Review Article

Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage

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BRCA1 (BReast-CAncer susceptibility gene 1) and BRCA2 are tumor suppressor genes, the mutant phenotypes of which predispose to breast and ovarian cancers. Intensive research has shown that BRCA proteins are involved in a multitude of pivotal cellular processes. In particular, both genes contribute to DNA repair and transcriptional regulation in response to DNA damage. Recent studies suggest that BRCA proteins are required for maintenance of chromosomal stability, thereby protecting the genome from damage. New data also show that BRCAs transcriptionally regulate some genes involved in DNA repair, the cell cycle, and apoptosis. Many of these functions are mediated by a large number of cellular proteins that interact with BRCAs. The functions of BRCA proteins are also linked to distinct and specific phosphorylation events; however, the extent to which phosphorylation-activated molecular pathways contribute to tumor suppressor activity remains unclear. Finally, the reasons why mutations in BRCA genes lead to the development of breast and ovarian cancers are not clearly understood. Elucidation of the precise molecular functions of BRCAs is expected to improve our understanding of hereditary as well as sporadic mammary carcinogenesis. (Cancer Sci 2004; 95: 866–871)

Approximately 5% of breast cancers show a familial pattern of occurrence.[1] Discovery of the genes conferring susceptibility to familial breast cancer and determination of their functional mechanisms would considerably enhance our understanding of the etiology and progression of breast tumors. In 1990, genetic studies provided initial evidence that the risk of breast cancer in some families is linked to chromosome 17q21.[2] This 17q-associated syndrome was characterized by autosomal dominant inheritance with incomplete penetrance. In fact, loss of heterozygosity (LOH) at 17q was found in most familial breast and ovarian tumors, suggesting the involvement of tumor suppressor gene(s).[3, 4] In 1994, the breast-cancer susceptibility gene, BRCA1, was identified by positional cloning; subsequently, this gene has been the subject of intensive research effort[5] (Fig. 1). BRCA1 is composed of 22 coding exons distributed over 100 kb of genomic DNA. This gene encodes 1863 amino acids, and more than 200 different germline mutations associated with cancer susceptibility have been identified. Many disease-predisposing alleles of BRCA1 have loss-of-function mutations, the majority of which result in premature truncation of the protein. Because only 45% of familial breast cancers showed evidence of linkage to BRCA1, the search for a second breast cancer susceptibility gene continued. In 1995, the BRCA2 gene was identified at chromosome 13q12.3[6, 7] (Fig. 1). Mutations in BRCA1 and BRCA2 are not simply associated with an elevated risk of breast cancer.[8] Mutation carriers also have increased susceptibility to ovarian, pancreatic, prostatic, and male breast cancers. Other associations may be revealed as more epidemiological information becomes available. Surprisingly, despite the inherited predisposition to cancer associated with BRCA1 and BRCA2, somatic disease-causing mutations in either of these genes are extremely rare in sporadic breast cancers.[9, 10]

Over the last 10 years, much has been learned about the structures, functions, and unique features of BRCA gene products. In particular, research into the functions of BRCA proteins has revealed that BRCA proteins interact with a number of regulatory proteins (Table 1). In this article, we review recent advances in our understanding of the roles of BRCA1 and BRCA2 in the biological response to DNA damage.

Role in DNA repair

1) BRCA1

Initial evidence suggesting a role of BRCA1 in the repair of damaged DNA was derived from the observation that BRCA1 is hyperphosphorylated in response to DNA damage and relocated to sites of replication forks marked by proliferating cell nuclear antigen (PCNA).[11, 12] In response to ionizing radiation, BRCA1 is bound and phosphorylated by an ataxia-telangiectasia mutated (ATM) kinase[13, 14] (Fig. 1). The major target for ATM phosphorylation after ionizing radiation is Ser1387 of BRCA1. In response to ultraviolet irradiation, Ser1457 is primarily phosphorylated, mainly by ATM-related kinase (ATR).[15] The G2/M control kinase, CHK2, has been shown to phosphorylate BRCA1 at Ser988 on exposure to ionizing radiation[16, 17] (Fig. 1). Other sites of BRCA1 that are phosphorylated in response to DNA damage, such as Ser1423 and Ser1524, have been reported.[13, 14] It is likely, therefore, that BRCA1 is phosphorylated at multiple residues by different kinases after DNA damage (Fig. 2). However, how each type of phosphorylation affects the functions of BRCA1 remains obscure.

