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The BRCA1 and BRCA2 Breast and Ovarian Cancer Susceptibility Genes — Implications for DNA Damage Response, DNA Repair and Cancer Therapy

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1. Introduction

Efficient intracellular DNA repair mechanisms are essential for preventing the accumulation of genetic mutations and protecting against genomic instability, which can lead to cancer development. This is reflected in the increased risk of breast and ovarian cancer conferred by mutations in the breast and ovarian cancer susceptibility genes 1 & 2 (BRCA1 and BRCA2), both of which have important roles in promoting the accurate repair of DNA damage and maintaining genomic integrity. BRCA1 was first identified in 1994 and mapped to chromosome 17q12 through linkage analysis in families with a strong family history of breast and/or ovarian cancer [1, 2]. BRCA2 was discovered a short time later when a second breast cancer susceptibility locus was mapped to chromosome 13q12, again by linkage analysis in similar families [3, 4]. BRCA1/2 mutations may be present in approximately 1/400-1/800 of the general population although a higher incidence of BRCA1 mutations have been observed in certain populations such as in Ashkenazi Jews. Studies estimate that inherited mutations in BRCA1 can increase the cumulative risk of developing breast cancer by age 70 to 80% and ovarian cancer risk to 30-40%, whereas BRCA2 mutation carriers have up to a 50% risk of breast cancer and 10-15% ovarian cancer risk by age 70. Additionally, mutations in BRCA2 also increase susceptibility to male breast cancer, prostate and pancreatic cancer [5, 6]. According to the Breast Cancer Information Core (BIC), over 1,700 distinct mutations have been identified in the BRCA1 gene to date, comprising inactivating truncations and deletions to missense mutations. While approximately 850 of BRCA1 mutations identified have been confirmed to increase cancer risk, the clinical relevance of the remaining mutations is unknown [7].

While BRCA1/2 mutations account for a relatively small proportion of all breast cancers (2.5-5%), mutations in these genes are responsible for approximately 20-25% of inherited breast cancer cases [8-10]. In addition, BRCA1 mutation carriers typically develop cancer before the age of 50 meaning that the number of years affected can be substantially greater than in most subtypes of sporadic breast cancer. This is likely due to the fact that both the BRCA1 and BRCA2 genes adhere to the Knudson "two-hit" hypothesis in which both alleles of a tumour suppressor gene must be mutated for the pathogenic phenotype to become apparent. Hence one inherited copy of mutant BRCA1/2 is the "first hit" and the "second hit" comes from acquiring a somatic mutation.

BRCA1 mutant breast tumours often fall into the basal-like breast cancer subtype, which typically exhibit low or absent expression of the oestrogen receptor α (ERα), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2) and are therefore commonly referred to as triple negative breast tumours. Due to the lack of expression of these receptors, there are no targeted treatments currently available for this type of cancer and as a result these patients tend to have a poor prognosis. Interestingly, in sporadic basal-like breast cancer patients with wildtype BRCA1, BRCA1 expression is often down-regulated possibly as a result of promoter methylation or over-expression of ID4, a negative regulator of BRCA1 expression. A term has been coined, known as "BRCAness", to describe sporadic basal-like tumours with low BRCA1 expression and/or a similar phenotype to BRCA1 mutant tumours. Both BRCA1 mutant and BRCA1-low tumour types are sensitive to DNA damaging agents suggesting a possible common pathogenesis involving dysfunction of BRCA1 or BRCA1-regulated pathways, such as DNA repair [11, 12].

Accordingly, the tumour suppressor function of BRCA1 and BRCA2 is mainly attributed to the role of these proteins in the regulation of conservative DNA repair pathways, thus maintaining genomic integrity. While the main function of BRCA2 identified to date is in promoting the error-free homologous recombination pathway, BRCA1 is a multi-functional protein with roles in many important cellular processes such as transcriptional regulation, ubiquitination, oestrogen metabolism, chromatin remodelling and mRNA splicing [13]. These additional functions of BRCA1 and how they relate to DNA repair will also be discussed, followed by an overview of BRCA2 function in the repair of damaged DNA and how the DNA repair defects in BRCA1/2 mutant related cancers can be exploited for treatment.

2. Structure of BRCA1

The BRCA1 gene encodes 24 exons translating into a 1863 amino acid protein which contains two main functional domains; a **r**eally **i**nteresting **n**ew **g**ene (RING) finger domain and two BRCA1 C-terminal (BRCT) domains (Figure 1). The RING finger domain, located at the N-terminus of BRCA1, is a zinc binding region with a conserved histidine and cysteine motif which is required for binding to the structurally similar **B**RCA1 **A**ssociated **R**ING **D**omain protein 1 (BARD1) which also has a RING finger domain and 2 BRCT domains. The BRCA1-BARD1 interaction is necessary for stability of both the BRCA1 and BARD1 proteins thus

BRCA1 generally exists in a heterodimeric complex with BARD1 *in vivo* [14]. Furthermore, binding of BARD1 to the RING finger domain of BRCA1 forms an E3 ubiquitin ligase complex, the function of which will be discussed later. A number of tumour associated mutations have been identified within the RING finger of BRCA1 such as C61G and C64G which abolish the ubiquitin ligase activity and confer sensitivity to ionising radiation, suggesting the RING domain of BRCA1 is important for regulating DNA repair [14, 15].
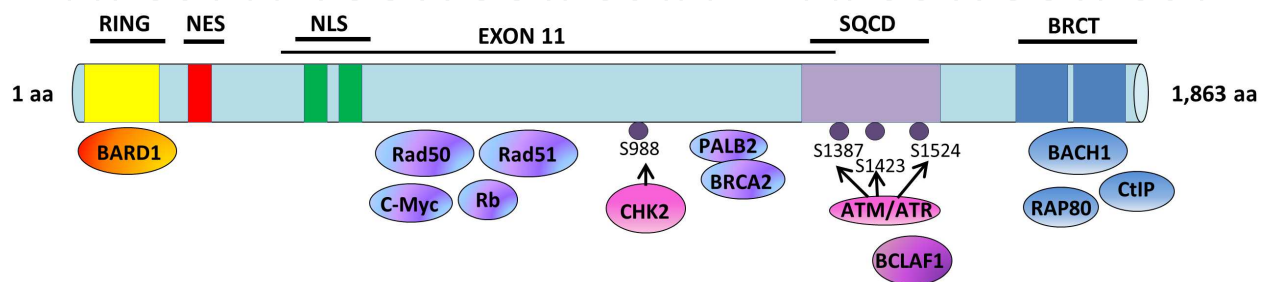

Figure 1. Structure and Binding Partners of BRCA1. Schematic diagram of BRCA1 and it's functional domains illustrating the position of; the RING finger responsible for BARD1 binding and E3 ligase activity, exon 11 which binds important HR proteins including Rad50 and Rad51, and the BRCT domains which mediate binding of RAP80, BACH-1 and CtIP. Phosphorylation sites important for DNA damage signalling are also shown, as well as the kinases responsible for their modification.

At its C-terminus, BRCA1 contains two conserved BRCT domains, each approximately 100 amino acids long. BRCT domains recognise and bind to phospho-peptides containing the pSer-X-X-Phe motif [16]. Phosphorylation is a major mechanism of signalling within the DNA damage response pathway and BRCA1 has been shown to bind to several phosphorylated DNA repair-related proteins through its BRCT domains such as BACH1 and CtIP [17]. As with mutations in the RING finger domain, mutations in the BRCT repeats of BRCA1 have been identified in cases of familial breast cancer. Furthermore, mouse embryonic fibroblast cells harbouring a BRCT mutation that disrupts BACH1 binding exhibit defective homologous recombination, increased sensitivity to genotoxic stress and develop tumours at a similar rate to those lacking BRCA1 [18].

The region of BRCA1 encoded by exons 11 – 13 comprises 65% of the BRCA1 peptide sequence and is also commonly mutated in breast cancer. It contains two nuclear localisation signals (NLS), a less structured central domain and an SQ cluster domain (SQCD) [7]. Mutations in NLS1 in particular, disrupt interactions between BRCA1 and importin-α, resulting in impaired nuclear localisation of BRCA1, which is detrimental to DNA repair [19]. BRCA1 also contains a nuclear export signal (NES) in its N-terminus, which contributes to subcellular shuttling of BRCA1. Numerous proteins with functions in different cellular processes bind the exon 11 – 13 region of BRCA1 including the important DNA repair proteins Rad50 and Rad51 as well as the transcription factor c-Myc and cell cycle regulator, Retinoblastoma (Rb) (reviewed in [7]). The SQCD is also relevant to the function of BRCA1 in DNA repair as it contains numerous serine-glutamine (SQ) or threonine-glutamine (TQ) residues which are targets for phosphorylation via the DNA damage-induced phosphatidylinositol 3-kinase-related kinases (PIKKs);

ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR) and are responsible for activating numerous functions of BRCA1 depending on the residue(s) phosphorylated [20].

3. BRCA1 in DNA repair

As previously mentioned, BRCA1 mutation or dysfunction has consistently been associated with genomic instability and it is proposed that this is mainly due to defective DNA damage repair pathways [6, 21]. DNA damage occurs frequently within cells due to by-products of normal metabolism such as reactive oxygen species (ROS) but can also occur following exposure to exogenous sources such as ionising radiation (IR), ultraviolet (UV) radiation or chemotherapy. In order to respond to different types of DNA damage, cells have several DNA damage repair pathways including base excision repair (BER) and nucleotide excision repair (NER) for repairing damaged bases and single strand breaks (SSBs) respectively, and non-homologous end joining (NHEJ) or homology-directed repair (HDR) for the repair of double strand breaks (DSBs). NHEJ is the most common form of DSB repair and can occur throughout all phases of the cell cycle. It involves binding of Ku70/Ku80 to the broken DNA ends followed by recruitment of the catalytic subunit of DNA-PK, which phosphorylates numerous sub-strates at and surrounding the break site thereby promoting the removal of single strand overhangs and subsequent re-ligation of the DNA ends by XRCC4/DNA ligase IV. Although this is the most commonly utilised DSB repair pathway, as it has no regard for sequence homology, it is a relatively error prone repair pathway and utilisation of this pathway is potentially mutagenic.

There are two types of homology-directed repair; single strand annealing (SSA) and homologous recombination (HR). SSA can repair DSBs at short repetitive sequences by annealing the complementary repeats in a Rad52-dependent manner. This always results in loss of genetic material and therefore SSA is the most mutagenic of the DSB repair pathways [22]. In comparison to both NHEJ and SSA, HR is relatively error-free since it uses a sister chromatid as a template to copy and replace damaged DNA. However, HR can only occur in the S and G2 phases of the cell cycle when a homologous sister chromatid is present and in close proximity [23]. Although the most extensively studied role of BRCA1 is its regulation of HR, BRCA1 has also been implicated in NHEJ, SSA and the repair of interstrand crosslinks (ICLs) and how BRCA1 is involved in each of these pathways will be discussed below.

