Role played by BRCA1 in regulating the cellular response to stress


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Abstract

BRCA1 (breast-cancer susceptibility gene 1) is a tumour suppressor gene that is mutated in the germline of women with a genetic predisposition to breast and ovarian cancer. In this review, we examine the role played by BRCA1 in mediating the cellular response to stress. We review the role played by BRCA1 in detecting and signalling the presence of DNA damage, particularly double-strand DNA breaks, and look at the evidence to support a role for BRCA1 in regulating stress response pathways such as the c-Jun N-terminal kinase/stress-activated protein kinase pathway. In addition, we examine the role played by BRCA1 in mediating both cell-cycle arrest and apoptosis following different types of cellular insult, and how this may be modulated by the presence or absence of associated proteins such as p53. Finally, we explore the possibility that many of the functions associated with BRCA1 may be based on transcriptional regulation of key downstream genes that have been implicated in the regulation of these specific cellular pathways.

Introduction

The tumour suppressor gene BRCA1 (breast-cancer susceptibility gene 1), which is mutated in the germline of women with a genetic predisposition to breast and ovarian cancers, was identified by positional cloning in 1994 and has been the subject of intensive research efforts ever since [1]. BRCA1 encodes a nuclear phosphoprotein of 1863 amino acids; germline mutations of BRCA1 are found in approx. 50% of breast/ovarian cancer pedigrees and in approx. 10% of women with early onset of breast cancer, irrespective of family history [2]. More than 200 different mutations in BRCA1 have been reported which are clearly associated with cancer susceptibility (http://www.nhgri.nih.gov/Intramural-research/Lab-transfer/BIC). Of the known mutations, 95% are predicted to result in premature termination of translation [3]. Somatic inactivation of BRCA1 is rare in sporadic breast cancers; however, the majority of sporadic tumours exhibit reduced expression of BRCA1 protein, indicating that epigenetic mechanisms may also play a role in regulating BRCA1 expression [3,4]. Although the exact function of BRCA1 remains to be defined, roles in transcriptional regulation, cell-cycle checkpoint control and DNA damage repair have been suggested.

The predicted amino-acid sequence and structure of the BRCA1 protein is consistent with these multiple roles.
pathway and in the activation of both the G1/S, S and G2/M cell-cycle checkpoints [10,11]. Moreover, evidence exists to suggest that BRCA1 may mediate some or all of these responses via its ability to transactivate specific subsets of target genes that are integral to the individual pathways.

Role in DNA repair

The first evidence to suggest a role for BRCA1 in the repair of DNA damage came from the observation that BRCA1 was hyperphosphorylated after exposure to the DNA-damaging agents UV, hydroxyurea and hydrogen peroxide, and was relocated to sites of replication forks marked by proliferating cell nuclear antigen [12,13]. The phosphorylation of BRCA1 and its subsequent effects on downstream signalling have been extensively studied since these initial observations were made.

In response to ionizing radiation, BRCA1 is bound and phosphorylated by the ATM (ataxia telangiectasia mutated) kinase, the product of the ataxia telangiectasia gene. The ataxia telangiectasia gene mutation carriers are predisposed to developing cancers, including breast cancer, and exhibit increased sensitivity to ionizing radiation [14,15]. It has been demonstrated that Ser1387 of BRCA1 is specifically phosphorylated in response to γ irradiation in an ATM-dependent manner, while Ser1457 is predominantly phosphorylated following UV irradiation, an effect that is mediated by the ATM-related kinase [16]. In addition, a number of other kinases have been shown to bind to and phosphorylate BRCA1 in response to DNA damage. Chk2, a kinase involved in the regulation of the G2/M checkpoint, which is mutated in a subset of Li–Fraumeni families, has been shown to phosphorylate BRCA1 on Ser1423 following γ irradiation [17,18]. Similarly, BRCA1 is phosphorylated on various other serine residues including Ser1432, Ser1457 and Ser1424 in response to both γ irradiation and UV irradiation [14,15].