Subsequent studies demonstrated the involvement of BRCA1 and BRCA2 in complexes that activate the repair of double-strand breaks (DSBs) and initiate homologous recombination (HR), linking the maintenance of genomic integrity to tumor suppression. BRCA1 and BRCA2 co-localize with Rad51 to form complexes.[18, 19] Eukaryotic Rad51 proteins are homologues of bacterial RecA and are required for recombination during mitosis and meiosis, as well as for HR repair of DSBs.[20] Rad51 coats single-stranded DNA to form a nucleoprotein filament that invades and pairs with a homologous region in duplex DNA, and then activates strand exchange to generate a

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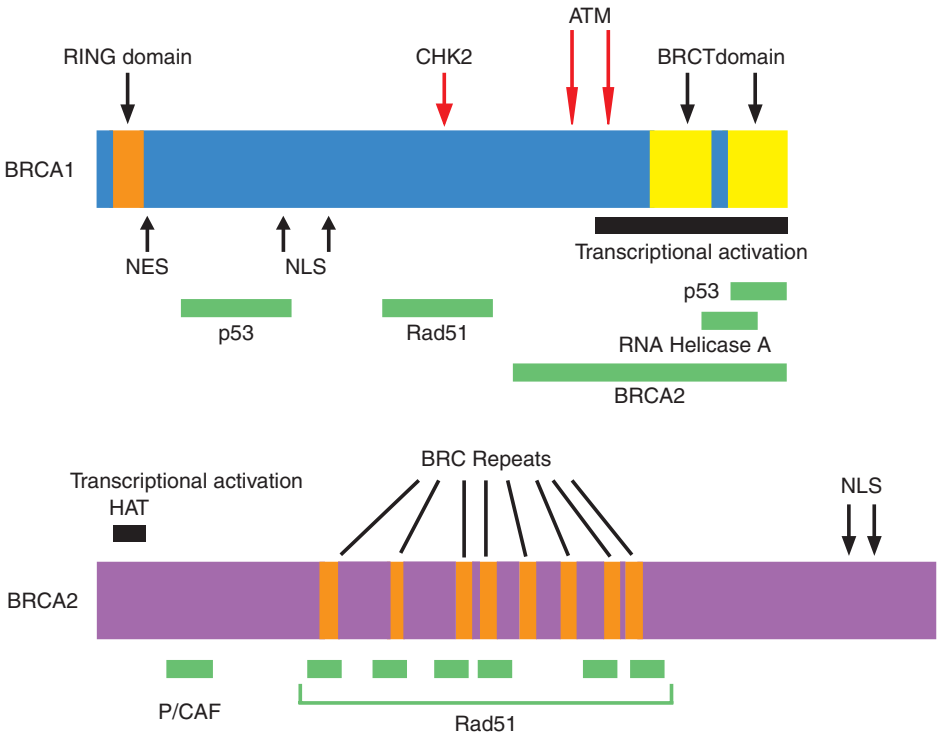

Fig. 1. Features of the BRCA proteins. BRCA1 contains the N-terminal RING domain, nuclear localization signals (NLS), and two C-terminal BRCT domains. Interacting proteins are shown below the binding regions (green bars). Sites phosphorylated by CHK2 or ATM are also indicated (red arrows). BRCA2 contains eight repeats of the BRC motif. Rad51 directly binds six of eight BRC repeats (green bars).

Table 1. Proteins interacting with BRCA1

DNA repair	ATM, CHK2, ATR, BRCA2, RAD51, RAD50/ MRE11/NBS1, BASC, PCNA, H2AX, c-Abl
Transcription	RNA polymerase II holoenzyme (RNA helicase A, RPB2, RPB10 α), HDAC1, HDAC2, E2F, CBP/p300, SWI/SNF complex, CtIP, p53, androgen receptor, ATF1, STAT1, estrogen receptor α, c-Myc, ZBRK1
Cell cycle	RB, CDK2, p21, p27, BARD1
Others	BAP1, BIP1, BRAP2, importin α

crossover between the juxtaposed DNA.[21, 22] Co-localization of BRCAs with Rad51 at sites of recombination and DNA damage-induced foci strongly suggests that BRCAs have a role in both the detection and the repair of DSBs[18] (Fig. 3). In this regard, focus formation of Rad51 is reduced after treatment with DNA-damaging agents and is deficient during repair of DSBs by HR in BRCA1-deficient cells.[23, 24] However, accumulating evidence suggests that BRCA1 might not directly regulate Rad51, since interactions between BRCA1 and Rad51 are indirect and stoichiometrically negligible.[25]