4. Homologous recombination

The process of HR includes several different stages. The MRE11-Rad50-Nbs1 (MRN) complex, in combination with the human single strand binding protein (hSSB1) is responsible for the initial sensing of DNA DSBs within the cell. MRN then binds to the break site leading to the recruitment and activation of ATM which in turn phosphorylates many substrates involved in DNA damage signalling. The histone H2AX is one such substrate of ATM which is phos-

phorylated at serine 139 (Ser139) forming γH2AX. ATM rapidly phosphorylates H2AX within the chromatin at, and flanking, the DSB site, thereby amplifying and propagating the DNA damage response. Additionally, γH2AX forms a docking site for numerous other proteins involved in DNA damage signalling and repair such as mediator of DNA damage checkpoint protein 1 (MDC1) which binds to γH2AX through its BRCT domain. MDC1 itself is able to recruit and anchor more MRN complexes to γH2AX surrounding the break site through interaction with Nbs1. MRN binding then amplifies ATM activation leading to a positive feedback loop which further amplifies the DNA damage response signalling cascade [24]. Once the necessary proteins have been recruited, DNA end resection must occur for HR to proceed and this involves the generation of 3′ single stranded DNA (ssDNA) overhangs at the DSB ends. MRN has a major role in end resection through the endonuclease and exonuclease activities of Mre11. The 3′ ssDNA overhang is then coated by replication protein A (RPA) which protects the single stranded DNA from degradation and prevents the formation of secondary structures. Next, partner and localiser of BRCA2 (PALB2) recruits BRCA2 which facilitates the displacement of RPA and subsequent loading of the recombinase Rad51 onto ssDNA forming a nucleoprotein filament which is responsible for homology searching and invasion of the homologous sister chromatid. This leads to formation of a transient displacement loop (D-loop) since once strand invasion has taken place, the second strand of the sister chromatid becomes displaced. Elongation by the DNA replication machinery and resolution of the D-loop then completes error-free repair of DNA [24].

BRCA1 was first implicated in DSB repair following the observation that murine embryos harbouring homozygous BRCA1 exon 11 deletions were hypersensitive to ionising radiation and exhibited both structural and numerical chromosomal aberrations in comparison to their heterozygous and wildtype counterparts [21]. Moynahan et al extended these findings by demonstrating that BRCA1-deficient embryonic stem cells were also highly sensitised to the DNA cross-linking agent mitomycin C (MMC) and importantly, correction of the BRCA1 exon 11 deletion restored normal levels of mitomycin C resistance [25] confirming that BRCA1 has a role in mediating resistance to DNA damaging agents. In 1999, the importance of BRCA1 specifically in homologous recombination was demonstrated in BRCA1$^{-/-}$ mouse embryonic stem cells which had 5 to 6 fold lower levels of homologous repair activity compared to BRCA1$^{+/-}$ cells while little effect was observed on non-homologous repair [26]. Over the past couple of decades, multiple roles for BRCA1 in HR have emerged, and BRCA1 appears to have distinct functions depending on its binding partners. Each of these functions will be considered below.

5. BRCA1 in DNA end resection

Processing of DSBs by DNA end resection is necessary to initiate the repair of DSBs by HR. A role for BRCA1 in promoting end resection was first observed when depletion of BRCA1 expression was shown to decrease the generation of ssDNA [27]. Chen et al showed that the interaction between BRCA1-CtIP-MRN (known as the BRCA1-C complex) facilitates end resection in S and G2 phases of the cell cycle and that this interaction is dependent on CDK

phosphorylating CtIP at serine 327. In agreement with this, when the CtIP S327A mutant (which cannot bind to BRCA1) is expressed in U2OS cells it leads to increased radiosensitivity in comparison to U2OS cells transfected with wildtype CtIP. Furthermore, the BRCA1-CtIP interaction was shown to be required for binding of MRN to BRCA1, which is essential for the synthesis of ssDNA overhangs [28, 29].

In direct contrast, a number of recent studies have demonstrated that BRCA1 is in fact dispensable for CtIP-mediated end resection. Reczek et al showed mouse embryonic fibro-blasts (MEFs) expressing the CtIP S326A mutation (equivalent to S327A in humans) displayed similar levels of Rad51 and RPA IRIF as CtIP wildtype cells. Accordingly, loss of the CtIP-BRCA1 interaction did not affect HR or tumour development in mice [30]. Polato and collea-gues reported similar findings showing that in contrast to the CtIP S327A mutant, mice harbouring the CtIP T847A mutation (which is essential for end resection but does not affect the BRCA1 interaction) had elevated levels of spontaneous chromosomal aberrations as well as decreased levels of IR-induced Rad51 indicating that CtIP functions independently of BRCA1 to promote end resection [31]. Further investigation of the role of the BRCA1-CtIP interaction in end resection was performed using a high resolution technique known as single molecule analysis of resection tracks (SMARTs) which allows visualisation of the length of the resected DNA in a single molecule. This revealed that although BRCA1-CtIP is expendable for the initiation of end resection, disruption of this interaction actually decreases the length and speed of resected DNA generated following IR or etoposide treatment [32]. Therefore, although BRCA1 is not essential for CtIP-mediated end resection it may facilitate the efficiency of the process.

In contrast to the role of BRCA1-CtIP in facilitating end resection, recent evidence suggests that BRCA1 in complex with receptor-associated protein 80 (RAP80) may actually prevent end resection with chromatin immunoprecipitation (ChIP) assays showing an increased abun-dance of HR proteins RPA and Rad51 on chromatin following RAP80 depletion. The BRCA1-RAP80 complex also contains ABRAXAS, BRCC36, BRCC45, MERIT40 and BARD1 and is known as the BRCA1-A complex, however it appears the BRCA1-RAP80 interaction is most important in the regulation of end resection. Decreased RAP80 has also been shown to increase the BRCA1-CtIP interaction, which may further enhance end resection [33, 34]. Although HR is a relatively error-free method of repair, poorly regulated HR can lead to recombination of inappropriate homologous sequences, which can produce genomic rearrangements and indeed depletion of RAP80 despite increasing HR leads to an increase in multiradial chromo-somes, as a result of improper recombination [34]. Thus the BRCA1-RAP80 complex, despite inhibiting end-resection required for HR, may also preserve genomic integrity by preventing excessive end resection, which can lead to chromosomal aberrations [33, 34].

6. BRCA1 in HR/NHEJ pathway choice

End resection is clearly a pivotal step in promoting HR, and the regulation of end resection has recently become an intense area of research in determining the choice between HR and

NHEJ in S and G2 phases of the cell cycle when both repair pathways are operational. In contrast to the role of BRCA1-CtIP in facilitating end resection in S and G2 phases thus allowing HR to proceed [35], 53BP1 has been shown to prevent end resection therefore inhibiting HR and promoting NHEJ. Several studies have shown that 53BP1 loss at least partially restores HR in BRCA1 deficient cells with Bunting et al observing increased IR-induced RPA phosphorylation in the absence of 53BP1 in BRCA1 mutant cells. Thus it has since been postulated that the antagonistic relationship between BRCA1 and 53BP1 may be responsible for mediating HR/NHEJ pathway choice [36, 37] (Figure 2). Bunting et al have also demonstrated that mice harbouring homozygous deletion of 53BP1 in combination with breast-specific homozygous deletion of BRCA1 exon 11 (BRCA1$^{\Delta11/\Delta11}$) display a greatly reduced breast tumour burden in comparison to BRCA1$^{\Delta11/\Delta11}$ mice with wildtype 53BP1 [36]. The authors therefore suggest that BRCA1 mutation carriers may benefit from inhibition of 53BP1 to alleviate the repair defect and thus genomic instability. Interestingly, when CtIP was depleted in BRCA1$^{-/-}$ 53BP1$^{-/-}$ MEFs, IR-induced ssDNA formation was decreased suggesting CtIP is necessary for the rescue of end resection observed in the absence of both BRCA1 and 53BP1. Furthermore, expression of CtIP in BRCA1 mutant cells decreases levels of genomic instability supporting a model whereby CtIP may partially overcome 53BP1-mediated inhibition of resection following loss of BRCA1 [31]. Despite the inhibitory role of 53BP1 in S phase, Kakarougkas et al propose that 53BP1 actually promotes HR in G2, specifically in DSBs occurring in heterochromatin regions due to 53BP1-dependent formation of phosphorylated KAP1 foci causing relaxation of the heterochromatin and allowing RPA loading in G2, thus 53BP1 can both promote and inhibit HR at different stages of the cell cycle [38].

The mechanism of the antagonism between BRCA1 and 53BP1 was investigated by super-resolution microscopy of IR-induced foci (IRIF), which enabled observations of the precise distribution of 53BP1 and BRCA1 following IR and showed enrichment of 53BP1 within IRIF in G0/G1 cells concomitant with the use of NHEJ mediated DSB repair in these stages of the cell cycle. However, in S phase 53BP1 was redistributed to the periphery of these foci while BRCA1 accumulated in the core of the IRIF. 53BP1 was not repositioned to IRIF margins following siRNA knockdown of BRCA1 showing BRCA1 is necessary for this process. This led the authors of this study to propose a process whereby BRCA1 may inhibit 53BP1 in S phase by preventing its interaction with chromatin at DSB sites allowing end resection and thus HR to proceed [39]. Following on from this, the deubiquitinating enzyme POH1 is also thought to be necessary for formation of the 53BP1 devoid IRIF core in a BRCA1-dependent manner. The suggested model involves BRCA1-mediated redistribution of 53BP1 from the core of the IRIF and this allows access to POH1 which removes RAP80 from the core allowing degradation of ubiquitin chains and complete clearance of 53BP1 from the DNA ends situated within the core of the IRIF thereby facilitating DSB end resection [40].

