

DNA Double Strand Break Repair and its Association with Inherited Predispositions to Breast Cancer

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Abstract

Mutations in *BRCA1* account for the majority of familial aggregations of early onset breast and ovarian cancer (~70%) and about 1/5 of all early onset breast cancer families; in contrast, mutations in *BRCA2* account for a smaller proportion of breast/ovarian cancer families and a similar proportion of early onset breast cancer families. *BRCA2* has also been shown to be associated with a much more pleiotropic disease spectrum compared to *BRCA1*. Since the identification of both *BRCA1* and *BRCA2* investigations into the functions of these genes have revealed that both are associated with the maintenance of genomic integrity via their apparent roles in cellular response to DNA damage, especially their involvement in the process of double strand DNA break repair. This review will focus on the specific roles of both genes and how functional differences may account for the diverse clinical findings observed between families that harbour *BRCA1* or *BRCA2* mutations.

Genomic integrity

The integrity of the genome is crucial for the correct functioning of the individual organism. Any perturbation that results in a loss of genomic integrity is likely to result in dysregulation and consequent uncontrolled cellular proliferation. Maintenance of genomic integrity is orchestrated by a number of different but interrelated DNA repair mechanisms that include DNA mismatch repair, nucleotide excision repair, base excision repair and DNA double strand break repair. In relation to human neoplasia all of these mechanisms have been implicated in disease including such illnesses as hereditary non-polyposis colorectal cancer (HNPCC), adenomatous polyposis, xeroderma pigmentosum and the focus of this review, inherited predispositions to breast cancer.

There are essentially two types of genes that have been associated with inherited predispositions to cancer, gatekeeper genes or caretaker genes [1]. Gatekeeper genes such as the adenomatous polyposis coli (*APC*) gene can be directly associated with disease by virtue of its involvement in the control of cell signalling pathway(s) that are associated with cellular proliferation. Caretaker genes, in contrast, are not involved directly with cellular proliferation but are intimately associated with the maintenance of genomic integrity. Loss of caretaker function will lead to an accelerated accumulation of change within the genome, which has the potential to affect cellular control and hence result in accelerated tumour development [2].

Initially it was considered that errors in genes associated with the maintenance of genomic integrity

could barely be tolerated and most resulted in severe symptoms that culminated in the early demise of the affected individual. Such conditions include Nijmegen Breakage Syndrome, Ataxia Telangiectasia, Fanconi Anaemia, Bloom's Syndrome, Werner's Syndrome and Rothmund Thompson Syndrome [3]. All of these conditions are extremely rare and are autosomal recessive diseases that are characterised by an enormous increase in the risk of developing malignancy. In 1993 the first hint that deficient DNA repair processes were more common than originally believed was revealed by the identification of the genetic basis of a relatively common inherited predisposition to colorectal cancer, namely HNPCC, where the underlying defect is in DNA mismatch repair [4]. In 1994 the first gene associated with the genetic basis of early onset breast and/or ovarian cancer was identified (*BRCA1*), which at the time could not be linked to any known cellular process [5]. Subsequently, *BRCA1* has been implicated in the cellular response to DNA double strand break damage [6].

Double strand break (DSB) repair

The mechanisms involved in the repair of DSBs are poorly understood as they are complex and require the accurate coordination of a large variety of proteins that are not only involved in the process of repair but also the recognition of repair, placing the cell cycle into a holding pattern until the repair is complete and transcriptional control.

Double strand breaks in DNA can be caused by a variety of environmental insults but most notably ionising radiation has been considered the most likely cause of such DNA damage. There are essentially three mechanisms of DSB, one involves nonhomologous end joining (NHEJ) repair, another is via homologous recombination repair (HRR) and the third is single strand annealing (SSA). There are differences between the three types of DSB repair in regards to the likelihood of errors. Both NHEJ and SSA are error prone as both mechanisms do not involve a sister chromatid in the repair process whereas HRR is essentially error free since this process relies on the use of the homologous chromosome for repair [7, 8].

