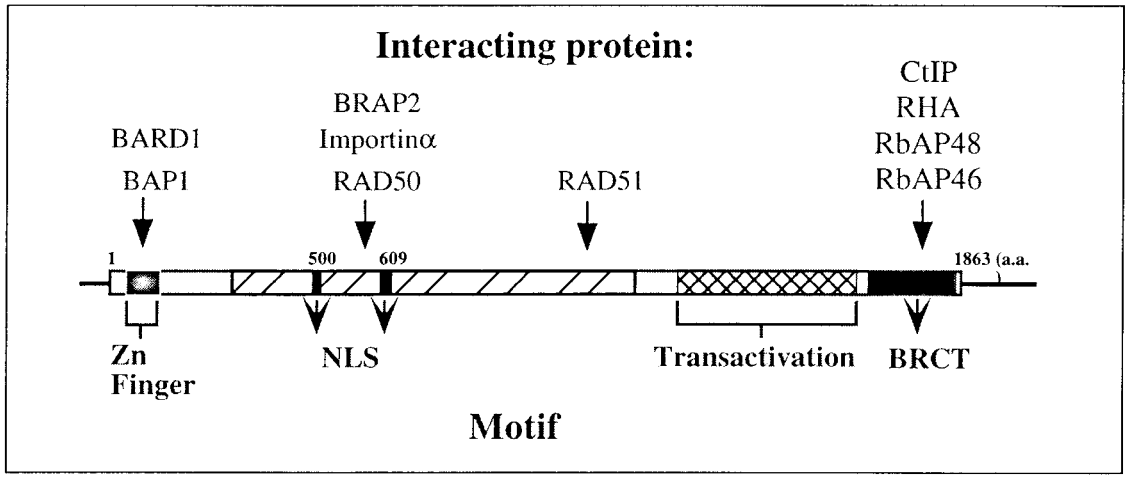

Fig. 2. The structure of BRCA1 demonstrating the N-terminal zinc finger, the C-terminal transactivation domain, and BRCT repeats. Interacting proteins and their approximate interacting sites are shown.

cells. Irradiation-induced Rad50-, Mre11-, and p95-containing foci can be restored by the ectopic expression of wild-type BRCA1. Furthermore, ectopic expression of wild-type, but not mutated BRCA1, confers resistance to HCC1937 cells that are hypersensitive to methyl methanesulfonate treatment. Together, these results suggest that BRCA1 is essential for the cellular responses to DNA damage, primarily mediated by the Rad50/Mre11/p95 complex. A crucial role of BRCA1 in DNA repair could explain its role in breast and ovarian tumorigenesis.

Recently, Harkin et al. (1999) constructed an inducible promoter to express full-length BRCA1 in human sarcoma and breast cancer cell lines. Using high-density oligonucleotide assays to analyze over 6,800 gene profiles after BRCA1 induction, GADD45 mRNA expression was induced 35-fold. GADD45, a DNA damage-responsive gene, was recently linked to the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) signal pathway leading to apoptosis (Takekawa and Saito, 1998). The induction of GADD45 was found to be independent of p53, when examined in breast cancer cells with mutated p53. Further characterization of BRCA1 induction of GADD45 will shed more light on its important role in DNA repair.