On the contrary, 53BP1 can also inhibit BRCA1 recruitment to DSBs in G1 phase of the cell cycle and if the 53BP1 effector protein RIF1 is reduced, BRCA1 IRIF form in G1. RIF1 accumulates at DSBs in a 53BP1-dependent manner and has been shown to bind to 53BP1 following activation of ATM. RIF1 IRIF are normally only formed in G1 but down-regulation of BRCA1 leads to a significant increase in RIF1 foci in S/G2. Moreover, this inhibitory effect of BRCA1
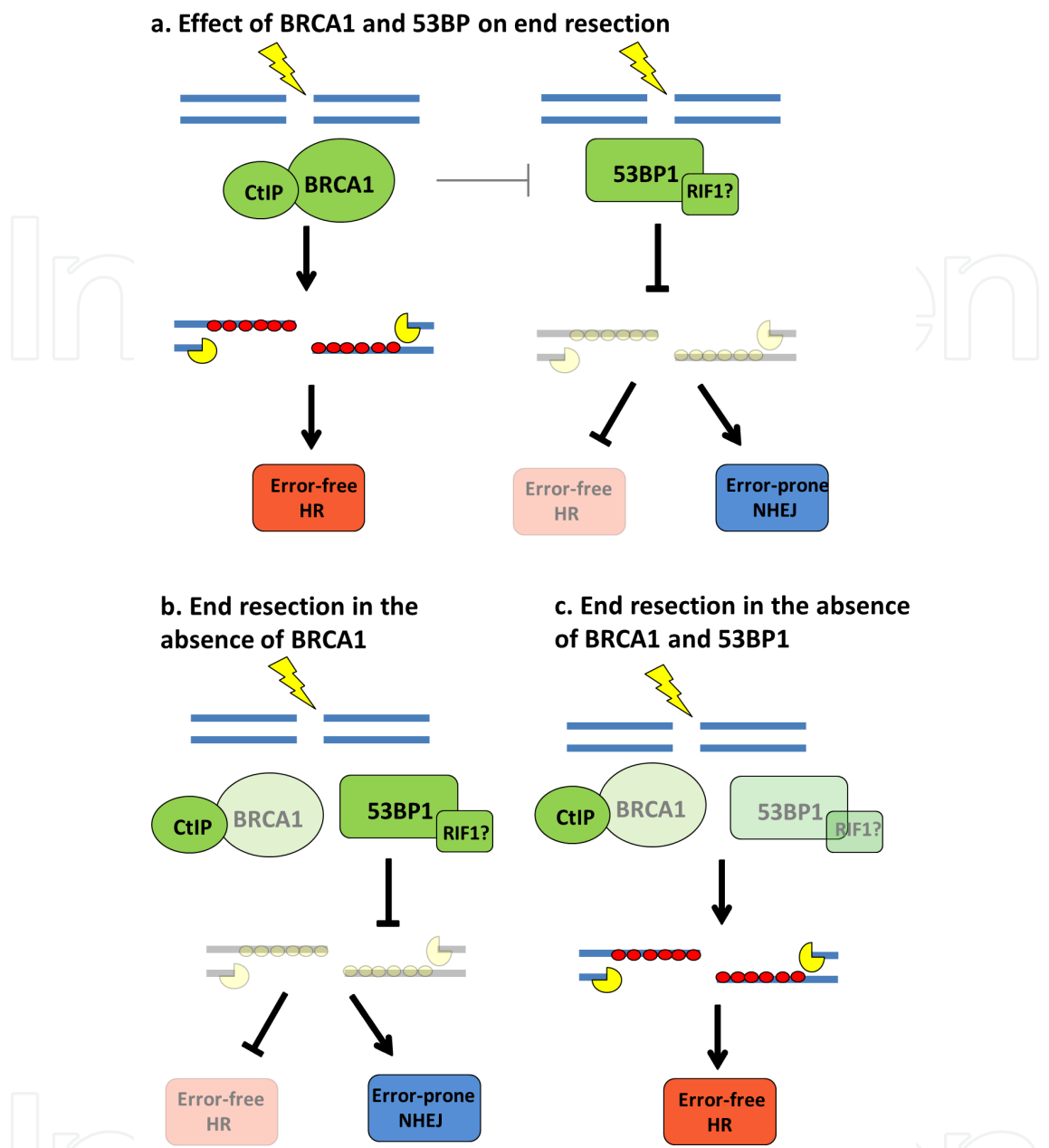
Figure 2. The Antagonistic Relationship of BRCA1 and 53BP1 on End Resection. Both the HR and NHEJ repair pathways can function in S and G2 phases of the cell cycle and end resection has an important role in the choice between HR and NHEJ. In wildtype cells, the BRCA1/CtIP complex promotes HR in S and G2 by stimulating end resection and by inhibiting 53BP1 (and possibly its effector protein RIF1), which acts as a barrier to end resection and thus HR (a). HR defects are observed in the absence of BRCA1, due to loss of its stimulatory effect on end resection and also loss of BRCA1-mediated inhibition of 53BP1 and RIF1. Together this blocks end resection and HR, and DNA is repaired by the potentially mutagenic process of NHEJ, such is the case in BRCA1 mutant breast cancers (b). In the absence of both BRCA1 and 53BP1 the barrier to end resection is removed and error-free HR can once more proceed via a CtIP-dependent mechanism. Therefore the status of 53BP1 in BRCA1 mutant tumours is pivotal for the regulation of HR (c).

on RIF1 IRIF is dependent on the BRCA1-CtIP interaction. Depletion of RIF1 rescues both the end resection and Rad51 loading defect caused by BRCA1 deficiency to a similar degree as loss of 53BP1. Therefore, in G1 RIF1 is recruited to DSBs by 53BP1 following ATM activation and

inhibits BRCA1 recruitment, while in G2/S RIF1 accumulation is inhibited by BRCA1-CtIP, suggesting that RIF1 and BRCA1 form a cell cycle-regulated circuit to favour NHEJ in G1 and HR in S/G2 [41].

Interestingly, loss of Ring finger Nucleotide Factor 168 (RNF168) which recruits both BRCA1 and 53BP1 to sites of DSBs seems to emulate the effects of 53BP1 loss, with depletion of RNF168 in BRCA1 deficient cells rescuing the homologous recombination defect. This study also demonstrated that expression of a dominant negative form of 53BP1 is able to restore HR in control cells but had no effect in RNF168 depleted cells, suggesting that 53BP1 and RNF168 inhibit HR through a similar mechanism [42].

7. BRCA1 in Rad51 loading

Another key role of BRCA1 in HR is in Rad51 loading which as discussed above is responsible for homologous strand invasion which then allows DNA polymerase to repair DNA using the sister chromatid as a template. BRCA1 has been reported to colocalise with Rad51 at nuclear foci within S phase of the cell cycle. Additionally, BRCA1 and Rad51 physically interact through regions within BRCA1 exon 11 [6, 43, 44]. Furthermore, depletion or mutation of BRCA1 has been shown to result in loss of Rad51 foci formation following DNA damage indicating that BRCA1 is required for Rad51 recruitment to DSB sites [27]. Following this, Sy and colleagues demonstrated that the displacement of RPA and subsequent loading of Rad51 filaments to single stranded DNA was dependent on the interaction between BRCA1, BRCA2 and PALB2 [45]. PALB2 was first identified as a binding partner of BRCA2 and is involved in the recruitment of BRCA2 to DSB sites but PALB2 was later shown to also interact with BRCA1 and this interaction is required for BRCA2-PALB2 localisation to sites of DNA damage. Additionally, depletion of PALB2 results in deficient HR and a PALB2 mutant unable to bind BRCA1 could not restore this repair defect in comparison to wildtype PALB2. This suggests that PALB2 acts as a scaffold between BRCA1 and BRCA2 [45, 46]. Considering the many and varied roles of BRCA1 in HR, it is therefore not surprising that defective HR is a characteristic of BRCA1 deficient cells and as a consequence DNA damage is repaired via error-prone mechanisms such as NHEJ resulting in a higher rate of genetic mutations which increases susceptibility to cancer [47].

8. Single Strand Annealing (SSA)

In comparison to the extensively studied functions of BRCA1 in HR, relatively little is known about the role of BRCA1 in the regulation of the SSA homology-directed repair pathway. Stark et al demonstrated that mouse embryonic stem (ES) cells harbouring homozygous deletion of BRCA1 exon 11 have decreased HR and SSA activity and the same effect was observed following disruption of the BRCA1-binding region of BARD1. Expression of wildtype BRCA1 in the BRCA1 mutant HCC1937 cell line model was also shown to promote SSA, although a

greater increase was observed in HR activity [48]. Although BRCA1 appears to positively regulate both HR and SSA, BRCA2 and Rad51 have opposing effects on these two DSB repair pathways, promoting HR while suppressing SSA. This suggests that BRCA1/BARD1 may function upstream of the branch point between HR and SSA regulating a step common to both pathways while BRCA2 and Rad51 act downstream of BRCA1 to inhibit SSA. Since BRCA1 is known to promote end resection which is required for both HR and SSA this is a possible mechanism by which BRCA1 augments both pathways. Furthermore, loss of the NHEJ factor Ku70, which limits resection of DSB sites, was able to rescue the SSA defect caused by disruption of BARD1, thus overcoming the barrier to resection and allowing SSA to proceed [49]. In contrast to BRCA1 regulating HR and SSA via the same mechanism, a study which analysed the effect of 29 different BRCA1 missense mutations on HR and SSA showed that several mutants with normal HR activity were defective in SSA. Interestingly all of the mutants showing differential regulation of the two pathways exhibited amino acid substitutions between residues 90 and 191 [50]. Therefore, BRCA1 may also have an additional function in the regulation of SSA that has yet to be discovered, possibly regulated by an undefined region within its N-terminus. This is supported by the fact that loss of Ku70 only partially restores SSA in BARD1-mutant cells suggesting BRCA1-BARD1 may also mediate SSA further down the pathway, independent of end resection regulation [49].

9. Non-homologous end joining

As previously mentioned, DNA DSBs can be repaired by homology-directed repair pathways or by NHEJ. However the significance of BRCA1 in the regulation of NHEJ is controversial and early studies produced conflicting results. Snouwaert et al showed a decrease in HR and an increase in NHEJ activity in mouse ES cells with homozygous deletion of BRCA1 between residues 223 and 763 compared to wildtype cells, providing evidence that BRCA1 suppresses NHEJ. Furthermore, expression of a BRCA1 transgene decreased NHEJ to normal levels in these cells [51]. In direct contrast, Zhong et al demonstrated a reduction in NHEJ activity in BRCA1 null mouse embryonic fibroblasts which could also be corrected by reintroduction of BRCA1 [52]. Another study showed no difference in NHEJ in BRCA1 deficient human breast tumour derived cells (HCC1937) compared to cell lines with wildtype BRCA1 [53]. More recent work suggests that the initial discrepancies observed may reflect different functions of BRCA1 depending on cell cycle phase and also on the subtype of NHEJ examined. There are 2 main subtypes of NHEJ – Ku80-dependent canonical NHEJ which is relatively precise and the alternative NHEJ pathway which is Ku80 independent and involves microhomology-mediated end joining (MMEJ) which is similar to SSA except MMEJ can anneal smaller homologous sequences (5-25 bps), but like SSA, MMEJ is extremely mutagenic. In accordance with the role of BRCA1 in tumour suppression, evidence suggests that ATM and Chk2 mediated phosphorylation of BRCA1 promotes precise or canonical end-joining while suppressing the mutagenic MMEJ [54, 55]. Additionally, the BRCA1-BACH1 complex is required for impeding error-prone MMEJ with expression of a BACH1 mutant defective in BRCA1 binding resulting in increased MMEJ activity [56].