Initially, evidence suggested that DSBs were primarily rejoined by NHEJ repair in mammalian cells [9]. More recent evidence has, however, indicated that HRR is active in mammalian cells [9] and that both mechanisms are important for the repair of DSBs. Apart from ionising radiation DSBs are frequently created during DNA replication, which are under normal circumstances very efficiently repaired via HRR [10]. Loss of either type of

repair process results in an increased frequency of chromosomal rearrangements, which can result in a loss or gain of genetic material, and/or recombination of the genome. The repair of DSBs is a complex process and involves not only the orchestration of a variety of proteins but also of different DNA repair processes. DSB repair can involve MLH1, a protein primarily involved in DNA mismatch repair and XPD, a protein involved in nucleotide excision repair. Furthermore, since the identification of genes associated with rare DNA repair disorders it has become apparent that they are interrelated and that there is considerable overlap in cellular phenotypes, for example Fanconi anaemia (group B and D) is related to deficiencies in *BRCA2* [11].

The repair of DSBs requires the formation of a protein complex that involves three different proteins, RAD50, MRE11 and NBS1 that interact with *BRCA1*.

A breakdown in DSB repair will result in chromosomal rearrangements that include deletions, duplications, inversions, and translocations. Since the genes involved in this process are integrally linked to cell cycle checkpoint control any perturbation in a cell ability to recognise DNA damage may adversely affect its ability to repair DSBs, which can result in adverse chromosomal change.

DNA damage and breast cancer

A small but significant proportion of breast cancer patients come from families where there are other 1st or 2nd degree relatives with disease and represents somewhere in the order of 10% of all breast cancer patients [12]. From this subset approximately two thirds of them can be accounted for by mutations in the breast cancer susceptibility genes *BRCA1* or *BRCA2*. It is now ten years since *BRCA1* was identified and during the intervening period much has been learnt about the functions of both *BRCA1* and *BRCA2* proteins [13]. The most striking finding about the function of the two genes has been the recognition of their roles in either sensing DNA damage or being intimately involved in its repair. *BRCA1* and *BRCA2* proteins are involved in either the recognition of DNA DSBs or the repair of these lesions, respectively.

BRCA1

BRCA1 appears to be unique to mammals and is likely to be relatively new in evolutionary terms as there are no orthologs found in yeast, *drosophila* or *c. elegans* genomes [14].

The function of *BRCA1* is complex and involves a series of proteins that have distinct binding sites on *BRCA1* (see Fig. 1) and there appears to be specific

functional activity localized in different regions of the protein. Evidence of additional proteins binding to BRCA1 is shown in Table 1. The majority of proteins that have been identified which interact with BRCA1 are also associated with some aspect of DNA repair or cell cycle checkpoint control [15]. Initial studies suggest that BRCA1 is involved in at least two types of DSB repair, NHEJ and HRR as well as the control of transcription [16]. A number of different facets to DNA repair have been associated with BRCA1 and these can be broadly categorised into those that are intimately involved in DNA damage sensing [17], DNA damage recognition [18], a contribution to S and G2 phase checkpoint control [19], transcriptional control [20] and X-chromosome inactivation [21]. The following summarises the functions that BRCA1 has been associated with:

1. BRCA1 and RAD51 involved in focus formation. Once a DNA break has been recognised one of the first events that can be visualized is the formation of foci around the DSB. Foci formation is mediated by RPA (replication protein A), which is a multifunctional molecule that not only functions in DSB repair but is also required for DNA replication [22]. If cells are irradiated RPA and BRCA1 can be observed collocalizing together. In BRCA1 deficient cells the number of damage-induced RPA foci is much greater than that compared to their wild-type counterparts [23].
2. BRCA1 is part of a large super protein complex known as BASC and evidence suggests that it could act as a damage sensor. BRCA1 appears to have an increased affinity for branched structures compared to double strand DNA implicated this protein as a damage sensor [24].
3. Phosphorylation of BRCA1 suggests that it acts as a signalling factor between damage sensing and recognition. After DNA damage BRCA1 is rapidly phosphorylated in dividing cells suggesting that it may function downstream of the checkpoint mechanisms that sense and signal DNA damage or problems with DNA replication during S-phase of the cell [25].
4. Routing DSB repair through HRR that are in appropriate phases of the cell cycle [26].
5. Mediates resectioning of broken termini to generate long single stranded tails required for RAD51 assembly. This is mediated by activating Chk1 kinase upon DNA damage [27].
6. During mitosis BRCA1 is clearly associated with the centrosome, and γ -tubulin associates preferentially with the hypophosphorylated form of BRCA1. This suggests that BRCA1 may direct

Table 1. Proteins that bind to BRCA1 or BRCA2

BRCA1	BRCA2
ATF1	P/CAF
BARD1	RAD51
BAP1	BRAF35
Rb	DSS1
TP53	BCCIP α
RAD50	EMSY
Mre1	
Nbs1	
ZBRK1	
Importin α	
BRAP2	
γ -tubulin	
STAT1	
P300/CBP	
BRCA2	
c-Abl	
RNA pol II	
HDAC1	
HDAC2	
CTIP	
LMO4	
BACH1	
ATM	
ATR	
c-Myc	
Nmi	
ERCC2	
CyclinD2	
Swi/Snf complex	
P300	
Oestrogen Receptor (ER)	

a role in centrosome regulation and chromosome segregation – an important point in relation to the maintenance of genomic integrity and the increase chromosomal errors observed in BRCA1 deficient cell lines [28].

7. Contribution of checkpoints in both S and G2 phases. BRCA1 is implicated in several different checkpoint events. After ionising radiation (IR) BRCA1 deficient cells fail to arrest scheduled DNA synthesis in S-phase [29]. G2 arrest does not occur in the absence of BRCA1 and it must be phosphorylated to initiate this checkpoint [30]. Both these functions of BRCA1 are in response to DSBs. Chromatid decatenation checkpoint control is also deregulated [31].
8. Possible role in down regulating mRNA 3' processing [32].

