Role played by BRCA1 in transcriptional regulation in response to therapy

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Abstract
BRCA1 (breast-cancer susceptibility gene 1) is a tumour suppressor, implicated in the hereditary predisposition to breast and ovarian cancer. BRCA1 has been implicated in a number of cellular processes including DNA repair and recombination, cell cycle checkpoint control, chromatin remodelling and ubiquitination. In addition, substantial data now exist to suggest a role for BRCA1 in transcriptional regulation; BRCA1 has been shown to interact with the Pol II holoenzyme complex and to interact with multiple transcription factors, such as p53 and c-Myc. We have previously identified a range of BRCA1 transcriptional targets and have linked these to specific cellular pathways, including cell cycle checkpoint activation and apoptosis. Current research is focused on the transcriptional mechanisms that underpin the association of BRCA1 deficiency with increased sensitivity to DNA damage-based chemotherapy and resistance to spindle poisons.

Introduction
BRCA1 (breast-cancer susceptibility gene 1) is a tumour-suppressor protein that was identified based on its linkage to familial breast and ovarian cancer [1]. The BRCA1 gene is mutated in up to 50% of inherited breast cancers, and women who carry a defective copy of the gene have a 50–85% cumulative lifetime risk of developing breast cancer and a 12–60% chance of developing ovarian cancer by the age of 80 [2]. Somatic mutations in BRCA1 are rare; BRCA1 mRNA and protein are down-regulated by epigenetic mechanisms, such as methylation of the BRCA1 promoter, and occur in approx. 30% of sporadic breast cancers and 70% of ovarian cancers [3]. Breast tumours arising from BRCA1 mutation exhibit a number of characteristics similar to those of basal breast tumours. In general, they occur at an early age of onset, are ER (oestrogen receptor) negative, PR (progesterone receptor) negative, Her2/Neu negative, p53 mutated and poorly differentiated with a poor prognosis [4]. In addition, they express many of the basal epithelial markers including cytokeratin 5, 6 and 17 as well as p-cadherin.

Substantial data now exists to suggest a role for BRCA1 in transcriptional regulation. The importance of BRCA1-mediated transcriptional regulation, within its role as a tumour suppressor, can be inferred from the fact that the majority of BRCA1 disease-associated mutations result in protein truncations [5]. Many of these have been formally proved, in synthetic reporter assays, to render the truncated protein transcriptionally inactive. Our group has demonstrated, using the HCC1937 breast carcinoma cell model which harbours the 5382insC mutation, that BRCA1 is not present on any of the many endogenous promoters tested using the chromatin immunoprecipitation assay [6]. BRCA1 has been shown to be a component of the core transcriptional machinery as it interacts with the Pol II holoenzyme complex, in part through binding to RNA helicase A [7]. Although BRCA1 does not appear to bind DNA in a sequence-specific manner, it has been established that it can interact with multiple transcription factors, such as p53, c-Myc and ERα [8–10]. More recently, BRCA1 has been shown to block initiation of mRNA synthesis by ubiquitinating the transcriptional pre-initiation complex, thereby sterically hindering the stable association of transcription factors TFIIH and TFIIE [11]. BRCA1 also plays a role in chromatin remodelling through its interactions with the histone deacetylases, HDAC1 and HDAC2, and the SWI/SNF chromatin remodelling complex [12,13]. This review will explore the role played by BRCA1 as a predictive marker of response to a number of therapeutic agents and how its role in transcriptional regulation may contribute to this.

Modulation of sensitivity to chemotherapeutic agents: DNA-damaging agents
Pre-clinical and clinical data have clearly demonstrated that functional BRCA1 is an important determinant of response to DNA-damaging agents (Figure 1). Initial studies examined the role of BRCA1 in response to DNA-damaging agents in MES cells (mouse embryonic stem cells) deficient in BRCA1. BRCA1−/− cells were consistently more sensitive to the effects of the alkylating agents mitomycin C and cisplatin than MES cells containing wild-type BRCA1 [14]. This work was complimented by Moynahan et al. [15] who showed that reconstitution of full-length BRCA1 into mouse embryonic

Key words: breast-cancer susceptibility gene 1 (BRCA1), cell cycle, checkpoint, chemotherapy, transcription.

Abbreviations used: APC, anaphase-promoting complex; BRCA1, breast-cancer susceptibility gene 1; Cdk, cell division cycle kinase; CDK, cyclin-dependent kinase; ER, oestrogen receptor; GADD, growth-arrest and DNA-damage-inducible protein; HDAC, histone deacetylase; MAD, mitotic arrest-deficient protein; MES cell, mouse embryonic stem cell; MSH, mutS homologue; Rb, retinoblastoma; siRNA, small interfering RNA.