Other studies have shown that BRCA1 co-localizes and co-immunoprecipitates with Rad50, together with its partners Mre11 and NBS1.[26, 27] BRCA1 apparently functions as a regulator of the Rad50-Mre11-NBS1 complex.[28] Mre11 encodes nuclease activity, which resects flush ends of DSBs to generate ssDNA tracts.[29] BRCA1 binds DNA directly and inhibits this Mre11 activity, regulating the length and the persistence of ssDNA generation at sites of DNA damage[30] (Fig. 3). Since ssDNA is a substrate for DNA repair by HR, BRCA1 might play an essential role in HR-mediated repair of DSBs by inactivating Mre11. Indeed, HR is defective in BRCA1-deficient cells.[31] Recent studies have shown that BRCA1 co-localizes with phosphorylated H2AX (γ-H2AX) in response to DNA damage. DSBs promote an extensive response in chromatin, demonstrated by the phosphorylation of Ser139 at the C-terminus of H2AX.[32, 33] This event extends for thousands of bases around a DSB and can be mediated by DNA damage signaling. γ-H2AX forms discrete foci within 10 min after DNA damage, and BRCA1 is detectable in these foci 30 min thereafter.[34] Importantly, in H2AX-deficient cells, BRCA1 fails to form DNA damage-induced foci, suggesting that at least part of the BRCA1 response to DSBs takes place on chromatin.[35] Forced entrapment of BRCA1 in chromatin causes phosphorylation of H2AX by co-localization with BRCA1 in a DNA damage-independent manner. BRCA1 might therefore recruit kinases responsible for H2AX phosphorylation to DNA lesions and nucleate repair foci.[36]

A recent study has revealed that BRCA1 contributes to the regulation of c-Abl activity.[37] c-Abl tyrosine kinase is ubiquitously expressed and localized in the cytoplasm and nucleus. Nuclear c-Abl is activated by diverse genotoxic agents and induces apoptosis mediated by p73 or Rad9.[38, 39] c-Abl is also implicated as a regulator of transcription and DNA repair. BRCA1 and c-Abl form a complex constitutively, and exposure to ionizing radiation triggers an ATM-dependent disruption of this BRCA1-c-Abl complex, coinciding with the activation of c-Abl kinase activity.[37] Loss of BRCA1 results in constitutively elevated c-Abl kinase activity, suggesting that BRCA1 is involved in the control of such activity. These findings suggest a route by which BRCA1 affects cellular responses to DNA damage, distinct from a direct role in DNA repair or a role in cell cycle checkpoint control.

2) BRCA2

The roles played by BRCA1 and BRCA2 in the repair of DSBs by HR appear to differ. Available evidence indicates a more direct role of BRCA2. BRCA2-deficient cells exhibit increased sensitivity to ionizing radiation, indicative of a defect in DSB repair, whereas the cell cycle checkpoint and apoptotic

responses to DNA damage remain intact.[40, 41] In addition, BRCA2-deficient cells accumulate chromosomal breaks and aberrant mitotic exchanges during culture. Rad51-deficient cells show similar phenotypes, providing genetic evidence that interactions of BRCA2 with Rad51 are fundamental for the maintenance of cell division and chromosome structure. Physiologically, interactions between BRCA2 and Rad51 are mediated by the BRC repeat and an unrelated domain located at the C-terminus (Fig. 1). Recent studies have shown that BRCA2 regulates the intracellular localization and function of Rad51.[42] In BRCA2-deficient cells, nuclear transport of Rad51 is impaired, suggesting that BRCA2 moves Rad51 from the site of synthesis to the site of DNA damage processing[42] (Fig. 3). These *in vitro* findings led to the hypothesis that BRCA2 plays an essential role in the repair of DSBs *in vivo*. One possible model is that the BRCA2-Rad51 complex resides in two states *in vivo*: an inactive state, which prevents the binding of single-strand DNA by Rad51, and an active state, in which Rad51 forms nucleoprotein filaments to be transferred to sites of DNA damage by BRCA2 (Fig. 3). Transition from the inactive to the active state is speculated to involve post-translational modification, such as phosphorylation induced by DNA damage, triggering a substantial structural change in the BRCA2-Rad51 complex to release Rad51 from BRCA2. Whether this model, based on *in vitro* biochemical observations, is relevant to the cellular function of full-length BRCA2 remains to be clarified. Structural characterization of the BRCA2-Rad51 complex might provide clues to this question.