Another study investigated differences in BRCA1 regulation of NHEJ subtypes throughout the cell cycle and interestingly BRCA1 was found to promote canonical NHEJ in G1 but not in G2/S [57]. Furthermore, depletion of BRCA1 increased the number of deletions acquired during NHEJ, confirming that BRCA1 favours precise NHEJ. BRCA1 was shown to interact with the canonical NHEJ factor Ku80, specifically in G1 and this interaction may be critical for stabilising Ku80 to sites of damage as ChIP assays show decreased Ku80 binding at I-Sce-mediated DSB sites following treatment with BRCA1 siRNA. Thus the authors conclude that BRCA1 may maintain genomic stability in G1 via promotion of precise end joining in addition to promotion of error-free HR in S and G2 [57].

10. BRCA1 in interstrand crosslink repair

In addition to the repair of DNA DSBs, BRCA1 has also been implicated in the repair of interstrand crosslinks (ICLs). DNA ICLs are caused endogenously by the by-products of lipid peroxidation and exogenously by DNA crosslinking agents such as the platinum-containing cisplatin and nitrogen mustard compound mitomycin C, commonly used for chemotherapy in the treatment of cancer. ICLs are extremely deleterious lesions due to the covalent bonding of DNA strands which inhibits strand separation and thus DNA replication and transcription. The repair of ICLs can be a complex process and varies depending on the stage of the cell cycle [58]. However, in brief, the presence of an ICL during DNA replication will lead to stalled replication forks and repair of the lesion will then progress by the formation of incisions on either side of the linked nucleotide via the action of NER endonucleases. Translesion synthesis (TLS) polymerases allow bypass of the ICL site, generating a DSB, which can then be repaired by HR. The FANC proteins have a major role in the repair of ICLs. FANC proteins are mutated in Fanconi Anaemia (FA), a syndrome associated with bone marrow failure, developmental defects and susceptibility to cancer. Activation of the FA pathway involves formation of a core complex of eight FANC proteins which together with accessory proteins form a ubiquitin ligase responsible for monoubiquitination of FANCD2 and FANCI which is essential for co-ordinating the incision step of ICL repair [59]. Since ICLs are ultimately repaired by HR, in general the functions of BRCA1 discussed above also apply to the repair of ICLs. The recruitment of BRCA1 in ICL-linked HR however, is dependent on FANC proteins since the BRCA1-RAP80 complex, which modulates HR repair of ICLs, has been shown to bind to K63-linked polyubiquitinated FANCG via the ubiquitin interacting motifs (UIMs) of RAP80 [58].

BRCA1 also regulates ICL repair independently of HR, evidenced by the observation that while loss of 53BP1 restores HR defects in BRCA1-depleted cells, depletion of 53BP1 does not rescue hypersensitivity of BRCA1 null cells to crosslinking agents [60]. Numerous reports suggest loss of BRCA1 impedes the recruitment of the FANCD2 complex to the ICL, but has no effect on the ubiquitination of FANCD2. Depletion of Ku70 rescues FANCD2 foci formation in BRCA1$^{\Delta11/\Delta11}$ MEFs following cisplatin or MMC treatment and also decreases the hypersensitivity to these agents suggesting that BRCA1 may recruit or retain FANCD2 at sites of ICLs via inhibition of Ku70/80 [60]. Recently, Long et al have reported another novel role of BRCA1 in ICL repair related to replication fork stalling. It has been shown that replication fork stalling

first occurs approximately 20 bp from the site of the ICL at which point the CMG DNA replicative helicase blocks extension of the leading strand and therefore needs to be removed from the DNA to allow approach towards the ICL, which triggers activation of the FANC pathway and thus ICL repair. Depletion of BRCA1 or defective BRCA1-BARD1 complex formation inhibits CMG 'unloading' in response to ICLs but not during normal DNA replication. As a result of BRCA1 loss, the extension of the leading strand towards the ICL was impeded and a defect in the generation of incisions at the site of the ICL was observed. Therefore localisation of the BRCA1-BARD1 complex is required at an early stage of ICL repair in addition to its later role in HR [61].

11. BRCA1 and the cell cycle

Cell cycle checkpoints are essential for repair of damaged DNA as cell cycle arrest affords time for the DNA to be repaired efficiently ensuring mutations or chromosomal aberrations are not maintained or replicated leading to genomic instability. Cell cycle regulation is mainly orchestrated by the balance of cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors. However, many other factors are involved in mediating these cell cycle regulators and the timely regulation of cell cycle checkpoints at different phases. Indeed, evidence exists of a role for BRCA1 in the regulation of the G1/S, S and G2/M checkpoints. Initial studies focussed on the role of BRCA1 in the intra-S and G2/M checkpoints, as the BRCA1 deficient cell line HCC1937 also harbours mutations within p53, a master regulator of the G1/S checkpoint, making it difficult to unravel the contribution of BRCA1 to this checkpoint in these cells. However, these cells, which have a defective G1/S checkpoint, also exhibit defective S and G2/M arrest following IR and both of these checkpoints are restored by expression of wildtype BRCA1. While ATM-mediated phosphorylation of BRCA1 at Ser1423 is necessary for IR-induced G2/M arrest, ATM-dependent phosphorylation of BRCA1 Ser1387 is required for S phase arrest [62, 63]. Following this, another study using siRNA mediated depletion of BRCA1 found that the BRCA1/BARD1 heterodimer is required for G1/S checkpoint arrest following IR induced DNA damage [64]. This study found that ATM dependent phosphorylation of BRCA1 on serine 1423 and 1524 is required to allow BRCA1 to function as a scaffold, facilitating the ATM dependent phosphorylation of p53 on Ser-15 thereby stabilising and activating p53 resulting in the transactivation of the cdk inhibitor p21 and activating the G1/S checkpoint. As in HR where BRCA1 function depends on its binding partners, different BRCA1 complexes regulate different cell cycle phases. BACH1 is activated in S phase and forms a complex with BRCA1 and BRCA2 necessary for cells to progress from G1 to S phase [65], and BRCA1 and TOPBP1 colocalise to foci specifically in S phase following treatment with different genotoxic agents, namely hydroxyurea, UV and zeocin [66].

Interaction of BRCA1 with the phosphorylated form of BACH1 is also required for the G2/M checkpoint as BACH1 depletion abolishes G2/M arrest following IR and while the expression of wildtype BACH1 rescues this defect, the BACH1 S990A mutant, which is unable to bind BRCA1, could not restore G2/M arrest [67]. BACH1 exerts cell cycle effects by binding to the BRCT domains of BRCA1 but exon 11 of BRCA1 also appears to play a role in G2/M checkpoint

activation as MEFs carrying homozygous BRCA1 exon 11 deletions display a defective G2/M checkpoint [68]. A mechanism for BRCA1-regulated G2/M checkpoint arrest was postulated by Yarden and colleagues who showed that in response to IR, ATM activates BRCA1 which inhibits cdk1/cyclinB1 (responsible for G2 to M progression) via activation of Wee1 kinase which antagonises cdk1/cyclinB1. This study also found that, following damage, BRCA1 activates Chk1 leading to inhibition of the cyclinB1 effector Cdc25c, ultimately resulting in G2/ M arrest [69]. This work has now been extended to show that BRCA1 E3 ligase activity may regulate this signalling cascade since BRCA1-mediated ubiquitination leads to degradation of Cdc25c and cyclinB1 thus arresting cells before progression into mitosis [70].

Other BRCA1-containing complexes have also been implicated in cell cycle regulation. BRCA1/ BARD1/MRN/CtIP is required for activation of the G2/M checkpoint with knockdown of BRCA1 or CtIP leading to increased accumulation of cells in mitosis following IR [29]. Additionally, loss of RAP80 inhibits BRCA1 recruitment to DNA break sites, leading to defective G2/M checkpoint arrest. RAP80 is likely to act upstream of BRCA1 which mediates G2/M arrest partially via Chk1 activation. Therefore, it is not surprising that depletion of RAP80 also results in decreased Chk1 phosphorylation [71], a finding which suggests that RAP80 does indeed regulate the G2/M checkpoint via the same pathway as BRCA1.

12. Post-translational regulation of BRCA1 in the DNA damage response

Many new pathways involved in the regulation of BRCA1 in response to DNA damage have recently emerged, including ubiquitination, SUMOylation and poly-ADP-ribosylation (PARylation) signalling. However, one of the first recognised signalling pathways integral to BRCA1 function in the DNA damage response was phosphorylation. In response to specific types of cellular insults BRCA1 is phosphorylated at different residues by different PIKKs in a cell cycle-dependent manner. Scully et al first demonstrated the phosphorylation of BRCA1 in response to DNA damage following observations of a mobility shift in BRCA1 gel migration. The phosphorylation of BRCA1 was shown to occur specifically in S-phase but not in G1 [72]. The 3 major PIKKs which activate BRCA1 include ATR, which phosphorylates BRCA1 primarily at Ser1423 in response to UV, and ATM and Chk2 which phosphorylate BRCA1 following IR-induced DSBs, with ATM phosphorylating Ser1387, Ser1423 and Ser1524 and Chk2 responsible for Ser988 phosphorylation [73-76]. Chk2-dependent BRCA1 phosphorylation is directly involved in the regulation of HR with expression of the S988A BRCA1 mutant in HCC1937 unable to restore HR activity in comparison to wild-type BRCA1. Similar effects were observed following expression of a dominant negative Chk2 protein which inhibited Chk2 kinase activity [77]. Phosphorylation of BRCA1 also allows formation of complexes with other phosphorylated proteins through interaction with the BRCT domains of BRCA1. Indeed, BRCA1s BRCT domains have been shown to be indispensable for the tumour suppressor functions of BRCA1 and its ability to promote HR [18].