BRCA1 IN GROWTH AND DIFFERENTIATION

The fundamental functions of BRCA1 in transcriptional regulation and the DNA repair process intertwine with its role in cellular proliferation and/or differentiation. In cell culture experiments, the phosphorylation and expression of BRCA1 is cell cycle regulated, with highest levels expressed as cells enter S phase (Chen et al., 1996b). In addition, the levels of BRCA1 mRNA appear to be reduced in both familial and sporadic breast cancers (Thompson et al., 1995). *Brca1*, the mouse homolog of *BRCA1*, is crucial for the growth and differentiation of the mouse embryo. Three groups have found that Brca1 homozygous deletions (−/−) are lethal early in embryonic development with failure to proliferate (Gowen et al., 1996; Hakem et al., 1996; Liu et al., 1996). Both Liu et al., who generated a mutation in exon 11, and Hakem et al., who generated a deletion of exons 5 and 6 containing the zinc finger, produced embryos that failed to gastrulate and which differentiated poorly. The mutant embryos were noted to have defects of neural development, including various degrees of anencephaly and spina bifida. These results imply that the Brca1 gene product likely plays a role in tissue growth and/or differentiation in mouse embryogenesis. However, the mutant embryos did have intact DNA synthesis and did not have increased apoptosis. The observation that the mutant embryos are much smaller than their wild-type littermates prior to their arrested development is in agreement with a proliferative defect.

Brca1 mutants expressed increased levels of the cyclin inhibitor p21 (Hakem et al., 1996). Interestingly, coexisting p53 or p21 mutations partially rescued the Brca1 null lethal phenotype (Hakem et al., 1997). Most p21-Brca1 double-mutant embryos were comparable in size to that of their wild-type littermates, suggesting that p21 may play a role in the impaired cellular proliferation observed in Brca1 mutant embryos. However, mutation of p21 or p53 cannot completely rescue Brca1 null embryos, which implies that there are multiple factors involved in their lethality.

These reports in mouse embryos appear to conflict with reports of BRCA1 transactivation of p21 in human cells. In humans, mutation or deletion of BRCA1 results in unregulated growth of tumors. In contrast, Brca1−/− mouse embryos failed to proliferate. There are several postulations to explain this apparent conflict. First, the human and mouse proteins share only a 58% homology and BRCA1 may function differently in these two species. Additionally, Brca1 may function quite differently during embryonic development. Nonetheless, BRCA1's roles in repair and growth and differentiation can be reconciled. As discussed above, BRCA1 plays an important role in the cellular response to DNA damage, leading to increased expression of p21, cell cycle arrest, and apoptosis or DNA repair. If mutated,