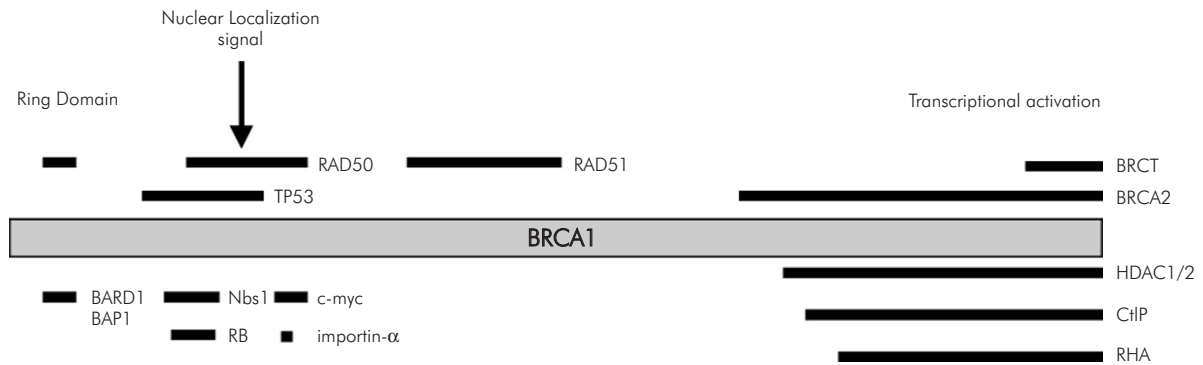


Fig. 1. Schematic representation of the various binding sites on BRCA1 protein

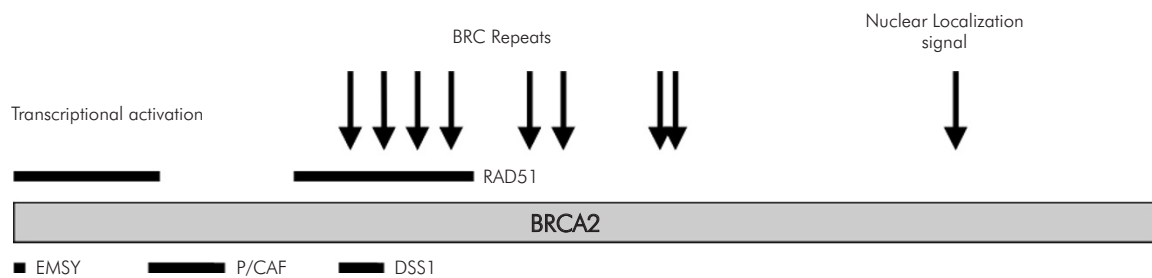


Fig. 2. Schematic representation of the various binding sites on BRCA2 protein

9. Role in transcriptional control
 - a) interacting with proteins to remodel chromatin structure [33]
 - b) transactivating genes through interaction with TP53 [34]
 - c) interacting with LMO4 repressing BRCA1's transcriptional activation function [35]
 - d) acting as a co-repressor of transcription [36].
10. X-chromosome inactivation. BRCA1, BARD1 and Xist co-localise on the inactive X-chromosome in female somatic cells and associates with Xist RNA. Perturbations of the relationship between BRCA1 and X-inactivation have the potential to destabilise silencing of the X-chromosome and thereby affect normal gene expression patterns [37].

BRCA2

Similar to *BRCA1*, *BRCA2* is evolutionarily speaking a relative newcomer, as it is only found in the same genomes as *BRCA1* [38]. This coupled with a high degree of similarity in disease expression between individuals harbouring changes in *BRCA1* and *BRCA2* suggests that their functions are either similar or that

they have unique and different functional specificity within the overall process [38].

Less is known about the function of *BRCA2* than *BRCA1*. Furthermore, what is known about *BRCA2* has been gained from studies on cell lines examining specific processes that can be observed as phenotypic differences compared to control cells.

Does a deficiency in DSB repair orchestrated by *BRCA1* or *BRCA2* result in an increased sensitivity to ionising radiation? The evidence is as follows:

1. Linked to homologous recombination. *BRCA2* directly interacts with *RAD51* and they in association with *BRCA1* can be identified in nuclear foci of undamaged replicating cells. Defective *RAD51* focus formation is observed in cells where *BRC* repeats are over-expressed, thereby inhibiting the self-association of *RAD51* and consequently the formation of *RAD51* nucleoprotein filaments on single strand DNA [39].
2. Gene expression of *BRCA2* is contingent on which phase of the cell cycle the cell happens to be in. This has led to difficulties in determining what

over-expression of *BRCA2* may confer but it appears that it may inhibit the cell cycle [40].

3. Mitotic checkpoint control. Thymic tumours from *BRCA2*^{1492T/T} mice had defective mitotic checkpoint control defects suggesting that there is a promotion of transformed cells that develop into malignancy [41].
4. Deficiency in the error free repair mechanism of DSBs. *BRCA2* mutant cells are phenotypically similar to mutants of the five *RAD51* paralogs strongly implicating *BRCA2* in DSB repair [42].
5. Reduced resistance to DNA-damaging agents. IR results in an increased (1.5 – 2 fold) rate of cell killing compared to control cells [43].
6. Missegregation of chromosomes. There is an excess of aneuploidy in *BRCA2* mutant cells caused by anomalies in the centrosome [44].
7. Reduced levels of HRR. There are gross defects in HRR in *BRCA2* mutant cells. HNEJ is not affected [45].
8. Reduced sister chromatid exchange (SCE). SCE is a consequence of HRR events where crossing over occurs between sister chromatids. The rate of SCE is reduced in *BRCA2* mutant cells implying a reduction in HRR efficiency [46].
9. Reduced efficiency of gene targeting but less than the 20-fold difference in *BRCA1* mutant cells [47].
10. Reduced numbers of *RAD51* foci after IR in *BRCA2* deficient cells. For HRR to occur one of the first events is the formation of *RAD51* foci. This implies that *BRCA2* is critical for the efficient formation of this initial step in HRR [48].