Over the past few years, much progress has been made on the role of ubiquitination in the DNA damage response and DNA repair pathways. In 2007, a number of independent research

groups identified RAP80 as an ubiquitin-binding protein that localises to DSBs following IR and promotes HR mediated DSB repair [78]. Translocation of RAP80 to regions of damage is dependent on 2 ubiquitin-interacting motifs (UIMs) within its N-terminus, which have specific affinity for K63-linked polyubiquitin chains generated at DSBs. RAP80 also binds to the BRCT domains of BRCA1 and loss of RAP80 results in loss of BRCA1 recruitment to sites of damage thus RAP80 targets BRCA1 to ubiquitinated structures at DSBs. BRCA1 is recruited to sites of DNA damage as part of the BRCA1-A complex which contains RAP80, ABRAXAS, BRCC36, BRCC45, MERIT40 and BARD1 [79]. Formation of RAP80 and BRCA1 positive IRIF is dependent on MDC1 therefore interest developed in the upstream signalling controlling this interaction. Phosphorylation of MDC1 by ATM was shown to recruit two ubiquitin E3 ligases, RNF8 and RNF168 and these proteins were shown to be necessary for RAP80-BRCA1 localisation to DSBs via generation of ubiquitin chains on histone 2A, recruiting the ubiquitin-binding protein RAP80 which binds to the K63 linked ubiquitin chains via it's UIM domains and recruits the rest of the BRCA1-A complex to DSBs (Figure 3). Other members of the BRCA1-A complex such as MERIT40 and BRCC45 are thought to not only facilitate accumulation of BRCA1 to sites of damage, but also stabilise and retain the binding of the BRCA1-A complex to DSB sites [80]. Additionally, BRCC36, which is a zinc-dependent metalloprotease and JAMM (JAB1/MPN/Mov34 metalloprotease) domain containing DUB with specific activity to K63-linked polyubiquitin, also forms a cytoplasmic complex known as BRISC, which contains BRCC36 and 45, MERIT40 and KIAA0157. Depletion of BRISC leads to an increase in BRCA1-A complex formation at sites of DNA damage suggesting a balance exists between the 2 complexes [81].

Another post-translational mechanism, SUMOylation, has also been shown to have a role in this signalling cascade and in localising BRCA1 to nuclear foci in response to IR. Small Ubiquitin-like Modifier (SUMO) isoforms 1, 2 and 3, as well as the SUMO-conjugating enzyme Ubc9, have all been shown to interact with BRCA1 in response to genotoxic stress. This interaction is dependent on the PIAS family of SUMO E3 ligases, namely PIAS1 and PIAS4 which are also found in DNA damage induced foci and when depleted inhibit the localisation of BRCA1 to γH2AX foci. The regulation of BRCA1 localisation by PIAS enzymes is thus indirect and it has been shown that PIAS1 and 4 both regulate upstream factors with PIAS1 depletion showing diminished localisation of RAP80, and PIAS4 regulating RNF168, K63-linked ubiquitination and RAP80 further up the damage signalling cascade [82, 83]. RAP80 contains a SUMO-interacting motif (SIM) as well as UIMs which mediate the interaction between the BRCA1-A complex and SUMO [84]. RAP80 can thus bind ubiquitin and SUMO simultaneously and both domains are required for RAP80 recruitment to DNA damage sites [85] (Figure 3). A further ubiquitin E3 ligase RNF4, which ubiquitinates SUMO chains, has been implicated in the recruitment of RAP80 and BRCA1 to DSBs and suggests that SUMOylation and ubiquitination act in concert in the recruitment of DNA repair factors to DSBs [84].

In 2013, a further post-translational modification, PARylation, was identified in the regulation of BRCA1 recruitment to DNA break sites. γH2AX has a major role in BRCA1 recruitment to foci in response to DNA damage but following the observation that γH2AX depletion inhibited maintenance of BRCA1 at foci but did not inhibit the initial accumulation of BRCA1, Li et al
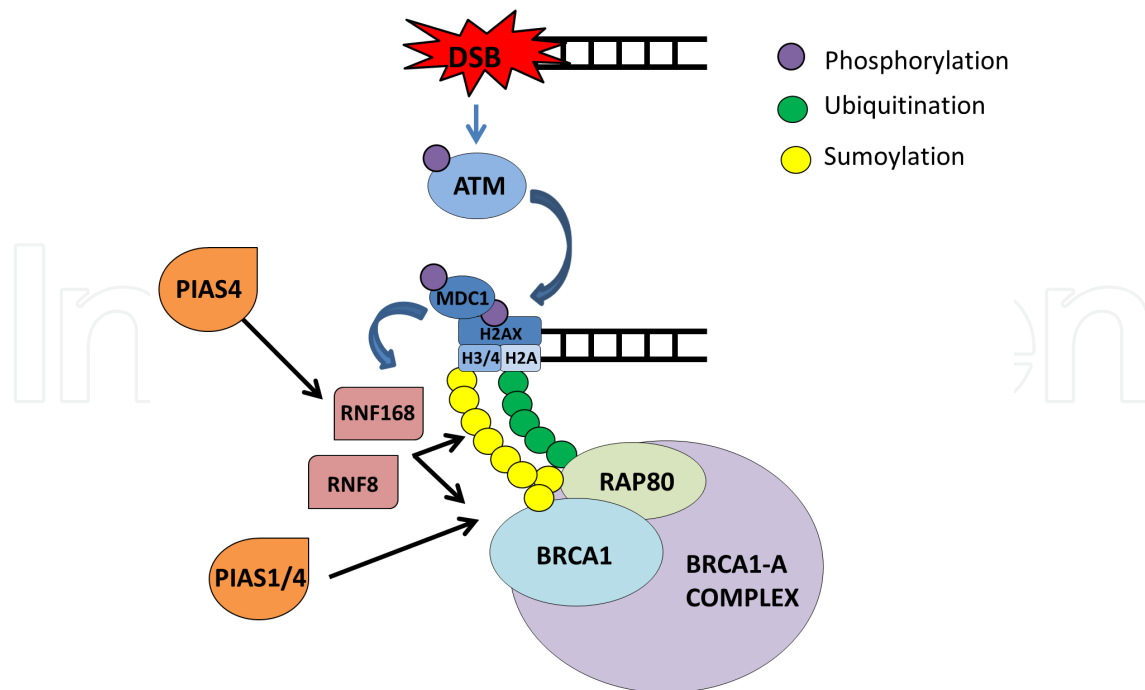
Figure 3. The Role of Post-translational Modifications in the Recruitment of BRCA1 to DNA DSBs. Following forma-tion of DSBs, ATM is activated and phosphorylates H2AX and MDC1 which stimulates RNF8 and RNF168 to generate K63-linked polyubiquitin chains on histone 2A. The UIMs of RAP80 then bind to the polyubiquitin chains and recruit BRCA1 and the rest of the BRCA1-A complex (ABRAXAS, BRCC36, BRCC45, MERIT40 and BARD1). PIAS1 and PIAS4 enzymes directly mediate BRCA1 SUMOylation, which is also required for BRCA1 localisation to DSBs and PIAS4 also indirectly regulates SUMOylation via RNF168. Additionally, RAP80 SIMs bind SUMO and facilitate the SUMOlyation of BRCA1.

postulated that γH2AX was responsible for stabilising BRCA1 at sites of damage but another factor regulated its initial recruitment. This group then showed that the RING finger domain, rather than the BRCT domain of BRCA1, was necessary for recruitment to DNA damage sites, with the BRCT domains of BARD1 also required. Following DNA damage, PARylation is induced and BARD1 was shown to interact with both PAR and the basic unit of PAR, ADP-ribose, through its BRCT domain. BRCA1 and PAR also interact but this interaction is BARD1-dependent. Moreover, BARD1 BRCT germline mutations identified in familial breast cancer patients fail to bind PAR, suggesting this is an important step in the recruitment of BRCA1 and its tumour suppressive functions. In agreement, PARP inhibitors were then shown to suppress early recruitment of BRCA1/BARD1 to DSBs [86].

13. The role of BRCA1 in transcription

The importance of BRCA1 in transcriptional regulation was highlighted by the discovery that the C-terminal domain of BRCA1 forms a complex with RNA polymerase II via interaction with RNA helicase A, which are both members of the core transcriptional machinery [87, 88]. Transcriptional regulation by BRCA1 can occur through either direct or indirect mechanisms. Direct regulation involves BRCA1 binding to the promoter of the gene, however, as BRCA1

does not contain any sequence specific DNA binding domains, specific DNA-binding transcription factors are required to recruit BRCA1 to the promoter regions of target genes [89]. BRCA1 is therefore able to act as either a co-activator or co-repressor of transcription depending on the transcription factor, and other members of the transcriptional complex, to which it is bound. The functional outcome of BRCA1 transcriptional regulation is wide and varied however many BRCA1 regulated genes play a role within the DNA damage response. For example, BRCA1 binds to p53 on the promoter of many p53-regulated genes where it co-activates their transcription. Intriguingly, BRCA1 was shown to selectively induce expression of p53 target genes involved in DNA repair such as p53R2 and Cyclin G2, as opposed to pro-apoptotic genes such as PIDD, PIG and KILLER/DR5. Additionally, BRCA1-mediated transcriptional activation of p53 target genes appears to be particularly important for cell cycle checkpoint control with BRCA1 also transactivating the p53 regulated genes 14-3-3σ and GADD45 both of which are involved in G2/M arrest following DNA damage. BRCA1 also interacts with c-Myc to form a transcriptional repressor complex. This complex binds to the promoters of a large number of genes including basal genes such as psoriasin and p-cadherin down-regulating gene expression [90]. This correlates with the low expression of BRCA1 in basal-like breast cancer.

More recently a comprehensive study by Gorski et al employed microarray analysis to identify almost 1,300 BRCA1-regulated genes in the MCF7 breast cancer cell line and also determined by ChIP-ChIP that BRCA1 was bound directly to promoters of over 600 genes. However, the majority of genes with BRCA1-bound promoters were not transcriptionally regulated by BRCA1 in unperturbed cells although a number of these genes such as MMP3, USP32 and CCL4L2 were commonly altered in response to DNA damaging agents. This implied a model whereby BRCA1 forms an inactive complex on gene promoters in the normal cellular context but in response to DNA damage can regulate the expression of genes involved in DNA repair and/or other DNA damage response processes. This is supported by observations that siRNA mediated knockdown of BRCA1 almost abolished the etoposide-induced activation of CCL4L2 transcription but had little effect on CCL4L2 mRNA expression in untreated cells [89]. Intriguingly, a different study showed that BRCA1 can negatively regulate its own expression by binding to the BRCA1 promoter and inhibiting transcription. However, in response to DNA damage, promoter binding may be inhibited releasing BRCA1 so it can be recruited to sites of DNA damage. The authors suggest that loss of BRCA1 from the promoter then increases BRCA1 transcription in order to replace BRCA1 protein consumed during DNA repair although this hypothesis requires further validation [91].