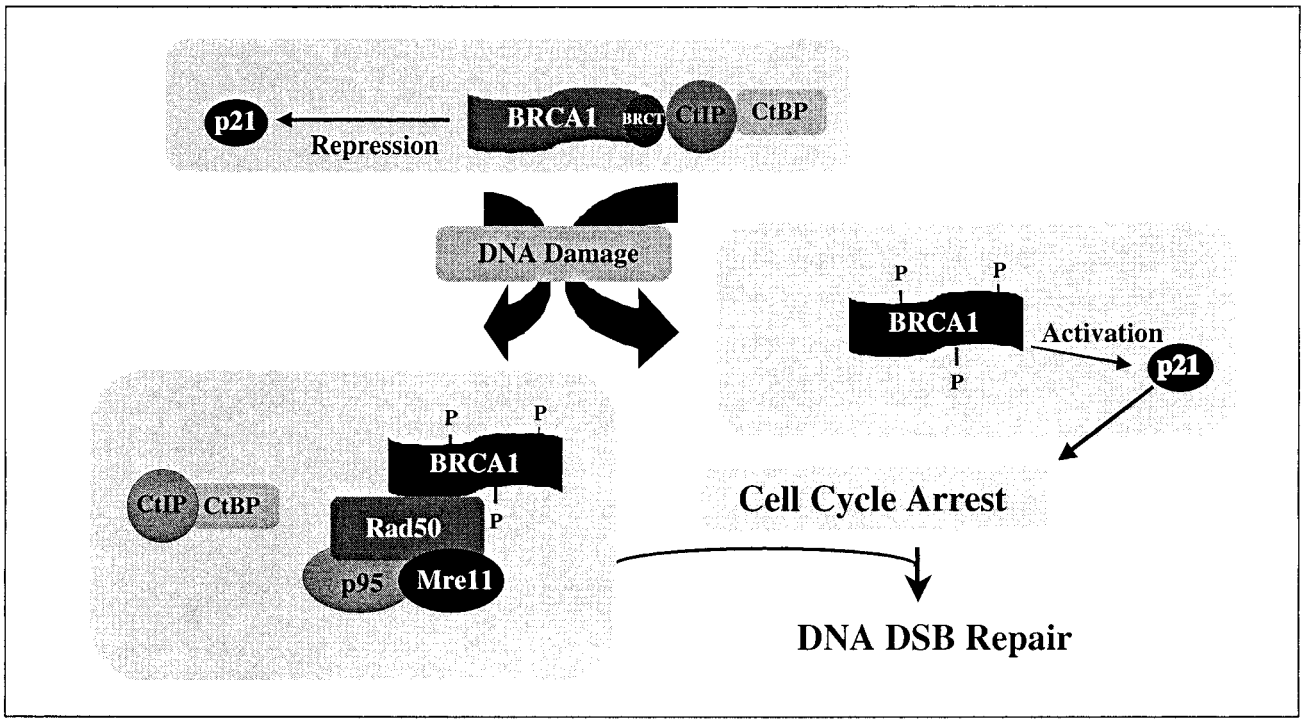

Fig. 3. BRCA1 appears to have dual roles in response to DNA damage leading to cell cycle arrest and/or repair through multiple mechanisms.

defects in other tumor suppressor genes are allowed to accumulate, which accelerates tumor formation and growth. In the developing mouse, the accumulation of such defects may overwhelm the organism and result in the inhibition of growth. Such a hypothesis is supported by the recent development of conditional *Brca1*−/− mutant mice (Xu et al., 1999a).

Conditional Brca1-null mice

The role of *Brca1* in mouse tumorigenesis, particularly breast cancer formation, has been difficult to study, limited by the embryonic lethality conferred by the *Brca1*−/− genotype. In addition, heterozygous *Brca1*+/− mice did not demonstrate a phenotype. Recently, the results of the successful conditional deletion of *Brca1* were reported. Wap-Cre or MMTV-Cre-mediated excision of *Brca1* exon 11 in mouse mammary epithelial cells resulted in increased apoptosis and abnormal ductal development (Xu et al., 1999a). Mammary tumor formation was observed in both strains (Wap-Cre and MMTV-Cre) but at low frequency and after a long latency. The tumors were associated with genetic instability characterized by aneuploidy, chromosomal rearrangements, or altered mRNA expression of p53. The formation of mammary tumors in these *Brca1* conditional mutant glands is accelerated in the background of *p53* null alleles. It suggests that the genetic instability due to disruption of *Brca1* may trigger further alterations, including the inactivation of *p53*, that lead to tumor formation. These observations are consistent with the recent findings that BRCA1 localized to the centrosome during mitosis and the hypophosphorylated form of BRCA1 can coimmunoprecipitate with gamma-tubulin, a centrosomal component essential for the nucleation of microtubules (Xu et al., 1999b). Moreover, mutant mouse embryo fibroblast (MEF) cells, which carry a Brca1 exon 11 isoform deficiency (Brca1 Δ11/Δ11), contain abnormal centrosome amplification, abnormal spindle formation, and unequal segregation of chromosomes in culture. These results suggest a direct link of the Brca1 Δ11/Δ11 phenotype to a cancer-related defect.

CONCLUSIONS

Our understanding of *BRCA1*'s role in tumor suppression has progressed significantly over the past 5 years. Gathering evidence implicates BRCA1 in transcriptional regulation as well as in the DNA repair pathway. BRCA1 is a large protein and undoubtedly has more than one functional domain and many interacting partners (Fig. 2).

Initial speculation as to BRCA1's involvement in the transcriptional process appears to be supported by the interaction of this protein with various transcription factors, including the repressor pair CtIP and CtBP. This particular interaction appears to repress the ability of BRCA1 to transactivate the p21 promoter. This interaction is disrupted upon DNA damage, thereby allowing p21-mediated cell cycle inhibition and possible damage repair. In addition, the induction of BRCA1 is followed immediately by the increased mRNA expression of GADD45, a DNA damage-response gene. BRCA1's repression of E2-responsive, ER-α-mediated transcription is perhaps the strongest evidence to explain BRCA1's role in predominantly breast and ovarian cancer. The biological significance of BRCA1's in-

teractions with other transcription factors remains to be proven. What additional downstream genes are activated or repressed, as a result of BRCA1's transcriptional regulation, will be of significant interest.

