Review

The pathology of familial breast cancer
How do the functions of *BRCA1* and *BRCA2* relate to breast tumour pathology?

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Abstract

Women with mutations in the breast cancer susceptibility genes, *BRCA1* and *BRCA2*, have an increased risk of developing breast cancer. Both *BRCA1* and *BRCA2* are thought to be tumour suppressor genes since the wild type alleles of these genes are lost in tumours from heterozygous carriers. Several functions have been proposed for the proteins encoded by these genes which could explain their roles in tumour suppression. Both BRCA1 and BRCA2 have been suggested to have a role in transcriptional regulation and several potential BRCA1 target genes have been identified. The nature of these genes suggests that loss of BRCA1 could lead to inappropriate proliferation, consistent with the high mitotic grade of *BRCA1*-associated tumours. BRCA1 and BRCA2 have also been implicated in DNA repair and regulation of centrosome number. Loss of either of these functions would be expected to lead to chromosomal instability, which is observed in *BRCA1* and *BRCA2*-associated tumours. Taken together, these studies give an insight into the pathogenesis of *BRCA*-associated tumours and will inform future therapeutic strategies.

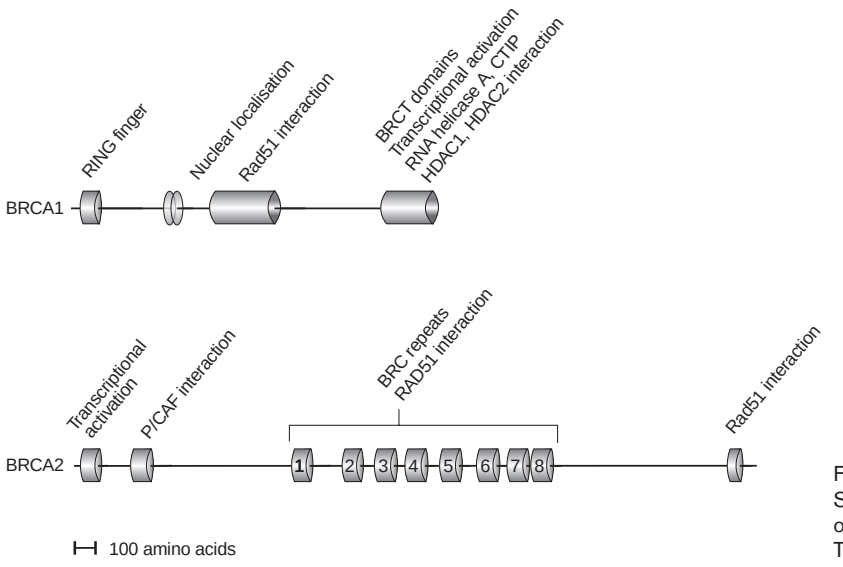
Keywords: *BRCA1*, *BRCA2*, breast cancer, cell cycle checkpoints, DNA repair, gene expression

Introduction

About one in 12 women in the Western world develop cancer of the breast, and at least 5% of these cases are thought to result from a hereditary predisposition to the disease [1,2]. Two breast cancer susceptibility genes in humans (*BRCA1* and *BRCA2*) have been mapped and cloned, and mutations in these genes account for most families with four or more cases of breast cancer diagnosed before the age of 60 years. (Note that '*Brca1*' and '*Brca2*' are used in the following discussion to denote the equivalent genes in mice, and that Roman text indicates the encoded protein.) Women who inherit loss-of-function mutations in either of these genes have approximately 85% risk of developing breast cancer by age 70 years [3]. Both *BRCA1* and *BRCA2* are thought to be tumour-suppressor genes, because the wild-type allele of the gene is observed to be lost in tumours of heterozygous carriers. As well as breast cancer, carriers of mutations in these genes are at elevated risk of cancer of the ovary, prostate and pancreas. Surprisingly, however, despite the association with inherited predisposition, somatic disease-causing mutations in *BRCA1* or *BRCA2* are extremely rare in sporadic breast cancers [1,2].