Given the plethora of binding partners and functions and the limited disease profiles observed in women with mutations in *BRCA1* why is there such specificity in disease expression?

Why do mutations in *BRCA1* and *BRCA2* result in such a specific disease pattern?

Mutations in *BRCA1* and *BRCA2* essentially predispose to an increase in breast and/or ovarian cancer although *BRCA2* changes confer an increased risk of a variety of other cancers, their relative frequency in comparison to breast or ovarian cancer is somewhat low. Given that the function of both genes is associated with the maintenance of genomic integrity and that this is a fundamental process that occurs in all cells it is surprising to observe such disease specificity. There are a number of possibilities that could account for the observed disease spectrum. During breast development at the time of puberty and again during pregnancy rapid clonal proliferation occurs that is retained within

the breast lobule [49-51]. Clonal expansion of any somatic cell runs the risk of amplifying genetic errors that may be harboured within the original set of cells. In this way it can be envisaged that women who harbour *BRCA1* or *BRCA2* germline mutations run the risk of amplifying a "second hit" and thereby alter the likelihood of developing disease [52]. Since both *BRCA1* and *BRCA2* are associated with genomic integrity why is there not an increased frequency of other malignancies in persons harbouring these changes? There are many possibilities with respect to the answer of this question and it should be emphasised that at this point in time there is insufficient knowledge about the functions of both *BRCA1* and *BRCA2*. Several different notions have been put forward but none have been unequivocally supported by experimental evidence. Evidence from one source has indicated that *BRCA1* can function as an inhibitor of oestrogen receptor signalling [53] suggesting that in cells deficient in this gene a loss of repression of oestrogen mediated mammary or ovarian epithelial proliferation occurs, thereby contributing to mammary and ovarian carcinogenesis [54]. Other more complex functions of *BRCA1* or *BRCA2* may also be tissue specific, not necessarily as a result of some intrinsic factor associated with only the breast or ovary but rather as a result of the growth and differentiation pathways of these organs [26, 55, 56].

BRCA1 and *BRCA2* and sporadic breast cancer

One of the most surprising results that became rapidly apparent after the identification of *BRCA1* and *BRCA2* was the absence of involvement of these genes in breast cancer patients who had no family history of disease (hereafter referred to as sporadic). Molecular analysis of sporadic breast tumours revealed that *BRCA1* or *BRCA2* did not behave as typical tumour suppressor genes by virtue of the fact that there appears to be an almost complete absence of 2nd *BRCA1* or *BRCA2* allele loss. This evidence implies that either haploinsufficiency is enough to initiate disease or that *BRCA1* or *BRCA2* are not involved in sporadic cases of breast cancer. Currently, this view for *BRCA1* remains intact whereas evidence has now emerged implicating a novel gene product that interacts with *BRCA2* to suppress its transactivational activity. The gene in question is termed EMSY and when overexpressed potentially mimics the effects of *BRCA2* inactivation [57]. Whether *BRCA1* will be implicated in sporadic breast and/or ovarian cancer remains to be determined.

Conclusions

There remains much to discover with respect to the function of BRCA1 and BRCA2, notwithstanding new mechanisms of disease are being discovered which will eventuate in a better understanding not only of inherited forms of breast cancer but also of sporadic breast cancer. A thorough understanding of the events that underlie this devastating disease will result in better outcomes for those women unfortunate enough to develop this malignancy.

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