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fibroblast cells with disrupted BRCA1 led to an increase in resistance to a number of different DNA-damaging agents, including the platinum agents. A number of other studies, using various techniques to inhibit expression of endogenous BRCA1, demonstrated that reduction of BRCA1 expression increased sensitivity to etoposide [16,17]. This suggests that reduction in BRCA1 expression, as observed in sporadic cancers, may also be important in modulating tumour response to DNA damage-based chemotherapy. In a screen to discover modulators of resistance to cisplatin, Husain et al. [18] identified that BRCA1 was overexpressed in cisplatin-resistant MCF-7 adenocarcinoma cells. Antisense inhibition of BRCA1 in this cell line reversed the phenotype; increased sensitivity to cisplatin was achieved through a decreased DNA repair capacity and enhanced apoptosis.

Studies in our laboratory have shown that HCC1937 cells harbouring a single copy of mutated BRCA1 were more sensitive to a range of DNA-damaging agents compared with HCC1937 cells reconstituted with wild-type BRCA1 [16]. These cells showed exquisite sensitivity to the topoisomerase II inhibitor, etoposide, and were 100–1000-fold more sensitive than cells reconstituted with a functional BRCA1. A similar increase in sensitivity to DNA damage was observed following the abrogation of endogenous BRCA1 expression by siRNA (small interfering RNA) in multiple additional cell line model systems. Numerous retrospective clinical studies support the pre-clinical data and demonstrate a notable increase in response to DNA damage-based therapy in BRCA1-linked breast and ovarian cancer. An initial study by Chappuis et al. [19] clearly demonstrated that BRCA patients are highly sensitive to and display a better clinical response to anthracycline-based chemotherapy than non-mutation carriers (90% complete response in BRCA1/BRCA2 carriers compared with 18% in matched sporadic controls). Further evidence came from a study by Delaloge et al. [20] who compared 15 BRCA1 mutation carriers with 57 matched control patients with locally advanced breast cancer who were treated with anthracycline- and cyclophosphamide-based chemotherapy. All BRCA1 mutation-carrying patients showed a clinical response to therapy in comparison with 63% of patients with sporadic breast cancer. Following surgery, 53% of patients with a BRCA1 mutation had a pathological complete response in comparison with 14% of control patients. As mentioned above, mutation of BRCA1 in sporadic breast and ovarian cancer is rare, and loss of BRCA1 function frequently occurs by down-regulation of BRCA1 mRNA expression. Retrospective studies to examine the correlation of BRCA1 expression and clinical outcome in response to chemotherapy in sporadic breast and ovarian cancer have not been published to date. However, a retrospective study by Taron et al. [21] in patients with non-small-cell lung cancer showed that low BRCA1 mRNA levels correlated with good clinical response, whereas high BRCA1 mRNA levels correlated with poor responses to DNA-damaging chemotherapy.

**Spindle poisons**

The role played by BRCA1 in modulating the therapeutic response to microtubule damage has been less well characterized. There are two main groups of microtubule damaging agents: the taxanes and the vinca alkaloids. Both classes of drugs inhibit cell division by interfering with microtubule spindle assembly in mitosis leading to checkpoint activation and ultimately apoptosis. The taxanes irreversibly bind to and prevent depolymerization of tubulin and the vinca alkaloids prevent tubulin polymerization. In contrast with the situation with DNA-damaging agents, BRCA1 expression appears to correlate with increased sensitivity to antimicrotubule agents (Figure 1). We initially demonstrated that inducible expression of exogenous BRCA1 dramatically sensitized breast cancer cells to the cytotoxic effect of taxol. Subsequently we extended these findings to other spindle poisons in other cell line model systems following modulation of BRCA1 expression utilizing a variety of experimental approaches [16]. Additional evidence came from work by Fedier et al. [22] who showed that ribozyme-mediated decrease in the BRCA1 expression resulted in resistance to paclitaxel and vincristine. More recently, Chabalier et al. [23] proposed that the mechanism of increased resistance to paclitaxel following BRCA1 down-regulation was mediated through premature inactivation of the spindle checkpoint, early sister-chromatid separation and chromosomal instability.

**BRCA1 transcriptional targets and their role in DNA-damage repair**

The DNA-damage repair process consists of a number of interdependent mechanisms: (i) detection of damaged DNA; (ii) cell cycle checkpoint activation to give the cell sufficient time to repair the DNA defect; and (iii) the ability to induce or inhibit apoptosis depending on the extent of
BRCA1 is involved in DNA damage detection, repair, cell cycle checkpoint activation and regulation of cell survival. Proteins that are regulated by BRCA1 at either the post-translational or transcriptional stage that have been discovered to date are shown.

Cell cycle control genes and checkpoint activation

During the G1 phase of the cell cycle, the Rb (retinoblastoma)-HDAC repressor complex binds to and inhibits the E2F-DP1 transcription factors. Phosphorylation of the Rb by CDK (cyclin-dependent kinase) 4/6-cyclin D and CDK2-cyclin A and -cyclin E dissociates the Rb-repressor complex, permitting transcription of S-phase genes. p21 serves as a potent inhibitor of the CDK2–cyclin A/E complex, thereby inhibiting the G1 to S-phase transition (Figure 3). BRCA1 has been shown to transcriptionally up-regulate p21 in a p53-independent manner, and consequently inhibit the G1 to S-phase transition. Indeed, BRCA1 could not prevent S-phase progression in p21-knockout cells. Furthermore, tumour-associated transactivation-deficient mutants of BRCA1 were defective in both transactivation of p21 and cell-cycle inhibition [24]. Li et al. [25] demonstrated that the BRCT (BRCA1 C-terminus) motifs of BRCA1 associated with CtIP, which in turn binds to the transcriptional corepressor CtBP. Interestingly, following treatment with DNA-damaging agents, hyperphosphorylated BRCA1 no longer forms a complex with CtIP/CtBP, which allows BRCA1 to transactivate the p21 promoter.