BRCA1 can also indirectly regulate transcription by binding to chromatin remodelling proteins such as the histone acetyltransferases (HATs) p300 and CBP [92] and the BRG1 and BRD7 subunits of the SWI/SNF chromatin remodelling complex which activates transcription by allowing transcriptional machinery to access DNA. Additionally, BRCA1 can ubiquitinate transcriptional preinitiation proteins which interferes with association of the transcriptional complex and subsequently represses mRNA synthesis [93]. According to Park et al, BRCA1 also binds to the histone deacetylases HDAC1 and HDAC2 and leads to histone deacetylation and transcriptional repression in a SUMO1-dependent manner. Following IR however,

SUMO1 repression of BRCA1-mediated transcription was alleviated via release of HDAC1 at BRCA1 bound promoters and this enhanced transcriptional activation [94]. BRCA1 mediated transcription is also inhibited by interaction with heterochromatin protein 1γ (HP1γ). Similar to the release of HDAC1 at BRCA1 regulated promoters, HP1γ is removed from the GADD45 promoter in response to etoposide treatment allowing BRCA1 to activate transcription. HP1γ is also attributed a role in recovery from BRCA1-mediated transcription. Following BRCA1 assembly at the promoter, HP1γ is then reassembled at the promoter and once again represses BRCA1 transcription [95]. These studies also propose regulatory mechanisms whereby BRCA1 differentially regulates genes in response to DNA damage.

14. BRCA1 in mRNA splicing

Our group recently characterised a novel function for BRCA1 in the regulation of pre-mRNA splicing of specific DDR genes via an interaction with BCLAF1 following DNA damage [96]. Phospho-peptide pulldown assays carried out with peptides mimicking BRCA1 phosphory-lated at serine-1423, revealed that BRCA1 and BCLAF1 interact and further studies went on to show that they only associate following treatment with DNA damaging agents. Further functional assays showed that BCLAF1 depletion, similar to the effects of BRCA1 knockdown, results in increased sensitivity to DNA damage, decreased DNA DSB repair capacity and genomic instability. Furthermore, decreased levels of BCLAF1 failed to sensitise BRCA1 mutant cells to IR suggesting the function of BCLAF1 in DNA repair is dependent on BRCA1.

BCLAF1 was previously identified as a member of a spliceosome complex containing numerous mRNA processing factors. This prompted investigation of the interactions of BRCA1 and BCLAF1 with proteins involved in mRNA splicing. While BCLAF1 is constitutively bound to the core splicing factors PRP8, U2AF64, U2AF35 and SF3B1, BRCA1 binding was only observed following DNA damage and the interaction was abolished when BCLAF1 was depleted. Conversely, BRCA1 was shown to be constitutively bound to the promoters of a large subset of genes (approx. 980) including a large group of DDR genes, such as ATRIP1, BACH1 and EXO1, whereas BCLAF1 and U2AF65 only bound after DNA damage and this was dependent on the presence of BRCA1. This supports a model whereby DNA damage induces BRCA1-mediated recruitment of splicing factors to the promoters of DDR genes via interaction with BCLAF1 and associated mRNA processing factors in order to promote mRNA splicing of these genes. Accordingly, mRNA splicing of BRCA1/BCLAF1 regulated genes is up-regulated following DNA damage, a process which is dependent on both BRCA1 and BCLAF1. Concurrently, protein levels of the target genes tested; ATRIP1, BACH1 and EXO1, were down-regulated following BRCA1 or BCLAF1 depletion after DNA damage. The study then went on to show that proteins encoded by targets of the BRCA1/BCLAF1 complex are turned over more rapidly following DNA damage and that the BRCA1/BCLAF1 mediated up-regulated splicing of these genes following DNA damage, functions to maintain the stability of these proteins, presumably by promoting the processing and subsequent stability of their transcripts.

This suggests that as well as playing a direct role in the repair of DNA DSBs, BRCA1 also regulates the transcription and mRNA processing of a large group of genes, many of which are involved in the DDR, including core DNA repair genes/proteins such as ATRIP, BACH1 and EXO1, in order to maintain the fidelity of the DNA damage response machinery.

15. BRCA1 and ubiquitination

The role of the E3 ubiquitin ligase activity of the BRCA1/BARD1 heterodimer in DNA repair and indeed the biological significance of this function of BRCA1 in general remains elusive. Ubiquitination involves the conjugation of the ubiquitin moiety to its target protein by the formation of a peptide bond between the C-terminal glycine 76 residue of ubiquitin and a lysine residue of the substrate [97]. Monoubiquitination can function as a form of post-translation modification that alters the function of a protein or it can target proteins for lysosomal degradation [98]. However, there is a further degree of complexity to the modification of proteins by ubiquitin owing to the fact that polyubiquitin chains can also be formed and the signal transduced as a consequence depends not only on the number of ubiquitin moieties added, but also on the lysine residue to which the ubiquitin molecule is attached. Since ubiquitin contains 7 lysine residues, there are several conformations a ubiquitin chain can exhibit, each conferring a unique signal to the conjugated substrate [99, 100]. For example, a K48-linked chain consisting of at least 4 ubiquitin adducts typically targets the protein for proteasomal degradation whereas a K63-linked chain may signal a conformational change in protein structure, form a docking site or transduce a signal in another way. K63-linked ubiquitin chains have been reported to be involved in DNA repair, activation of signalling pathways and protein trafficking [97]. The conjugation of ubiquitin to 'tag' proteins involves a cascade of 3 classes of enzymes; E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzymes) and E3 (ubiquitin-protein ligase enzymes). It is generally accepted that E3 enzymes such as BRCA1/BARD1 are responsible for specific substrate recognition of the protein [101]. In fundamental terms, the process of ubiquitination begins when E1 activates the C-terminal glycine of ubiquitin in an ATP-dependent reaction. E2 transfers the activated ubiquitin from E1 to E3, which is bound to the substrate protein. E3 then facilitates the formation of an isopeptide bond between ubiquitin and an internal lysine residue of the substrate [102].

The BRCA1/BARD1 E3 ligase was first shown to polyubiquitinate in a K6-linked manner, which does not target proteins for proteasomal degradation however, the biological significance of the K6-linked ubiquitination is unknown. Nevertheless, BRCA1/BARD1 has been shown to autoubiquitinate itself via K6 linkage and this enhances the ubiquitin ligase activity of the complex [103, 104]. Although most E3 ligases only conjugate with one E2 enzyme, BRCA1/BARD1 has been shown to interact with at least 8 different E2 enzymes which determine mono- or polyubiquitination and also the linkage specificity of the ubiquitin chains, therefore BRCA1/BARD1 does not only induce the originally identified K6-linkage mediated via the UbcH5c E2 enzyme [105]. One of the biggest challenges in the BRCA1 field has been the identification of bona-fide BRCA1 ubiquitination targets. This is due to the fact that BRCA1/BARD1 is a relatively promiscuous ubiquitin E3 ligase when studied *in-vitro.* As a result numerous *in-vitro* ubiquitination targets have been identified, including H2AX, RNA Pol II,

CtIP, ERα, γ-tubulin and NPM1, however few bona fide BRCA1/BARD1 substrates have been confirmed *in-vivo* [106].

Nevertheless, a number of pathogenic mutations in the RING finger of BRCA1/BARD1 such as C61G and C64G have been identified in cases of familial breast cancer suggesting the ubiquitin ligase function of BRCA1/BARD1 may be important for its tumour suppressor functions. Additionally, many of these studies have reported that these mutations result in increased genomic instability, again suggesting that the ubiquitin ligase function of BRCA1 is important for its role in tumour suppression. However, controversy exists over whether this is the case or not. Many studies have employed cells expressing the synthetically engineered RING finger mutation I26A. This mutation inhibits binding of BRCA1 to E2 enzymes but doesn't alter the formation of BRCA1/BARD1 complex therefore allowing separation of the functions of BRCA1 due to ubiquitin ligase activity from those dependent on BARD1 association. Shakya et al have shown that in response to DNA damage, mice harbouring the BRCA1 I26A mutant exhibit similar phenotypes to mice expressing wildtype BRCA1 with comparable levels of chromosomal abnormalities, mitomycin C resistance and ubiquitin foci at sites of DNA damage [18]. Furthermore, Reid et al reported similar findings and extended their study to show the I26A mutation had little effect on Rad51 recruitment at damage-induced foci or on levels of HR activity. However, ubiquitin ligase deficient cells exhibited increased numbers of chromosomal abnormalities following treatment with mitomycin C compared to BRCA1 wildtype ES cells, although the chromosomal aberrations were much more pronounced in cells with deletions of BRCA1 exon 11 [107]. These studies suggest BRCA1 E3 ubiquitin ligase activity is dispensable for its role in DNA damage repair.

On the contrary, several findings support a role for BRCA1-dependent ubiquitination in DNA repair. First, CtIP ubiquitination was shown to be specifically dependent on BRCA1 following DNA damage and this was required for localisation of CtIP to damage-induced foci and association with chromatin as well as G2/M checkpoint regulation [108]. More recently, Shabbeer et al have shown that while re-expression of wildtype BRCA1 rescues cell survival in cell line models with decreased levels of functional BRCA1, introduction of the I26A BRCA1 mutant or the C61G RING domain mutation failed to increase cell survival. This study also demonstrated BRCA1-dependent ubiquitination of cell cycle proteins Cyclin B and Cdc25c in response to IR and HU, in a K48-linked manner via the E2 enzyme UbcH1. This tags these proteins for proteasomal degradation which enables efficient G2/M arrest following DNA damage [70]. Furthermore, mouse models have shown that introduction of the C61G mutation increases genomic instability to a similar level of that observed in BRCA1 null mice and *in-vitro* experiments showed low levels of HR activity in C61G mutant cells. However, in comparison to BRCA1 null mice, mice expressing the BRCA1 C61G mutation displayed a greater number of DNA damage-induced Rad51 foci and γH2AX-positive cells and were less responsive to PARP inhibition indicative of residual HR activity despite loss of ubiquitin ligase activity [109]. Thus, although there are conflicting reports on the role of the BRCA1/BARD1 ubiquitin E3 ligase in DNA repair pathways *per se*, evidence seems to suggest BRCA1/BARD1 E3 ligase activity may be important in HR and cell cycle checkpoint regulation after DNA damage, but the significance of this function remains an active area of research.