CDK = cell division kinase; HAT = histone acetyltransferase; NHEJ = nonhomologous end joining.

Figure 1



Features of the BRCA1 and BRCA2 proteins. Structures, features and regions of interaction with other proteins discussed in this review are indicated. The relevant references are given in the text.

The *BRCA1* gene is made up of 22 coding exons and encodes a protein of 1863 amino acids [4••]. Most of the coding region shows no sequence similarity to previously described proteins, apart from the presence of a RING zinc finger domain at the N-terminus of the protein and two 'BRCT' repeats at the C-terminus [2] (Fig. 1). The *BRCA2* gene has 26 coding exons and encodes a protein of 3418 amino acids, with an estimated molecular weight of 384 kDa [2,5••]. The only obvious feature of the BRCA2 protein is the presence of eight copies of a 30–80 amino acid repeat (the BRC repeat) in the part of the protein encoded by exon 11 [6•,7•] (Fig. 1). These repeats are able to bind the RAD51 protein implicated in DNA repair and recombination [8•,9•].

Functions for the BRCA proteins in both transcriptional regulation and DNA repair/recombination have been suggested [2]. It is still unclear, however, how loss of *BRCA* gene function leads to tumourigenesis. Important clues to this process are provided by the pathological features of breast tumours in women who are carriers for mutations in *BRCA1* or *BRCA2*. These tumours are clearly distinct from each other, as well as from sporadic tumours [10••]. This indicates that, despite many similarities in these genes [2], they must have at least some nonredundant functions. The evidence for the proposed functions of the BRCA1 and BRCA2 proteins are reviewed and related to some of the pathological features of *BRCA*-associated tumours.

Transcriptional regulation

The presence in BRCA1 of a RING finger domain, which are frequently found in transcriptional regulatory proteins, led to the tacit assumption that BRCA1 might be involved in the transcriptional regulation of genes [4••]. Subsequently, however, it has become clear that RING finger domains are found in a wide variety of proteins of different function, and are indicative of protein–protein interaction rather than interaction with DNA. Nevertheless, there is significant accumulating evidence for a role in transcriptional regulation for BRCA1, and to a lesser extent for BRCA2. Disregulation of target genes consequent to the loss of *BRCA* genes is a plausible mechanism to explain the pathological features of *BRCA*-associated tumours. As described below, however, the exact function of the BRCA proteins in transcriptional regulation is not yet understood.

A C-terminal fragment of BRCA1, which is fused to the GAL4 DNA-binding domain, activates transcription of reporter genes, suggesting that BRCA1 might regulate transcriptional activation [11•–13•]. The finding that endogenous, full-length BRCA1 copurifies with the RNA Pol II holoenzyme complex via an interaction with RNA helicase A supports such a role for BRCA1 [14•,15,16]. It is worth noting, however, that RAD51 can also be found in this complex despite having no known role in transcriptional regulation [17].

Several groups have used overexpression of BRCA1 in various cell lines to find targets of BRCA1 transcriptional regulation (Table 1). Most work has concentrated on the cell division kinase (CDK) inhibitor, $p21^{WAF1}$, which acts to arrest the cell cycle at the G_1 to S phase and G_2 to M phase transitions [18]. In COS cells, 293T cells and human colon cancer cells, reporter constructs containing portions of the $p21^{WAF1}$ promoter are activated by overexpression of

Table 1

Suggested transcriptional targets of BRCA1

Gene	Regulation	Function
p21^{WAF1}	↑↓*	inhibitor of cyclin dependant kinases
BAX	↑	proapoptotic
GADD45	↑	G2/M checkpoint
MDM2	↑	regulation of p53
CDC25A	↓	phosphatase/cell cycle regulation
Synthetic estogen receptor responsive promoter	↓	

*Overexpression of BRCA1 activates *p21^{WAF1}* expression [19•,20–22]. However, *p21^{WAF1}* is also activated in *BRCA1*-null mice [29••].