It is likely, therefore, that BRCA1 is phosphorylated on multiple residues by a variety of different kinases following different types of DNA damage. How all of these different phosphorylation events modulate BRCA1 function remains to be defined.

The relocation of hyperphosphorylated BRCA1 to sites of DNA damage has, in addition, implied a more direct role for BRCA1 in DNA repair. A number of subsequent studies have demonstrated the presence of BRCA1 in protein complexes that are involved in the homologous recombination (HR) repair of double-strand breaks (DSBs) induced by ionizing radiation, free radicals and during replication of a single strand break. Cells typically repair DSBs by the HR, or non-homologous end-joining (NHEJ) pathways. HR is the more accurate of the two mechanisms and predominates in S and G2 phases of the cell cycle when the DNA has been replicated [19]. It provides a perfect second copy of the sequence (sister chromatid) for aligning the breaks. The more error-prone mechanism of NHEJ occurs most often in the G1 phase of the cell cycle. Cells in G1 have only the homologous chromosome for recombination repair, an unacceptable template for HR owing to the likelihood of generating homozygosity for recessive mutations. Instead, the cell chooses to utilize NHEJ, which simply links broken ends together without the benefit of a template. To do this, the cell uses the end-binding Ku70/80 complex and DNA-dependent protein kinase (DNA-PK) followed by XRCC4–ligase 4. Recent reports have suggested a role for BRCA1 in inhibiting this error-prone pathway, which explains why BRCA1–deficient tumours exhibit a characteristic karyotype of duplications, inversions, translocations and deletions [20–22].

Upon detection of a DSB, a signalling cascade is initiated aimed at halting cell cycle progression and recruiting repair factors. The RAD50–MRE11–NBS1 protein complex is involved in both NHEJ, where its role is to expose both 3′ ends of the broken DNA, and in HR to facilitate strand invasion into homologous sequence [23]. Activation of RAD50–MRE11–NBS1 occurs via phosphorylation of the NBS1 protein by ATM, which is also known to phosphorylate BRCA1 (see above). In normal cells, the RAD50–MRE11–NBS1 localizes rapidly to breaks in DNA after exposure to ionizing radiation, and BRCA1 has been shown to interact directly with RAD50 at these damaged regions [24,25].

In addition, both ATM and the RAD50–MRE11–Nijmegen breakage syndrome gene 1 (NBS1) complex are components of a large complex of proteins, the BRCA1–associated genome surveillance complex, which contains BRCA1 and at least 15 other proteins involved in tumour suppression and sensing DNA damage, which are thought to mark sites of abnormal DNA structure and activate repair mechanisms [26]. BRCA1 has also been shown to co-localize with RAD51, the human homologue of bacterial RecA at sites of DSBs in DNA [27,28]. RAD51 plays an integral part both in normal mitosis and meiosis, and in DSB repair through its ability to bind single-stranded DNA and facilitate DNA strand exchange [29,30]. RAD51 forms a nucleoprotein filament that most likely includes the related proteins XRCC2 and XRCC3, which exchange the broken single strand with the same sequence from a double-stranded DNA molecule (reviewed in [31]).

BRCA1 co-localizes with RAD51 at sites of recombination and in ionizing radiation-induced foci, which strongly implicates BRCA1 in both the detection and repair of DSBs [28]. Consistent with these observations, the HCC1937 cell line, which expresses a single, mutated copy of BRCA1, is hypersensitive to ionizing radiation and exhibits a 2–3-fold increase in radiation resistance when wild-type BRCA1 is reintroduced to the cells [32]. A recent report has further demonstrated the significance of the BRCA1–RAD51 association by showing that mouse embryonic stem cells lacking BRCA1 exhibit reduced RAD51 focus formation after exposure to a DNA-damaging agent and are deficient in the repair of DSBs by HR [33].