BRCA1 has also been linked to G2/M checkpoint regulation. The Cdc2 (cell division cycle 2)-cyclin B kinase is pivotal in regulating the G2 to M-phase transition. As cells approach M-phase, Cdc25 is activated, which in turn activates Cdc2. Negative regulators of the system include 14-3-3 protein which binds to Cdc2–cyclin B kinase and exports it from the nucleus, GADD45 (growth-arrest and DNA-damage-inducible protein 45), which sequesters Cdc2 from cyclin B, and finally p21, which inhibits Cdc2 kinase activity [26]. GADD45 has been shown to be highly inducible upon overexpression of BRCA1, through a p53-independent mechanism [27,28]. Furthermore, Fan et al. [29] demonstrated that the transcriptional activation of GADD45 by BRCA1 occurs through additional regulatory regions, which contain two OCT-1 motifs and one CAAT box, which directly bind to Oct-1 and NF-Y (nuclear factor-Y) respectively. Mullan et al. [30] extended this work into the pre-clinical setting and demonstrated that expression of BRCA1 in the presence of taxol and vincristine induced GADD45 expression, acute G2/M arrest and ultimately increased cell death, compared with control cells.

The kinase activity of the Cdc2-cyclin B complex can also be modulated in a BRCA1-dependent manner by transcriptional repression of cyclin B1 and Cdc2 and activation of 14-3-3σ protein and p21 [31–33] (Figure 3). In addition, BRCA1, in combination with the transcription factor Oct-1, has been shown to mediate the transcriptionactivation of MAD2 (mitotic arrest-deficient protein 2) [34] (Figure 3). MAD2 is a key component of the spindle assembly checkpoint and inhibits the APC (anaphase-promoting complex). It is postulated that loss of transcriptionally active
BRCA1 would down-regulate MAD2 expression, leading to loss of mitotic checkpoint activity and ultimately account for the higher levels of genomic instability seen in BRCA1 mutant tumours. Indeed, in this mouse model, BRCA1 (del 11/del 11) cells failed to arrest at metaphase in the presence of nocodazole and underwent p53-dependent apoptosis.

**Pro-survival compared with apoptosis**

BRCA1 interacts with and modulates the transcriptional activity of p53 [35]. More specifically, wild-type BRCA1 increases transcription of p53-responsive promoters, including p21 and BAX (Bcl-2-associated X protein), whereas cancer-associated transactivation-deficient BRCA1 mutants do not. Interestingly, although p53 can drive the expression of a number of pro-apoptotic genes, BRCA1 overexpression did not lead to apoptosis in most cell lines tested. This may in part be due to the fact that BRCA1 appeared to preferentially up-regulate p53-dependent genes involved in DNA repair and growth arrest [36]. c-Myc is preferentially amplified in BRCA1-linked breast tumours in comparison with sporadic breast tumours (53% compared with 23% respectively) and has therefore been postulated to contribute to tumour progression in BRCA1-associated breast cancers [37]. Although c-Myc is generally considered to be a transcriptional activator, binding of BRCA1 can inhibit the transcriptional and transforming activity of c-Myc [9,38]. Recently we have shown in our laboratory that BRCA1 in conjunction with c-Myc down-regulated a number of transcriptional targets including psoriasin [6]. Psoriasin expression was induced following treatment with a number of DNA-damaging agents. Furthermore, modulation of psoriasin expression using siRNA or overexpression studies increased and decreased survival following etoposide treatment respectively. Although the pathway by which psoriasin sensitizes cells to etoposide is unclear, it may be related to the putative role of psoriasin in regulating pro-survival genes through its interaction with the transcription co-factor JAB1 (reviewed in [39]). This again highlights the relationship between BRCA1 expression and chemotherapy responses.

**Summary**

BRCA1 is a multifunctional protein that is involved in tumour suppression. Pre-clinical and clinical data indicate that BRCA1 can modulate the response to chemotherapeutic agents. More specifically, loss of BRCA1 function leads to increased sensitivity of tumour cells to DNA-damaging chemotherapeutic agents such as cisplatin. In contrast, loss of BRCA1 function appears, in the pre-clinical setting, to render cells more resistant to antimicrotubule agents. However, this requires further study in the clinical setting. The mechanism whereby BRCA1 modulates the therapeutic response to spindle poisons and DNA-damaging agents is in part mediated by transcriptional regulation. Key components of the DNA damage-repair pathway, particularly those involved in cell cycle checkpoint activation and pro-apoptotic survival genes, are transcriptionally regulated by BRCA1.

**References**


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