The involvement of BRCA1 in repair pathways is further underscored by its association with the Rad50/MRE11/p95 complex and with its colocalization with Rad51. Rad51 is implicated in yeast homologous recombination repair and a similar role is suspected in mammalian cells. In addition, BRCA1 also associates and coimmunoprecipitates with the Rad50 complex involved in the nonhomologous DNA double-strand break repair pathway. Precisely how BRCA1 participates in various pathways of DNA repair, through its interaction with proteins in response to DNA-damaging agents, remains to be determined. Nevertheless, BRCA1 appears to have dual roles in response to DNA damage leading to cell cycle arrest by upregulation of p21 expression and DNA repair by forming repair foci (Fig. 3). Inactivation of BRCA1 may lead to the accumulation of other genetic insults, such as in p53, which increases sensitivity to DNA-damaging agents and accelerates tumor formation. Novel therapeutic strategies can be developed based on these basic findings. Mammary tumors from Brca1−/− conditional knockout mice provide an excellent model to study breast cancer treatment.

Whether such mutations in *BRCA1* are the threshold events in breast cancer formation appear unlikely, but this remains to be determined. Mechanisms other than mutation have been proposed to account for inactivation or reduction in BRCA1 protein expression in sporadic tumors, including cytoplasmic mislocation (Chen et al., 1995) and decreased mRNA expression (Thompson et al., 1995). Certainly, loss of BRCA1 function in sporadic tumors is a late event, not present in all tumors. Why a mutation in a gene involved in DNA repair would affect primarily the female sex organs is complex, but may be better understood by the findings that wild-type BRCA1 serves to repress E2-responsive ER-α-mediated transcription. However, understanding a larger role for this gene in the more common nonhereditary breast cancer remains a formidable challenge.

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LITERATURE CITED

Anderson SF, Schlegel BP, Nakajima T, Wolpin ES, Parvin JD. 1998. BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. Nat Genet 19:254–256.

Bienstock RJ, Darden T, Wiseman R, Pedersen L, Bennet JC. 1996. Molecular modeling of the amino-terminal zinc ring domain of BRCA1. Cancer Res 56:2539–2545.

Bork P, Hofmann K, Bucher P, Neuwald AF, Altschul SF, Koonin EV. 1997. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. FASEB J 11:68–76.

Callebaut I, Mornon JP. 1997. From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. FEBS Lett 400: 25–30.

Chapman MS, Verma IM. 1996. Transcriptional activation by BRCA1. Nature 382:678–679.

Chen Y, Chen CF, Riley DJ, Allred DC, Chen PL, Von Hoff D, Osborne CK, Lee WH. 1995. Aberrant subcellular localization of BRCA1 in breast cancer. Science 270:789–791.

Chen CF, Li S, Chen Y, Chen PL, Sharp ZD, Lee WH. 1996a. The nuclear localization sequences of the BRCA1 protein interact with the importin-α subunit of the nuclear transport signal receptor. J Biol Chem 271:32863–32868.

Chen Y, Farmer AA, Chen C-F, Jones DC, Chen P-L, Lee W-H. 1996b. BRCA1 is a 220 kDa nuclear phosphoprotein that is expressed and phosphorylated in a cell cycle dependent manner. Cancer Res 56: 3168–3172.

Easton DF, Bishop DT, Ford D, Crockford GP. 1993. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet 52:678–701.

Fan S, Wang JA, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID, Rosen EM. 1999. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 284:1354–1356.