One effect of genomic lesions can be to block transcription of genes on a damaged DNA strand. Transcription-coupled repair (TCR) has evolved to ensure high-priority repair of these types of lesions by removing the stalled RNA polymerase. It has been reported previously that mouse embryonic stem cells deficient in BRCA1 are defective in
the ability to carry out TCR of oxidative DNA damage and are hypersensitive to ionizing radiation and hydrogen peroxide [34]. The involvement of BRCA1 in TCR is possibly mediated through its ability to bind to the RNA polymerase II holoenzyme complex and RNA helicase A, thus mediating degradation of the stalled polymerase machinery via a BARD1 ubiquitylating pathway [35–37].

**Role in activation of stress kinase pathways**

Stress kinase pathways can be activated by events occurring at the cell membrane and cytoplasm as a result of cytotoxic stress. Activation of these pathways can direct the phosphorylation of multiple cellular targets, leading to the activation of DNA repair pathways or cell-cycle arrest via transcriptional activation of specific subsets of genes (reviewed in [9,38]).

A link between BRCA1 expression and activation of the stress-activated protein kinase (SAPK) cascades has been established. JNK is so-called for its ability to bind and phosphorylate Ser63 and Ser73 of the N-terminal transactivation domain of the transcription factor c-Jun, thereby inducing its activation [39]. Depending upon the specific cellular context, activation of the JNK pathway can lead to cell-cycle arrest or apoptosis. For example, JNK phosphorylates p53 on Ser34, resulting in the dissociation of murine double minute-2 (mdm2) from p53, thus preventing the targeted ubiquitylation of p53 and enhancing p53-induced transcription and apoptosis [39]. In addition, JNK has been implicated in the phosphorylation of the transcription factor c-Myc, which is also a BRCA1-binding partner, and in the inhibition of the apoptotic suppressors, bcl-2 and bcl-x [39,40]. In order to examine the effects of BRCA1 on cellular signalling pathways, we generated cell lines with inducible expression of BRCA1 (Figures 1A and 1B). We have demonstrated that over-expression of BRCA1 induces activation of JNK-mediated apoptosis (Figure 1C), an effect that correlates with growth arrest and DNA damage-inducible (GADD) 45 induction by BRCA1 [10]. The suggestion that GADD45 may regulate the JNK pathway came from the observation that GADD45 associates with, and mediates the activation of, MEKK4, an upstream regulator of the JNK kinase cascade [41]. Further delineation of this pathway demonstrated that BRCA1 modulates stress-induced apoptotic signalling through a pathway that sequentially involves the H-ras oncogene, MEKK4, JNK, Fas and Fas ligand interactions and the activation of caspase-9 [41,42].

In addition to JNK, activation of p38 mitogen-activated protein kinase (MAPK) has also been shown to induce either apoptosis or cell-cycle arrest, depending on the nature of the signal and the cellular context [43]. Downstream transcriptional targets of p38 include the transcription factors myocyte enhancer factor 2 (MEF2)/C, Elk1, stress activated protein 1 (SAP-1), activating transcription factor 2, GADD153 and STAT1 (signal transducer and activator of transcription) [39,44,45]. STAT1-mediated transcriptional activation is enhanced by assorted DNA-damaging agents, including UV irradiation, an effect that is dependent on p38/MAPK-induced phosphorylation on Ser277 [46,47]. A recent report has demonstrated that BRCA1 and STAT1 can cooperate to regulate the cyclin-dependent kinase (CDK) inhibitor, p21, and that this regulation is dependent on a physical interaction between BRCA1 and STAT1 [48]. We have shown recently that, in response to interferon γ (IFN-γ) treatment, BRCA1 synergistically upregulates expression of a subset of IFN-γ-regulated genes [49]. The exact mechanism by which this occurs is yet to be defined, but it is likely to reflect the ability of BRCA1 to associate with STAT1, the major effector of the IFN-γ-signalling pathway. The association between BRCA1 and STAT1 may be enhanced following specific types of cellular stress. This mechanism is supported by the observation that STAT1-mediated transcriptional activation in response to UV radiation is dependent on p38/MAPK-induced phosphorylation on Ser277, and that BRCA1 preferentially associates with Ser277-phosphorylated
STAT1 [46–48]. In addition to STAT1 homodimers, BRCA1 may also associate with other STAT1-associated complexes in the nucleus. The observation that BRCA1 specifically associates with STAT1 following phosphorylation on Ser727 raises the intriguing possibility that BRCA1 acts as an ‘integrator’ protein, linking the DNA damage and stress response pathways to effect specific cellular responses such as cell cycle arrest or apoptosis.