16. BRCA1 in tissue-specific tumourigenesis

As mentioned earlier, the majority of BRCA1 mutant tumours do not express the oestrogen receptor. Despite this, the notion that oestrogen may contribute to the development of BRCA1-related tumours is supported by the fact that the risk of breast cancer in BRCA1 mutation carriers is reduced by approx. 50% following oophorectomy, which decreases circulating oestrogen levels. Additionally, pregnancy has been reported to increase the risk of breast cancer in BRCA1 carriers, in contrast to non-carriers for whom pregnancy is protective. This suggests a potential ERα-independent mechanism by which oestrogen may promote tumourigenesis. One such mechanism may be through the conversion of oestrogen to semi-quinone and quinone forms during normal oestrogen metabolism, a process which also results in the release of free radicals. Indeed, our group has recently demonstrated that exposure to the predominant endogenous oestrogen, estradiol (E_2), or its metabolites 2-hydroxyestradiol (2-OHE$_2$) or 4-hydroxyestradiol (4-OHE$_2$) induces DNA DSBs in breast cell lines [110]. Additionally, depletion of BRCA1 leads to decreased repair of DSBs generated by treatment with oestrogen metabolites and results in genomic instability marked by increased levels of chromosomal aberrations. Interestingly, cells with decreased BRCA1 expression also exhibited elevated numbers of DSBs at early time-points following 2-OHE$_2$ and 4-OHE$_2$ exposure which could not be attributed to a repair defect. Since BRCA1 was previously shown to mediate the transcriptional repression of the CYP1A1 gene, which encodes an enzyme responsible for metabolising androgens to bioactive oestrogens, it appeared plausible that loss of BRCA1 may enhance the production of oestrogen metabolites [111]. Indeed this study then went on to confirm that BRCA1 loss leads to up-regulation of oestrogen metabolising enzymes CYP1A1 and CYP3A4 and down-regulated expression of the detoxification enzyme NQO1 leading to an increase in the production of 2-OHE$_2$ and 4-OHE$_2$. Thus BRCA1 has a role in repressing the production of oestrogen metabolite induced DSBs as well as mediating the repair of DSBs in response to 2-OHE$_2$ and 4-OHE$_2$ exposure. This finding is particularly significant in explaining why BRCA1 mutation carriers predominantly develop tumours in hormonal tissues such as the breast or ovaries where levels of oestrogen are particularly high [110].

17. Role of BRCA2 in the DNA damage response

Like BRCA1, BRCA2 was identified as a breast/ovarian cancer susceptibility gene by linkage analysis and to date approximately 2000 distinct BRCA2 mutations, polymorphisms or variants have been catalogued in BIC. In comparison to BRCA1, much less is known regarding the functions of BRCA2 owing mainly to the large size of the BRCA2 protein (3418 amino acids), which has been difficult to express and/or purify, hampering functional studies. Additionally, the structure of BRCA2 shares limited homology to other proteins and it's most distinguishing feature is the presence of conserved BRC repeats which are repeated regions of approximately 30 amino acids [112], the number of which varies by species. Human BRCA2 contains eight BRC domains which can mediate interaction with 6-8 Rad51 molecules [113]. BRCA2 also contains a DNA binding domain (DBD) capable of associating with both ssDNA and dsDNA

and it was the combination of these two properties (Rad51 and DNA binding) that proposed a role for BRCA2 in HR [112, 114, 115]. BRCA2 also contains an N-terminal region, which interacts with PALB2 and is also involved in transcriptional activation and a C-terminal region which can bind multimeric Rad51, in comparison to BRC domains which interact with monomeric Rad51 [116] (Figure 4).
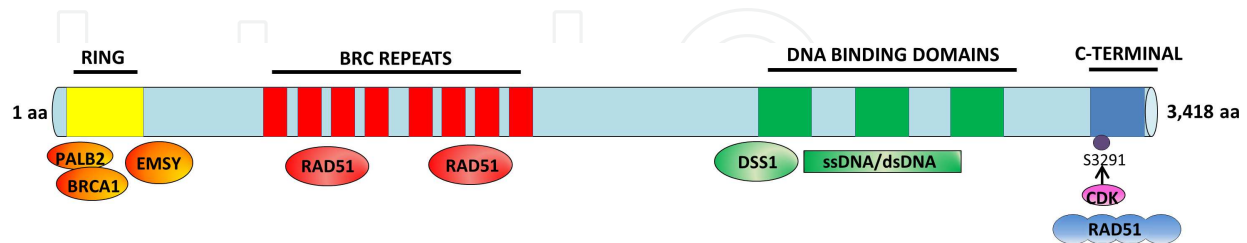

Figure 4. Structural Features and Binding Partners of BRCA2. Schematic diagram of BRCA2 and the functional domains mediating important protein interactions including; PALB2 binding to the RING domain, binding of Rad51 monomers and Rad51 filaments at BRC repeats 1-4 and 5-8 as well as polymeric Rad51 binding at the C-terminus, which is dependent on phosphorylation of Serine3291 by CDK. Also shown are the DNA binding domains and DSS1 binding region which allow BRCA2 to recruit Rad51 to sites of RPA-coated ssDNA thereby promoting HR.

Early phenotypic studies demonstrated that BRCA2 depletion led to increased sensitivity to DNA damaging agents, impaired homologous recombination and decreased formation of Rad51 foci following DNA damage [117, 118]. More recent investigations, following the purification of the full-length BRCA2 protein, have shed light on the mechanism of BRCA2 in HR showing that BRCA2 is responsible for Rad51 nucleation and filament formation by overcoming the inhibitory effects of RPA to allow Rad51 binding to ssDNA at DSB sites [119]. This process is enhanced by Deleted in Split Hand/Split Foot protein 1 (DSS1), which associates with BRCA2 to promote Rad51 nucleofilament formation potentially through stabilisation of the BRCA2 protein [120, 121]. Not only does BRCA2 mediate Rad51-ssDNA interaction but it also inhibits Rad51 binding to dsDNA, which impedes Rad51-mediated DNA strand exchange. Furthermore, BRCA2 can stabilise the Rad51 nucleoprotein filaments by blocking Rad51 ATP hydrolysis, which maintains the active ATP-bound form of Rad51-ssDNA necessary for efficient HR [114].

Carreira et al have demonstrated that the BRC repeats are required for the function of BRCA2 in Rad51-mediated HR and have investigated the functions of individual BRC domains. While all BRC domains bind Rad51 and facilitate the formation of Rad51-ssDNA nucleofilaments, they function via slightly different mechanisms, with BRC domains 5-8 having higher affinity for Rad51-ssDNA filaments and BRC domains 1-4 preferentially binding free Rad51 as well as mediating the inhibition of ATPase activity and preventing binding to dsDNA in order to stimulate DNA strand exchange [122, 123]. In contrast to BRC binding of Rad51 monomers, the C-terminal region of BRCA2 binds only oligomeric Rad51 and the role of this interaction in HR is more controversial than that of BRC repeats. Specifically, serine 3291 of BRCA2 is required for Rad51 association and this residue is phosphorylated by CDK in a cell cycle dependent manner, which abolishes the BRCA2-Rad51 interaction. In response to DNA damage, S3291 phosphorylation is reduced and the affinity of the BRCA2 C-terminal region

for Rad51 is increased which stabilises the Rad51 nucleoprotein filament and may even protect against nucleofilament disassembly. Thus a model is proposed in which IR stimulates ATM-dependent inactivation of CDK, which maintains S3291 in a non-phosphorylated form, promoting HR by allowing the formation and stabilisation of Rad51-ssDNA. On the contrary, it has been suggested that S3291 phosphorylation, which is detected at the highest levels in G2/M, could be involved in the termination of HR, therefore allowing progression into mitosis following efficient repair in S and G2 [124-126].

The CAPAN-1 pancreatic cancer cell line is commonly used for studies of BRCA2 function, as it contains a naturally occurring BRCA2 mutation in which one allele is lost and the other contains the 6174delT frameshift mutation resulting in a truncated BRCA2 protein with loss of 1416 amino acids at the C-terminus. Consequently, CAPAN-1 cells show defective Rad51 foci formation in response to DNA damage and thus decreased HR activity and hypersensitivity to PARP inhibitors [127] which are currently in clinical trials for the treatment of BRCA1/2 deficient tumours (discussed in next section). Intriguingly, Edwards et al were able to produce PARPi resistant clones following treatment of CAPAN-1 cells with the PARPi KU0058948. Edwards et al. then went on to show that many of these cell line clones contained further deletions within the *BRCA2* gene, resulting in restoration of the BRCA2 open reading frame. These restored BRCA2 ORFs always contained the N-terminus of BRCA1 fused to the C-terminus, however, they contained large deletions of other BRCA2 regions thought to be functionally important such as the BRC repeats and the DBD. Despite the loss of these domains, the PARPi resistant clones regained the ability to localise Rad51 to nuclear foci and to repair cells by HR [128]. This adds confusion to the significance of specific BRCA2 regions and suggests possible redundancy of BRCA2 domains. Similarly, Siuad and colleagues demonstrated that deletion of the entire BRCA2 DBD had minimal effects on HR providing PALB2 was present. However, when PALB2 was not bound, mutation of the DBD significantly abrogated HR. Additionally, mutation of the DSS1 binding region within the DBD also decreased HR despite tolerance of the DBD deletion. Additionally "micro-BRCA2" constructs less than 20% of full length BRCA2 were also sufficient for HR providing the C-terminus was intact [116]. Together these studies suggest plasticity of the BRCA2 protein in enhancing HR and also indicate the functional importance of the C-terminal region of the protein. This is in contrast to a number of other studies which claim the BRCA2 C-terminus may be dispensable for HR, therefore the significance of BRCA2 domains in HR requires further clarity [129, 130].

In addition to its role in HR, BRCA2 also maintains genomic integrity by preventing the Mre11 mediated degradation of stalled replication forks. A recent study conducted by Schlacher et al confirmed in a number of mammalian cell lines that the absence of BRCA2 led to shortened nascent DNA strands at stalled replication forks in response to hydroxyurea (HU). Cells with mutations in the C-terminal Rad51 binding region of BRCA2 were defective in protecting nascent DNA strands from Mre11 mediated fork degradation thus stabilisation of Rad51 by the C-terminal region of BRCA2 is essential in the maintenance of stalled replication fork stability. Importantly, degradation of stalled replication forks due to loss of BRCA2 had little effect on cell survival but significantly increased chromosomal aberrations, indicating another mechanism whereby BRCA2 maintains genomic stability. This finding also has clinical

implications as drugs which elicit replication fork stalling such as HU may actually increase the mutagenic potential of BRCA2 deficient cells and thus may be contraindicated in these patients [129-131].