Futreal PA, Cochran C, Marks JR, Iglehart JD, Zimmerman W, Barrett JC, Wiseman RW. 1994a. Mutation analysis of the THRA1 gene in breast cancer: deletion/fusion of the gene to a novel sequence on 17q in the BT474 cell line. Cancer Res 54:1791–1794.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Soderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG, Barrett JC, Skolnick MH, Kamb A, Wiseman R. 1994b. BRCA1 mutations in primary breast and ovarian carcinomas. Science 266:120–122.

Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH. 1996. *Brca1* deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. Nature Genet 12:191–194.

Gowen LC, Avrutskaya AV, Latour AM, Koller BH, Leadon SA. 1998. BRCA1 required for transcription-coupled repair of oxidative DNA damage. Science 281:1009–1012.

Haile DT, Parvin JD. 1999. Activation of transcription in vitro by the BRCA1 carboxyl-terminal domain. J Biol Chem 274:2113–2117.

Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW. 1996. The tumor suppressor gene *Brca1* is reguired for embryonic cellular proliferation in the mouse. Cell 85:1009–1023.

Hakem R, de la Pompa JL, Elia A, Potter J, Mak TW. 1997. Partial rescue of Brca1$^{5-6}$ early embryonic lethality by p53 or p21 null mutation. Nature Genet 16:298–302.

Hall PA, Lane DP. 1997. Tumour suppressors: a developing role For P53? Curr Biol 7:R144–R147.

Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, King MC. 1990. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250:1684–1689.

Harkin PD, Bean JM, Miklos D, Song Y-H, Truong VB, Englert C, Christians FC, Ellisen LW, Maheswaran S, Oliner JD, Haber DA. 1999. Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. Cell 97:575–586.

Hu Y-F, Hao ZL, Li R. 1999. Chromatin remodeling and activation of chromosomal DNA replication by an acidic transcriptional activation domain from BRCA1. Genes Dev 13:637–642.

Iwabuchi K, Bartel PL, Li B, Marraccino R, Fields S. 1994. Two cellular proteins that bind to wild-type but not mutant p53. Proc Natl Acad Sci USA 91:6098–6102.

Koonin EV, Altschul SF, Bork P. 1996. BRCA1 protein products: functional motifs. Nature Genet 13:266–267.

Li S, Chen PL, Subramanian T, Chinnadurai G, Tomlinson G, Osborne CK, Sharp ZD, Lee WH. 1999. Binding of CtIP to the BRCT repeats of BRCA1 involved in the transcription regulation of p21 is disrupted upon DNA damage. J Biol Chem 274:11334–11338.

Liu CY, Flesken-Nikitin A, Li S, Zeng YY, Lee WH. 1996. Inactivation of the mouse *Brca1* gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. Genes Dev 10:1835–1843.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher J, Yakumo K, Gholalmi Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FJ, Helvering L, Morrison P, Rostek P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71.