Transcriptional regulation by BRCA1
Compelling evidence exists to suggest a role for BRCA1 in transcriptional regulation. In general, the genes that are known to be transcriptional targets of BRCA1 are either involved in cell-cycle regulation, DNA repair or apoptosis. The ability of BRCA1 to modulate gene expression appears to be mediated in part by the C-terminal acidic domain, which has been shown to mediate transcriptional activation when fused to a heterologous DNA-binding domain [50,51]. Consistent with a role in transcriptional regulation, the BRCT motifs have been shown to mediate interactions with a number of proteins implicated in these processes, including RNA pol2/RNA helicase A (RHA), the histone deacetylases (HDACs) HDAC1 and HDAC2, and a range of sequence-specific DNA-binding transcription factors including c-Myc, activating transcription factor 1, p53 and STAT1 [49,52].

Direct transcriptional targets of BRCA1 that are involved in cell-cycle control include the CDK inhibitor p21, which is involved in regulating the G1/S transition [53]. p21 functions, in part, to inhibit cyclin D–cdk4–cdk6 complex formation, which, in turn, prevents phosphorylation of Rb and release of E2F, thereby maintaining cells in G1/S. Regulation of p21 expression by BRCA1 has been shown to occur in both a p53-dependent and -independent manner. BRCA1 has been shown to associate with, and potenti ate the activation of, p53 target genes including p21 [54]. It has been suggested that p53-independent induction of p21 by BRCA1 may be mediated through the interaction of BRCA1 with the transcriptional co-repressor complex CtBP interacting protein (CtIp) that may normally inhibit BRCA1-mediated transactivation by the formation of a BRCA1–CtIp–C-terminal binding protein (CtBp) repressor complex. Dissociation of this complex in response to DNA damage frees BRCA1 to transactivate targets, such as p21, and induce a G1/S arrest [55].

Figure 2 | BRCA1-mediated G2/M arrest in response to taxol correlates with BRCA1-induced activation of GADD45

(A) Northern-blot analysis of the time course of induction for BRCA1, GADD45, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a loading control. Panels represent separate hybridizations to the identical blot. (B) Cell-cycle distribution analysis of propidium iodide-stained MBR62-bcl2 cells in the presence and absence of 10nM Taxol and when cells are induced (−tet) or non-induced (+tet) to express BRCA1 for 24 h. Reproduced with permission from [59], “BRCA1 and GADD45 mediated G2/M cell cycle arrest in response to antimicrotubule agents”, P.B. Mullan, J.E. Quinn, P.M. Gilmore et al. (2001) Oncogene 20, pp. 6123–6131 © Nature Publishing Group.

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Two genes involved in regulation of the G2/M boundary are also known to be transcriptional targets of BRCA1; these are the DNA damage inducible gene, GADD45 and 14-3-3σ [10,56,57]. GADD45 has been shown to induce G2/M cell-cycle arrest through its ability to bind cdc2 and disrupt the cyclin B–cdc2 complex, which is essential for progression into mitosis [58]. Consistent with this, we have demonstrated recently that BRCA1 regulates both the G2 and mitotic checkpoints following treatment of cells with the spindle poison, taxol, an effect that correlates with BRCA1-mediated induction of GADD45 [59] (Figures 2A and 2B). Furthermore, inducible expression of GADD45 also activated G2 and mitotic arrest following taxol treatment, suggesting a role for GADD45 in the regulation of the G2/M checkpoint following disruption of the mitotic spindle [59]. A potential model through which BRCA1 regulates expression of GADD45 has come from a number of related sources. A BRCA1-responsive sequence has been identified in the proximal GADD45 promoter and shown to contain a potential CCAAT/enhancer-binding protein-binding site (60). It has previously been demonstrated that BRCA1 can repress c-Myc-mediated transactivation and, in addition, that c-Myc can repress GADD45 expression through the CCAAT/enhancer-binding protein element within the GADD45 promoter [60]. It is postulated therefore that BRCA1-mediated induction of GADD45 may be achieved through the ability of BRCA1 to relieve transcriptional repression induced by c-Myc at the GADD45 promoter. Similarly, the G2/M checkpoint gene 14-3-3σ has also been identified as a BRCA1 target gene [57]. 14-3-3σ regulates the G2/M checkpoint by binding to, and sequestering, cdc25 in the cytoplasm, thereby preventing activation of cyclin B–cdc2, resulting in G2/M arrest. These studies suggest that BRCA1 can regulate multiple cell-cycle checkpoint pathways through transcriptional activation of key target genes.