BRCA1 [19•,20–22]. Furthermore, in colon cancer cells, BRCA1 overexpression leads to an increase in p21^{WAF1} protein levels, accompanied by cell cycle arrest [19•]. There may be some cell-type specificity in this, however, because overexpression of BRCA1 in osteosarcoma cells does not induce increased p21^{WAF1} protein levels [23•]. Other studies suggest that BRCA1 may also be able to activate the promoters of the proapoptotic gene *BAX*, the G_2 to M phase checkpoint gene *GADD45*, and the p53 regulatory gene *MDM2* [20,21,23•].

There are also some indications that BRCA1 may act as a repressor of transcription. BRCA1 overexpression has been shown to inhibit transcription from the promoter of the cell-cycle regulatory gene *CDC25A* and from a synthetic oestrogen receptor-responsive promoter [24,25•]. This may involve the interaction of BRCA1, via CtIP, with the transcriptional corepressor CtBP [26•,27•]. CtBP can inhibit transcription by binding the histone deactylase HDAC1, which has been found in BRCA1-containing complexes [28•]. When BRCA1 alone is overexpressed in 293 cells, a *p21^{WAF1}* promoter–reporter construct is activated, but when CtIP and CtBP are coexpressed it is not [27•]. Interestingly, interaction of BRCA1 with CtIP and CtBP is disrupted upon DNA damage, suggesting a role for BRCA1 in the DNA damage-dependent induction of p21^{WAF1} [27•]. Because loss of Brca1 in mouse embryos also leads to induction of p21^{WAF1} [29••], however, the role of BRCA1 in p21^{WAF1} regulation is not clear.

There are, as yet, no reports of BRCA1 binding DNA and acting directly as a transcription factor. Rather, BRCA1 appears to exert its influence on transcription as a cofactor or adaptor, because it can interact with both DNA-binding transcription factors and the RNA Pol II holoenzyme. The influence of BRCA1 on the *p21^{WAF1}*, *BAX* and *MDM2* promoters is mediated, at least in part, by p53, with which BRCA1 has been shown to physically interact [20–22].

Transcriptional regulation has also been proposed as a function of BRCA2, because sequences encoded by exon three, when fused to the GAL4 DNA-binding domain, can activate transcription of a reporter gene [30•]. It is not clear whether these sequences can function similarly in the context of the intact protein, however. Other evidence supporting a role for BRCA2 in transcriptional regulation is that it can associate with histone acetyltransferase (HAT) activity. The exact nature of this association is not clear, because one study suggested that BRCA2 has intrinsic HAT activity [31•], whereas another suggested that it is associated with HAT activity by virtue of an interaction with P/CAF, which possesses HAT activity [32•]. Association with HAT activity does not necessarily denote a role in transcriptional regulation, however. It could also reflect a role in chromatin remodelling, which might be required for the proposed role of BRCA2 in homologous recombination, which is discussed below.

The promoters that BRCA1 has been shown to activate are those for stress-induced genes involved in cell cycle checkpoints, whereas those that BRCA1 inhibits are for cell division promoting genes. This is consistent with BRCA1 regulating checkpoints and cell proliferation. Particularly interesting in this context is the report that overexpression of BRCA1 can inhibit oestrogen receptor signalling [25•]. This suggests a model whereby loss of BRCA1 could lead to an increased proliferative capacity of the breast epithelium in response to oestrogen. However, this appears to be at odds with clinical findings that show loss of the oestrogen receptor in a high proportion of BRCA-associated breast tumours [33,34].

A major problem with many of the studies described above is that overexpression of BRCA1 itself, especially at inappropriate phases of the cell cycle, could cause genotoxic stress and thus lead to expression of stress-induced genes as a secondary effect. Experiments using *BRCA*-null cells in which exogenous BRCA expression is induced to wild-type levels at the appropriate time in the cell cycle would rule out this possibility.