Monteiro ANA, August A, Hanafusa H. 1996. Evidence for a tran-

scriptional activation function of BRCA1 C-terminal region. Proc Natl Acad Sci USA 93:13595–13599.
Nibu Y, Zhang H, Levine M. 1998. Interaction of short-range repressors with *Drosophila* CtBP in the embryo. Science 280:101–104.
Ouchi T, Monteiro ANA, August A, Aaronson SA, Hanafusa H. 1998. BRCA1 regulates P53-dependent gene expression. Proc Natl Acad Sci USA 95:2302–2306.
Schaeper U, Subramanian T, Lim L, Boyd JM, Chinnadurai G. 1998. Interaction between a cellular protein that binds to the C-terminal region of adenovirus E1A (CtBP) and a novel cellular protein is disrupted by E1A through a conserved PLDLS motif. J Biol Chem 273:8549–8552.
Scully R, Anderson SF, Chao DM, Wei WJ, Ye LY, Young RA, Livingston DM, Parvin JD. 1997a. BRCA1 is a component of the RNA polymerase II holoenzyme. Proc Natl Acad Sci USA 94:5605–5610.
Scully R, Chen JJ, Ochs RL, Keegan K, Hoekstra M, Feunteun J, Livingston DM. 1997b. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. Cell 90:425–435.
Scully R, Chen JJ, Plug A, Xiao YH, Weaver D, Feunteun J, Ashley T, Livingston DM. 1997c. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 88:265–275.
Somasundaram K, Zhang HB, Zeng YX, Houvras Y, Peng Y, Zhang HX, Wu GS, Licht JD, Weber BL, El-Deiry WS. 1997. Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor $P21^{WAF1/CiP1}$. Nature 389:187–190.
Takekawa M, Saito H. 1998. A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell 95:521–530.
Thomas JE, Smith M, Tonkinson JL, Rubinfeld B, Polakis P. 1997. Induction of phosphorylation on BRCA1 during the cell cycle and after DNA damage. Cell Growth Differ 8:801–809.
Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT. 1995. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. Nature Genet 9:444–450.
Tomlinson GE, Chen T-L, Stastny VA, Virmani AK, Spillman MA, Tonk V, Blum JL, Schneider NR, Wistuba II, Shay JW, Minna JD, Gazdar AF. 1998. Characterization of a breast cancer cell line derived from a germ-line *BRCA1* mutation carrier. Cancer Res 58:3237–3242.
Wang Q, Zhang H, Kajino K, Greene MI. 1998. BRCA1 binds c-Myc and inhibits its transcriptional and transforming activity in cells. Oncogene 17:1939–1948.
Wong AK, Ormonde PA, Pero R, Chen Y, Lian L, Salada G, Berry S, Lawrence Q, Dayananth P, Ha P, Tavtigian SV, Teng DH, Bartel PL. 1998. Characterization of a carboxy-terminal BRCA1 interacting protein. Oncogene 17:2279–2285.
Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, Fields P, Marshall G, Narod S, Lenoir GM, Lynch H, Feunteun J, Devilee P, Cornelisse CJ, Menko FH, Daly PA, Ormiston W, McManus R, Pye C, Lewis CM, Cannon-Albright LA, Peto J, Ponder BAJ, Skolnick MH, Easton DF, Goldgar DE, Stratton MR. 1994. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. Science 265:2088–2090.
Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregrory S, Gumbs C, Micklem G, Barfoot R, Hamoudi R, Patel S, Rice C, Biggs P, Hashim Y, Smith A, Connor F, Arason A, Gudmundsson J, Ficenec D, Kelsell D, Ford D, Tonin P, Bishop DT, Spurr NK, Ponder BAJ, Eeles R, Peto J, Devilee P, Cornelisse C, Lynch H, Narod S, Lenoir G, Egilsson V, Barkadottir RB, Easton FE, Bentley DR, Futreal PA, Ashworth A, Stratton MR. 1995. Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792.
Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX. 1999a. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation [In Process Citation]. Nature Genet 22:37–43.
Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX. 1999b. Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol Cell 3:389–395.
Yarden RI, Brody LC. 1999. BRCA1 interacts with components of the histone deacetylase complex. Proc Natl Acad Sci USA 96:4983–4988.
Yu X, Wu LC, Bowcock AM, Aronheim A, Baer R. 1998. The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. J Biol Chem 273:25388–25392.
Zhang H, Somasundaram K, Peng Y, Tian H, Bi D, Weber BL, El-Deiry WS. 1998a. BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene 16:1713–1721.
Zhang X, Morera S, Bates PA, Whitehead PC, Coffer AI, Hainbucher K, Nash RA, Sternberg MJ, Lindahl T, Freemont PS. 1998b. Structure of an XRCC1 BRCT domain: a new protein-protein interaction module. EMBO J 17:6404–6411.
Zhong Q, Chen C-F, Li S, Chen Y, Wang C-C, Xiao J, Chen P-L, Sharp ZD, Lee W-H. 1999. BRCA1 is essential for DNA damage response mediated by the Rad50/Mre11/p95 complex. Science 285:747–750.