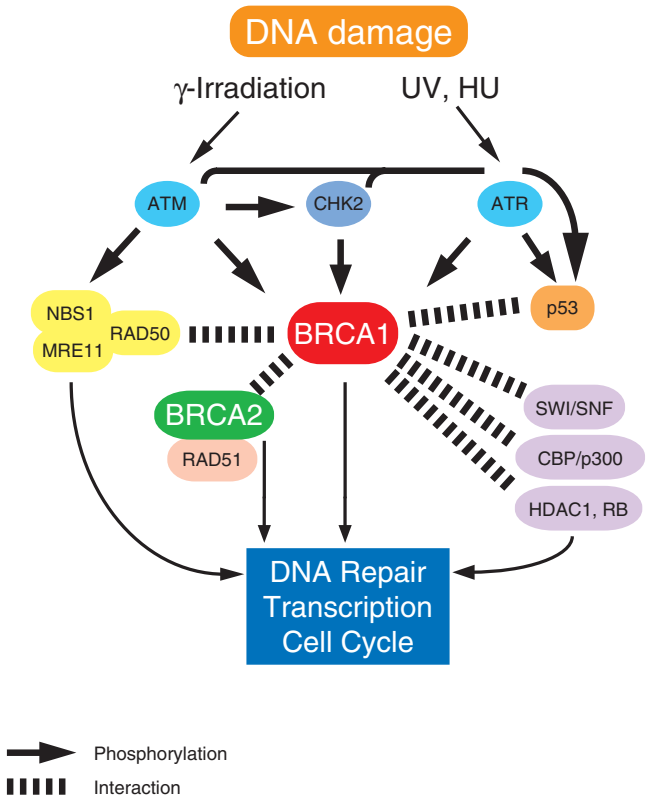

Fig. 2. Functions of BRCA proteins in response to DNA damage. On DNA damage, BRCA proteins interact with numerous other proteins to modulate DNA repair, transcription, and the cell cycle.

Role in transcriptional response to DNA damage

1) BRCA1

BRCA1 has been implicated in the transcriptional regulation of several genes activated in response to DNA damage. The first line of evidence came from an observation that the C-terminus of BRCA1 binds and activates the basal transcriptional machinery.[43, 44] A subsequent series of studies demonstrated that the C-terminus of human BRCA1 (amino acids 1528–1863) complexes with RNA polymerase II through RNA helicase A.[45] This interaction appears to involve several proteins associated with the core polymerase complex. In fact, BRCA1 protein is a component of the RNA polymerase II holoenzyme, and deletion of the C-terminal 11 amino acids of BRCA1 attenuates the association with this holoenzyme. BRCA1 was also shown to regulate transcription in a purified *in vitro* system. More recently, other regions of BRCA1 have also been shown