DNA repair

Mouse cells with *Brca1* or *Brca2* mutations are hypersensitive to ionizing radiation, a genotoxic treatment that causes primarily double-strand breaks in DNA [35••–38••]. This suggests that Brca1 and Brca2 play a part in the cellular response to DNA double-strand breaks. This function may be restricted to part of the cell cycle, however, because *Brca2* mutant cells can repair the double-strand breaks that arise during V(D)J recombination [38••] (Bertwistle D, Ashworth A, unpublished observations); these occur during the G_1 phase of the cell cycle, when nonhomologous end joining (NHEJ) is thought to repair most double-strand breaks [39]. Expression of BRCA1 and BRCA2 is very low during G_1 phase, but is induced as

cells enter S phase [40•,41•]. During the late S and G_2 phases of the cell cycle, when there are two copies of chromosomes, one copy can be used as a template to repair double-strand breaks in the other by homologous recombination [39]. In dividing mammalian cells as many as 30–50% of DNA double-strand breaks may be repaired this way [42]. RAD51, a protein that plays a key role in homologous recombination, has been shown to associate with both BRCA1 and BRCA2 [8•,9•,35••,43,44••]. Furthermore, BRCA1 also associates with the RAD50–MRE11–nibrin complex, which is thought to process DNA double-strand breaks for repair by both NHEJ and homologous recombination [45••]. Together, these data suggest that BRCA1 and BRCA2 are involved in homologous recombination-mediated repair of DNA double-strand breaks. To complicate matters, however, there is also some evidence that BRCA1 may have a role in the mechanistically independent process of the transcription-coupled repair of oxidative DNA damage [46].

Spontaneous chromosomal abnormalities are observed at high frequency in untreated *Brca1* and *Brca2* mutant cells, implying that these genes act to repair DNA damage that occurs as a consequence of normal cell division, as well as that caused by genotoxic agents [36••,38••]. The nature of the chromosomal aberrations suggests a defect in the repair of DNA damage sustained during the S or G_2 phase of the cell cycle. Because BRCA1 and BRCA2 are associated with RAD51, it is significant that loss of RAD51 causes chicken DT40 cells to spontaneously accumulate DNA double-strand breaks, arrest at the G_2/M phase transition and die [47]. A possible explanation for such severe phenotypes comes from studies in bacteria, which suggest that double-strand breaks occur frequently as a normal consequence of DNA replication and are repaired by homologous recombination [48]. The role of homologous recombination in DNA replication in eukaryotes is less clear. Holliday junction recombination intermediates do spontaneously arise in normal yeast cells during S phase, however [49]. This suggests that homologous recombination repairs DNA damage that occurs spontaneously during DNA replication in eukaryotic as well as in prokaryotic cells. The embryonic lethality observed in *Rad51* null mice [50] and many *Brca1* and *Brca2* mutant mouse strains may therefore be due to a failure of DNA replication.

Cell cycle checkpoint activation and loss

Cells respond to DNA damage by activating checkpoints that prevent their progression through the cell cycle. The spontaneous DNA damage observed in *Brca1* and *Brca2* mutant cells would be predicted to activate such checkpoints. Indeed, these cells suffer from a proliferative defect, probably due to checkpoint activation caused by upregulation of the CDK inhibitor $p21^{WAF1}$ [29••,37••, 38••]. $P21^{WAF1}$ is induced in response to DNA damage by p53, which is upregulated in *Brca2* mutant cells. Therefore, loss of the p53 pathway would be predicted to alleviate the proliferation defect observed in *Brca1* and *Brca2* mutant cells. Although this may be the case for some mutations [51••], others are only partially rescued [52••] (Connor F, Ashworth A, unpublished observation), suggesting that this is not the only checkpoint that is activated. Nevertheless, loss of the p53 pathway appears to be important for the progression of *BRCA*-associated tumours. Supporting this hypothesis is the finding that *p53* mutations are found at a higher frequency in *BRCA*-associated breast tumours than in sporadic breast tumours [53••,54••].

Checkpoints exist at G_1/S, S, G_2/M and M in the cell cycle, and all these appear to be operational in *Brca2* mutant cells [38••,51••]. In contrast, cells in which exon 11 of *Brca1* is deleted have an intact G_1/S checkpoint, but are defective in an ionizing radiation-induced G_2/M checkpoint [55••]. Furthermore, cells overexpressing a C-terminal fragment of BRCA1 fail to arrest when treated with the spindle inhibitor colchicine [56]. These results suggest that BRCA1 may also be involved in the G_2/M and spindle checkpoints.