BRCA2 can also function independently of Rad51 to promote genomic stability through a role in the maintenance of G2/M checkpoint arrest after DNA damage. Depletion of BRCA2 or PALB2 leads to premature recovery of the G2/M checkpoint via Aurora A/PLK1 activation, causing unrepaired cells to enter mitosis. Thus, BRCA2 and PALB2 halt activation of Aurora A/BORA/PLK1 until DNA damage is repaired and it is appropriate for cell cycle progression to occur [132]. A role for BRCA2 in transcriptional regulation and chromatin remodelling has also been reported. In 1997, Milner and colleagues showed that a region of BRCA2 exon 3 fused to the GAL4 DNA binding domain stimulated transcriptional activity in U2OS cells [133]. It was later demonstrated that BRCA2 coactivates androgen receptor (AR) mediated transcription via binding to GRIP1 and P/CAF1 both of which possess histone acetyltransferase activity, also suggesting a role for BRCA2 in chromatin modulation. AR signalling is anti-proliferative and it was therefore postulated that decreased AR-mediated transcription following loss of BRCA2 may contribute to tumourigenesis in BRCA2-linked cancer [134]. On the contrary, the nuclear protein EMSY binds to exon 3 of BRCA2 and silences the transcriptional activation function of BRCA2. EMSY also associates with two additional chromatin remodelling proteins, HP1β and BS69 and localises to sites of DNA repair, potentially implicating BRCA2 in the modulation of access to chromatin during repair. EMSY is overexpressed in a number of sporadic breast and ovarian cancer cases and it has been postulated that increased EMSY expression may emulate BRCA2 mutant familial cancers but this requires further validation [135].

As mentioned earlier another BRCA2 interacting protein, PALB2, is an important mediator of BRCA2 function within HR mediated DSB repair. PALB2 was first identified as a nuclear interactor of BRCA2 following mass spectrometric analysis of protein bands immunoprecipitated in HeLa lysates using a BRCA2 antibody [136]. PALB2 is functionally similar to BRCA2 in several ways. Biallelic mutations in both BRCA2/FANCD1 and PALB2/FANCN have been identified as the cause of Fanconi Anaemia complementation groups D1 and FA-N, respectively. There are now 13 different subtypes of FA attributed to mutations in 12 unique genes but the BRCA2 and PALB2 related FA groups differ from the other identified subgroups, exhibiting a more severe phenotype and increased incidence of solid tumours such as Wilms tumours and medulloblastomas at an early age, suggesting a possible common functionality [137]. Indeed, PALB2 colocalises with BRCA2 in nuclear DNA repair foci and depletion of PALB2 leads to decreased Rad51 localisation to foci, abrogated HR activity and increased sensitivity to the DNA crosslinkers such as MMC [136]. Additionally, 3 of 8 mutations within the PALB2-binding N-terminal region of BRCA2 were shown to disrupt the BRCA2-PALB2 interaction and only these 3 mutations exhibited defective HR when introduced into BRCA2 deficient V-C8 cells [138]. As discussed earlier, PALB2 and BRCA2 recruit BRCA1 to sites of DSBs and promote Rad51 loading and HR. However, the BRCA2/PALB2 complex has also been shown to interact with DNA polymerase η at DNA DBSs induced by collapsed replication

forks and this complex is required for completing efficient HR via initiation of DNA synthesis following Rad51-dependent formation of a D-loop structure [139].

Additionally, mutations within PALB2 itself have now been identified in 0.4 - 4.8% of familial breast cancer patients depending on the population examined [140, 141]. Initial studies estimated that pathogenic PALB2 mutations increase breast cancer risk by ~2.3 fold [142, 143]. However, subsequent analysis of PALB2 mutation carriers estimate that the relative breast cancer risk is approximately 17.6 for 20 to 39 year olds and 8.7 for mutation carriers between 40 and 79 years. The same study found PALB2 mutation carriers with no family history had a 33% risk of breast cancer by 70 years of age whereas two or more cases of early onset breast cancer amongst first degree relatives increased this risk to 58%, comparable to the risk associated with BRCA2 mutation [144]. Therefore, PALB2 is now considered a *bona-fide* breast cancer susceptibility gene.

18. Clinical and therapeutic implications of BRCA1/2 dysfunction

As this chapter reveals, BRCA1 and BRCA2 are involved in the regulation of various DNA damage response and DNA repair pathways at the cellular level, but what are the clinical implications of this? The majority of BRCA1 mutant tumours are of triple negative breast cancer subtype (75%), present at young age (less than 50 years) and typically have a poor prognosis due to lack of targetable receptors. BRCA1 dysfunction has also been noted in over 30% of sporadic breast and ovarian cancers marked by low expression of BRCA1. On the contrary, BRCA2 mutant tumours are normally ERα positive. Although the DNA repair defects in BRCA1/2 mutation or dysfunction increase genomic instability and are associated with breast and ovarian cancer susceptibility, the same repair defect may also be exploited in the treatment of BRCA1/2-related cancers. Breast and ovarian cancer patients harbouring BRCA1/2 mutations are highly sensitive to treatment with platinum compounds, such as cisplatin and carboplatin, as well as alkylating agents as these agents cause ICLs, which are normally repaired by BRCA-dependent HR. On the other hand, BRCA1 mutant tumours are relatively resistant to treatment with taxanes and investigations in mouse models have suggested this may be due to an up-regulation of the drug efflux transporter P-gP, although this is yet to be confirmed in human BRCA1-mutant tumours [145-147]. Based on this it is clear that knowledge of the BRCA status of a tumour may be used to inform treatment regimes.

While platinum agents are relatively effective in treating BRCA-related cancer cells, they also cause a high level of toxicity in normal cells, resulting in severe side effects and intolerance in many patients. Thus the identification of PARP inhibitors as a potential treatment specifically targeting BRCA deficient cells was welcomed [148-150]. Poly(ADP-ribose) polymerase (PARP) is an enzyme responsible for catalysing the transfer of ADP-ribose to target proteins, including itself and many histone proteins, at single strand break sites within DNA. Poly-ADP-ribosylated (PARylated) proteins form docking sites for a number of BER proteins such as XRCC1, DNA ligase III and DNA polymerase β thereby mediating their recruitment to sites of damage [151]. Therefore, when PARP is inhibited,

SSBs are unrepaired which results in stalled replication forks during S-phase of the cell cycle. As mentioned above the rescue of stalled forks requires the HR machinery including BRCA1 and BRCA2. Because of this, inhibition of PARP in the absence of BRCA1/2 leads to accumulation of DNA DSBs during S-phase (due to collapse of stalled replication forks), resulting in synthetic lethality of these cells. It was therefore initially thought the effectiveness of PARPi's in BRCA-associated cancer was due to the synthetic lethality caused by the lack of HR to repair excessive damage caused by loss of PARP. Accordingly, PARP inhibitors have been well tolerated because normal cells with at least one functional allele of BRCA1/2 can overcome the effects of PARP inhibition [152]. Other mechanisms of action for the effectiveness of PARP inhibitors have since been demonstrated. For example, PARP inhibition has been shown to increase mutagenic NHEJ in HR deficient cells via a DNA-PK-dependent pathway, with inhibition of various NHEJ factors able to abrogate sensitivity to PARPi in cells lacking BRCA1, BRCA2 or ATM. Based on this, it has been suggested that up-regulation of the NHEJ pathway may mediate cytotoxicity of PARPi in an HR-deficient setting [153]. Another model of sensitivity to PARP inhibition involves trapping PARP1 and PARP2 on the damaged DNA causing cytotoxic lesions that cannot be repaired in the absence of an efficient HR pathway. PARP-DNA lesions had a greater effect on cell viability than the accumulation of SSBs due to inactivation of PARP suggesting this may be the primary mechanism of cell death in HR-deficient cells following PARP inhibition [154].

Phase I and II clinical trials treating BRCA mutant patients with the PARPi olaparib have in general been successful with one study showing clinical benefit in 12 out of 19 patients with BRCA-related breast, ovarian or prostate cancer [155, 156]. However, the progression of PARP inhibitors into the clinic suffered a setback in 2011 when Phase III trials investigating the use of iniparib in TNBC failed to prolong the survival of study participants [157]. Although numerous studies have since confirmed that iniparib did not actually inhibit PARP activity and had a different mechanism of action from the other PARP inhibitors currently in trials, the failure of iniparib did hamper the advancement of PARP inhibitors [158, 159]. Nevertheless, Phase III clinical trials are now underway to determine whether PARP inhibitors should be approved in a combination regime with platinum agents or even as single agents for the treatment of BRCA deficient breast and ovarian cancer patients. There are also trials underway in other cancer types with mutations or dysfunction of DNA repair genes. Thus it is hoped that PARP inhibitors will not only be an effective treatment for BRCA-linked breast and ovarian cancers but also for other cancer types with defective HR pathways [160].

19. Conclusion

In conclusion, BRCA1 and BRCA2 both have essential roles in numerous DNA repair pathways and the importance of efficient DNA repair mechanisms is illustrated by the dysfunctional repair observed when BRCA1 or BRCA2 are mutated leading to genomic instability and thus susceptibility to breast and ovarian cancer. While BRCA1 is a multifunctional protein mediating HR, NHEJ, SSA, ICL repair and cell cycle regulation via a variety of mechanisms including

transcriptional regulation, ubiquitination and mRNA splicing, the role of BRCA2 is more straightforward, facilitating Rad51 loading to ssDNA to promote HR as well as protecting stalled replication forks from degradation. Different binding partners of both proteins can modulate their function in repair pathways and therefore the identification of novel interactors of BRCA1 and BRCA2 is likely to shed further light on their mechanism of action. Additionally, the identification of these interactors may identify novel therapeutic targets for the treatment of BRCA-associated breast and ovarian cancers. Furthermore, the regulation of BRCA1 in response to DNA damage is becoming increasingly complex as more signalling pathways such as SUMOylation and PARylation have been shown to mediate recruitment of BRCA1 to DSBs with the intricate control of BRCA1 potentially reflecting it's prominent and varied role in DNA repair. Recent studies have also demonstrated the significance of specific regions of BRCA1 and BRCA2 in mediating different repair functions and therefore it is likely that not all cancer-associated mutations within these genes affect repair in the same way and further investigation of the role of different mutations may be useful, particularly in the case of BRCA2 where PARPi resistant clones have arisen still harbouring BRCA2 mutations albeit different from the original BRCA2 mutation. However, in general PARP inhibitors so far appear promising for the treatment of some BRCA1/2 mutant tumours and provide an example of how DNA repair defects, normally harmful to the cell, may actually be utilised for treatment benefit. Overall, BRCA1/2 mutations lead to highly dysfunctional DNA repair pathways, the catastrophic effects of which are revealed by phenotypic investigations demonstrating accumulation of genetic mutations and chromosomal instability, ultimately predisposing to cancer.

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