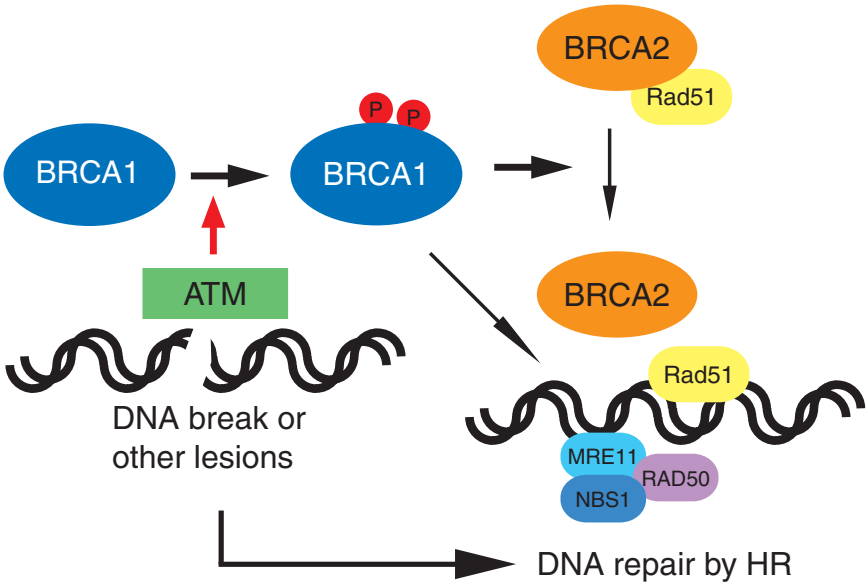

Fig. 3. A model for the role of BRCA proteins in repairing damaged DNA. BRCA1 is phosphorylated by ATM in response to DSBs. Phosphorylated BRCA1 activates DNA repair through HR, in cooperation with BRCA2 and Rad51. BRCA1 also recruits Rad50-Mre11-NBS complex to the sites of DNA damage.

to contribute to transcriptional regulation in concert with RNA polymerase II holoenzyme.[46] It is now clear that BRCA1 bound to holoenzyme is present as a heterodimer with BARD1 (BRCA1-associated RING domain protein 1), suggesting that the N-terminal RING-finger domain of BRCA1 provides another pool for the holoenzyme component.[47] The internal portion of BRCA1 binds to a large number of transcriptional factors, which may mediate signals to RNA polymerase II. Indeed, transcriptional activation by BRCA1 is supported by its ability to interact directly or indirectly with several transcriptional factors.

Finding target genes regulated by BRCA1 would shed considerable light on the transcriptional role of BRCA1. Studies using microarray technology have shown that p53-responsive cell cycle progression inhibitor and stress-response factors such as p21 and GADD45 are stimulated by BRCA1 overexpression[48] (Fig. 4). Subsequent investigations have revealed that BRCA1 serves as a co-activator for p53.[49] Co-immunoprecipitation experiments have also demonstrated that BRCA1 interacts with p53. Deletion of the N-terminus (amino acids 224–500) impairs *in vitro* interactions with p53. Furthermore, a truncated mutant of BRCA1 that retains the p53-binding site exhibits a dominant negative effect in p53-mediated transcription, thereby substantiating a pivotal role for interactions of BRCA1 and p53 *in vivo*. A recent study shows that p53 is stabilized by overexpression of BRCA1, suggesting that BRCA1 functions to stimulate p53 pathways.[48]

BRCA1 also binds to ZBRK1, a transcriptional factor binding specifically to the DNA sequence GGGXXXCAGXXXTTT.[50] This binding motif is present in the promoter region of many transcriptional targets for BRCA1, such as p21, GADD45, and EGR1. Indeed, coexpression of BRCA1 and ZBRK1 was found to repress GADD45 promoter, contrary to previous findings that BRCA1 activates GADD45 expression. One explanation for this discrepancy is that overexpression of BRCA1 may titrate ZBRK1 away from the promoter, allowing transcription to occur. In addition, phosphorylation induced by DNA damage and binding of BRCA1 to other repressors such as CtIP may modify this regulatory mechanism. Previous studies have indicated that BRCA1 activation is attenuated by the CtIP-CtBP complex, which binds to the BRCT domain of BRCA1. The interaction of BRCA1 with CtIP is partially abrogated by DNA-damage-induced, ATM-dependent phosphorylation of CtIP, thus relieving suppression of the transactivation potential of BRCA1[51] (Fig. 4).