The spindle checkpoint acts to monitor the fidelity of chromosome segregation during mitosis [57]. Chromosome segregation is controlled by the mitotic spindle, a system of microtubules organized by the centrosome. During mitosis, there are two centrosomes, one at either end of the mitotic spindle, and one diploid set of chromosomes segregates to each. The spindle checkpoint prevents chromosome missegregation by ensuring that each chromosome pair has recruited microtubules from both poles of a bipolar spindle, before chromosome segregation occurs at anaphase.

At the end of mitosis each daughter cell inherits one of the two centrosomes, and duplicates this at the G_1/S phase transition so that it has two centrosomes during mitosis [58]. Recent studies have found that a high proportion of *Brca1* and *Brca2* mutant cells contain supernumerary centrosomes [55••,59••]. There is thought to be no checkpoint monitoring the number of centrosomes in a cell, and therefore cells with excess centrosomes can enter mitosis [60]. Some of these cells might be predicted to arrest during mitosis due to the activation of the spindle checkpoint, however. Indeed, the growth of *Brca2* mutant cells can be rescued by the expression of a dominant negative mutant of the spindle checkpoint gene *BUB1* [51••]. This implies that some *Brca2* mutant cells activate the spindle checkpoint.

Under certain circumstances, however, mitoses with supernumerary centrosomes can fail to activate the spindle checkpoint [60] and this could lead to chromosome missegregation [61]. The consequence of this would be chromosome gain or loss and therefore aneuploidy, which is

observed in a large proportion of *Brca1* and *Brca2* mutant cells [55••,59••]. These results suggest that Brca1 and Brca2 may regulate centrosome duplication. The finding that BRCA1 associates with centrosomal proteins supports this hypothesis [62]. Alternatively, the excess of centrosomes in *Brca1* and *Brca2* mutant cells may be a secondary effect; centrosomes can undergo multiple rounds of duplication during S-phase arrest leading to supernumerary centrosomes [60,63]. Whatever the mechanism of centrosome amplification in *Brca1* and *Brca2* mutant cells, this phenomenon suggests that mutations in *BRCA1* and *BRCA2* might cause chromosomal instability in human tumours. It will be interesting to know whether these tumours have a high frequency of mutations in spindle checkpoint genes.

The chromosomal aberrations and aneuploidy found in mouse cells mutant for *Brca1* or *Brca2* are in agreement with the pathological data showing chromosomal instability in *BRCA*-associated breast tumours [64•]. In the light of these profound and irreversible changes, therapeutic strategies involving the reintroduction of *BRCA1* or *BRCA2* into tumours [65] may well be viewed as closing the stable door after the horse has bolted.

Conclusion

Our understanding of the precise functions of the BRCA1 and BRCA2 proteins and how their loss leads to tumourigenesis is still incomplete. However, we now have several clues as to the general cellular processes in which these proteins are involved, and this understanding has already illuminated some of the pathological features of *BRCA*-associated breast tumours. Activation or repression of transcriptional targets of the BRCA1 protein might play a role in tumour initiation or progression. These changes in gene expression may underpin some of the pathological changes associated with the tumours. Lack of activation of stress-induced checkpoint genes could contribute to aneuploidy. Failure to inhibit growth promoting genes could increase and disregulate proliferation. The genetic instability observed in mouse models and human tumours most likely reflects a role for BRCA1 and BRCA2 in homologous recombination and possibly in centrosome regulation. This effect may manifest itself relatively early in breast cancer progression, increasing the probability of tumourigenesis rather than directly promoting tumour growth. The synergy between mutations in *p53* and the *BRCA* genes in tumours indicates a role for loss of checkpoint control in tumour progression. Taken together these findings give an insight into the pathogenesis of *BRCA*-associated tumours and will inform future therapeutic strategies.

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