Genes that are involved in DNA repair are also transcriptional targets of BRCA1. These include the nucleotide excision repair (NER) genes xeroderma pigmentosum complementation group C (XPC), damaged-DNA binding protein 2 (DDB2) and GADD45, which are transactivated by BRCA1 in a p53-independent manner and are integral to the global genome repair pathway [61]. This study suggests that BRCA1 can activate global genome repair in the absence of p53 and implicates GADD45 in nucleotide excision repair as well as cell-cycle arrest. It has also been demonstrated that BRCA1 can influence p53-dependent transcriptional activation. Specifically, it has been reported that BRCA1-mediated stabilization of p53 results in activation of DNA repair genes such as DDB2 and p53R2 [56]. In contrast, DNA damage-induced stabilization of p53 results in activation of apoptotic genes such as p53-induced gene (P1G3) [56]. BRCA1-induced stabilization of p53 is mediated by its ability to transactivate p14/alternative reading frame (ARF) and subsequent phosphorylation of p53 on Ser15 [53]. The authors concluded that BRCA1 preferentially directs cells towards repair rather than apoptosis.

We have identified a series of BRCA1 target genes that are synergistically up-regulated by BRCA1 in the presence of IFN-γ. These include interferon regulatory factor-7 (IRF-7), MxA and interferon stimulated gene-54 (ISG54) (Figure 3A). The physiological significance of these findings was underscored by the observation that IFN-γ-mediated induction of IRF-7 and MxA was abrogated in the BRCA1 mutant cell line HCC1937, an effect that could be recovered by exogenous expression of wild-type BRCA1 [49]. A previous study has reported that BRCA1 and STAT1 cooperate to regulate the CDK inhibitor, p21, and that this effect is mediated by a physical interaction between BRCA1 and STAT1 [48]. In contrast to this report, we failed to observe p21 induction or G1/S arrest following BRCA1 induction in our model cell system; rather, we observed a dramatic apoptotic cell death phenotype in the presence of BRCA1 and IFN-γ which correlated with IRF-7 induction [49] (Figure 3B). It is possible that these conflicting observations may have arisen because the MBR62-bcl2 cell line used in our study is p53 mutant, whereas the 2F1GH, U3A and G8 cell lines utilized by Ouchi et al. [48] are p53 wild-type.

It has been suggested that IRF-7 may function as a tumour suppressor gene, based on the observation that lack of IRF-7...
expression in the 2TGH fibrosarcoma cell line is due to methylation of the IRF-7 promoter and that IRF-7 is activated through the JNK/SAPK stress response pathway in response to various DNA-damaging chemotherapeutic agents [62]. Our results support a role for IRF-7 in mediating growth suppression and suggest that specific genes, such as IRF-7, may be co-ordinately regulated in response to different stimuli such as IFN-γ or through a BRCA1-dependent DNA damage response pathway. Moreover, it can be speculated that the transcriptional profile of genes regulated by BRCA1 may undergo a ‘switch’ depending on the presence or absence of p53. When p53 is present, BRCA1 activates genes involved in DNA repair, whereas when p53 is absent or mutated in cells, BRCA1 fulfils the normal p53 role by transcriptionally activating genes, such as IRF-7, that drive the cell towards apoptosis.

References