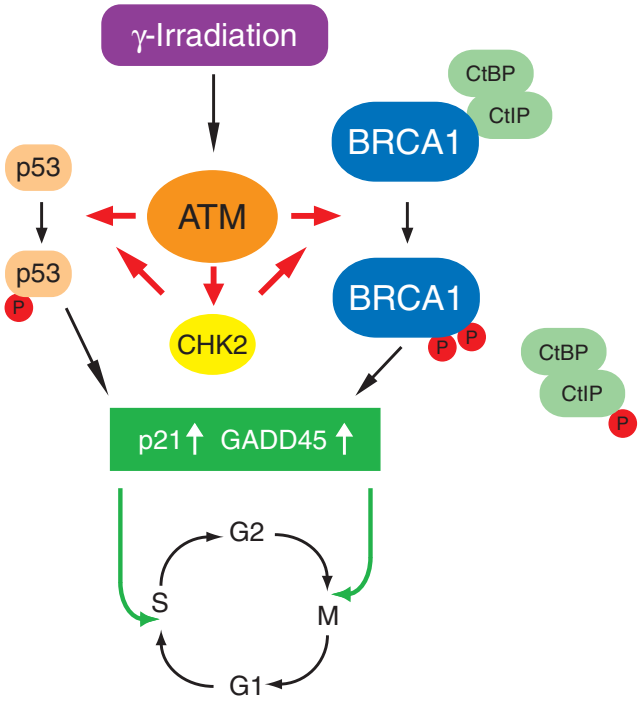

Fig. 4. Role of BRCA1 in transcriptional regulation after exposure to ionizing radiation (IR). ATM is activated by IR and phosphorylates CtIP to disrupt the CtIP-CtBP-BRCA1 complexes. BRCA1 is then released and activates p21 and GADD45. Activation of cell cycle checkpoints induces replication arrest to allow repair of DNA damage.

A recent study has reported that BRCA1 and STAT1 co-operate to regulate p21. BRCA1 binds to the transcriptional activation domain of STAT1.[52] The binding of BRCA1 to STAT1 leads to the induction of a subset of IFN-γ regulated genes. STAT1-mediated transcriptional activation by UV radiation depends on Ser727 phosphorylation by p38/MAPK, and BRCA1 mainly associates with Ser727-phosphorylated STAT1. These findings raise the intriguing possibility that BRCA1 functions as a bridging protein, connecting DNA damage and stress response pathways to execute specific cellular responses, such as cell cycle arrest or apoptosis.

2) BRCA2

The possible function of BRCA2 as a transcriptional regulator is far less certain. Available evidence suggests that the product of BRCA2 exon 3 (amino acids 23–105) activates transcription and that a missense mutation (Tyr42Cys) of BRCA2 reduces the transactivation potential. The basis for this mutation and its relevance to carcinogenesis remain to be defined. Other studies have shown that overexpression of BRCA2 is associated with down-regulation of basal p53 transcriptional activity. In contrast, BRCA2 might activate transcription by modulating histone acetylation. BRCA2 interacts with the transcriptional co-activator protein P/CAF (p300/CBP-associated factor) and its associated p300/CBP, both of which possess histone acetylase activity. BRCA2 might recruit these histone modifiers to the transcription complex to induce transcriptional activity.

Recent work has demonstrated that a novel protein, EMSY, binds to exon 3 of BRCA2.[53] EMSY is amplified in some sporadic breast cancers and appears to negatively regulate BRCA2 function in transcriptional activation. A role for ENSY in the DNA damage response is supported by its co-localization with γ-H2AX and BRCA2 in irradiated cells. EMSY also has a basal promoter suppressive function, suggesting that it functions as a general transcriptional repressor. Moreover, EMSY is implicated in DNA repair and chromatin remodeling. In sporadic breast cancer, EMSY amplification correlates with a poorer outcome, specifically for node-negative breast cancer. In addition, overexpression of EMSY is associated with high-grade sporadic ovarian carcinomas, suggesting involvement of the BRCA2 pathway in sporadic breast and ovarian cancers. Although the mechanistic implications of BRCA2-EMSY interactions remain to be fully delineated, further analysis of this novel BRCA2-associated protein might provide important insights into pathways that are disrupted in sporadic breast and ovarian cancers.

Role in DNA damage-responsive cell cycle checkpoints

1) BRCA 1

Cell cycle checkpoints play an essential role in cell survival by preventing the propagation of DNA damage through cell cycle progression before DNA repair. Recent studies using cells defective for different DNA damage-responsive proteins have demonstrated that both ATM and BRCA1 are required for effective S-phase and G2/M-phase checkpoints. Expression of BRCA1 variants defective for ATM-mediated phosphorylation is associated with a defect in G2/M arrest, suggesting that

BRCA1 phosphorylation by ATM is indispensable for G2/M checkpoints in the DNA damage response[13] (Fig. 4). Other work has indicated that BRCA1 regulates G2/M DNA damage-induced checkpoints through its ability to activate Chk1 kinase and thereby induce signaling cascades downstream of Chk1.[54] In this context, the finding that BRCA1-deficient cells exhibit defective G2/M arrest in response to ionizing radiation further supports a role of BRCA1 in the regulation of G2/M checkpoints.

As mentioned above, BRCA1 functions as a co-activator of p53-mediated gene transcription. In BRCA1-deficient cells, the expression of 14-3-3σ, which is regulated by p53, is significantly diminished.[55] Since 14-3-3σ is a major G2/M checkpoint control gene, 14-3-3σ induction by BRCA1 may also be involved in BRCA1-mediated G2/M checkpoints. Other studies have shown that overexpression of BRCA1 results in the transcriptional activation of GADD45 in a p53-dependent manner.[56, 57] As GADD45 has been implicated in G2/M checkpoints, BRCA1 may in part activate G2/M checkpoints by induction of GADD45 protein (Fig. 4). Interestingly, another p53 target gene, G1 cyclin-dependent kinase inhibitor p21, is also transactivated by exogenous expression of BRCA1 to block S-phase entry in a p53-independent manner[58] (Fig. 4). Importantly, cancer-associated mutant BRCA1 failed to activate the p21 promoter. BRCA1 has also been found to transactivate the cyclin-dependent kinase inhibitor p27KIP1.[59] The induction of G1 arrest by exogenous BRCA1 expression is likely to be associated with activation of p27KIP1.

2) BRCA2

It remains unclear whether BRCA2 participates directly in cell cycle regulation or checkpoint functions. Available evidence suggests that BRCA2 mediates G2/M-phase control by interacting with a novel protein, BRCA2-associated factor 35 (BRAF35), which binds to branched DNA structures.[60] Nuclear staining has revealed a close association of BRAF35/BRCA2 complex with condensed chromatin, coincident with histone H3 phosphorylation. Importantly, antibody microinjection experiments suggest a role of BRCA2/BRAF35 complex in modulation of metaphase progression.[60] However, it is premature to conclude that BRCA2 is directly involved in mitotic progression. Since BRCA2 has a major role in DNA repair, its suppression is thought to induce unrepaired DNA lesions, which cause cell cycle arrest by activating checkpoint signaling, including mitotic progression.

Another line of evidence suggests that genetic instability caused by loss of BRCA2 function could trigger mutations, including those in checkpoint genes such as p53.[61] Furthermore, previous work has shown that tumors from BRCA2-deficient animals exhibit dysfunction of the spindle assembly checkpoint, accompanied by mutations in p53.[62] In this context, dysfunction of p53 as a result of mutation leads to uncontrolled cell cycle checkpoints, inducing uncontrolled proliferation and invasive growth. These observations clearly indicate an indirect role of BRCA2 in cell cycle regulation. Obviously, more work will be required to determine whether BRCA2 regulates cell cycle control, as distinct from its role in DNA repair.

Conclusions

BRCA1 was identified about 10 years ago. Mutations in BRCA genes have been established to predispose women to breast and ovarian cancers, the endpoint of BRCA protein dysfunction. Although previous studies have implicated both BRCA1 and BRCA2 in the cellular response to DNA damage, little is known about the mechanism by which BRCA proteins modulate this response. Extensive research has revealed that BRCA proteins bind and interact with a number of regulatory proteins (Table 1). Accumulating evidence suggests that BRCA1 and BRCA2 participate in multiple functions, including DNA repair, transcription, and cell cycle control. In the near future, numerous other proteins that bind to BRCA proteins will probably be identified, leading to the discovery of new functions.

It is unclear why a BRCA-related predisposition to cancer is apparently site-specific, affecting the breast and ovary, despite the fact that the known functions of BRCA proteins are essential to all cell types. A possible explanation is that breast or ovarian epithelia are particularly vulnerable to transformation when heterozygous for BRCA gene mutations. This increased vulnerability could be attributed to tissue-specific effects of the haploinsufficiency involved in the hormone-responsive proliferative changes unique to these cells. At present, however, the roles of BRCA proteins in epithelial cell biology and transformation remain uncertain.

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