Homologous DNA repair: safeguarding genome territories from knives

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DNA damage agents constantly target the genome integrity causing lethal damages. Homologous DNA repair as universal error-free repair pathway constitutively acts to remove double strand breaks. Tumour suppressor genes are the major candidates and mediators of this pathway. In this context, we review the emerging role of BRCA1/PALB2/BRCA2/RAD51 complex and show how BRCA1 interact with different protein partners to be the first-line mediator of homologous DNA repair pathway at the site of DNA damage. A defect anywhere in this BRCA1/PALB2/BRCA2/RAD51 assembly halts formation and stabilization of nuclear foci of BRCA2 at DNA damage sites compromising HDR repair progression.

Keywords: Breast cancer susceptibity gene 1, hereditary breast cancer, phosphorylation, replication protein A, strand invasion.

GENOTOXIC agents constantly threaten genome integrity throughout the cell's life span and cause damages[1–4]. If unrepaired, these damages disrupt genetic stability leading to apoptosis or carcinogenesis[1,5–7]. Thus to cope with this damage, a complex network of processes called DNA damage response (DDR) comes into action to ensure a collaborative coordination between cell cycle fate and DNA repair pathways[7–10]. Increasing evidences confirm that hereditary breast cancer (HBC) is a result of impaired control over genome stability[11,12]. The genes, known to be linked to familial breast cancer, code for DNA repair molecules that participate in DDR and have been proposed as candidates of tumour suppression[6,13,14]. Thus, identification of these genes is important for understanding damage repair pathways[15].

In the present review, we summarize the emerging role of BRCA1/PALB2/BRCA2/RAD51 complex in homologue-directed recombination (HDR) repair pathway which is critical for the maintenance of genome stability[14,16]. We review how BRCA1 interacts with different protein partners to be the first-line mediator of error-free HDR pathway and to provide a base for positioning and recruitment of other mediators of BRCA1/PALB2/BRCA2/RAD51 complex to the site of DNA damage[13,14,17,18].

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Double strand breaks and choice of HDR repair

The most deleterious initiators and mediators of cancer, among many DNA lesions, are double strand breaks (DSBs) that halt cell cycle progression[1,4,6,8,19,20]. Clearly, an error-free attempt to address the challenge of genome integrity maintenance is made principally through two evolutionarily conserved and distinct DNA repair pathways: non-homologous-direct end joining and homologue-directed recombination (HDR) repair pathways[6,9,15,19,21,22]. DSBs are specifically repaired by HDR repair pathway and it is estimated that more than 16 genes are involved in this repair[15,23].

The importance of repair pathways lies in the fact that failure to repair damaged DNA accelerate the likelihood of tumour genesis. Thus, to find out the origin of many cancers, understanding DSB repair is important. Further, the repair of DSBs modulates the therapeutic response to radiation and to a variety of chemotherapeutic agents, many of which induce DSBs due to collapse of replication fork or as an intermediate of repair.

DNA damage and MRN complex-mediated signalling

DSBs are initially detected by chromatin associated fractions of MRN (**M**RE11/**R**AD50 (DNA recombinase)/ **N**BS1) complex[5,24,25]. This primary sensor sends signals and centrally governs the activation of ATM (Ataxia-Telangiectasia mutated) which subsequently phosophorylates a large cassette of downstream mediators including PAR (Poly-ADP Ribose)[24,25], RAD17, MDC1, Ctlp, ChK1 (Checkpoint Kinase 1) ChK2 (Checkpoint Kinase 2), H2AX, BRCA1 (Breast Cancer susceptibity gene 1), CDK (Cyclin-Dependant Kinases), ATR (ATM-Rad3 related) and RPA (Replication Protein A), TOPB1 (DNA topoisomerase BPI) (Figure 1). Subsequent retention and accumulation of MRN complex at damaged site is considered critical for HDR repair and is dependent on H2AX bound MDC1, ctlp, PAR and RAD17. Moreover, several evidences show that interaction with clamp loader RAD17 not only facilitates retention of MRN complex but also plays an important role in activation of ATR and CHK1 that along with CHK2 plays a role in cell cycle

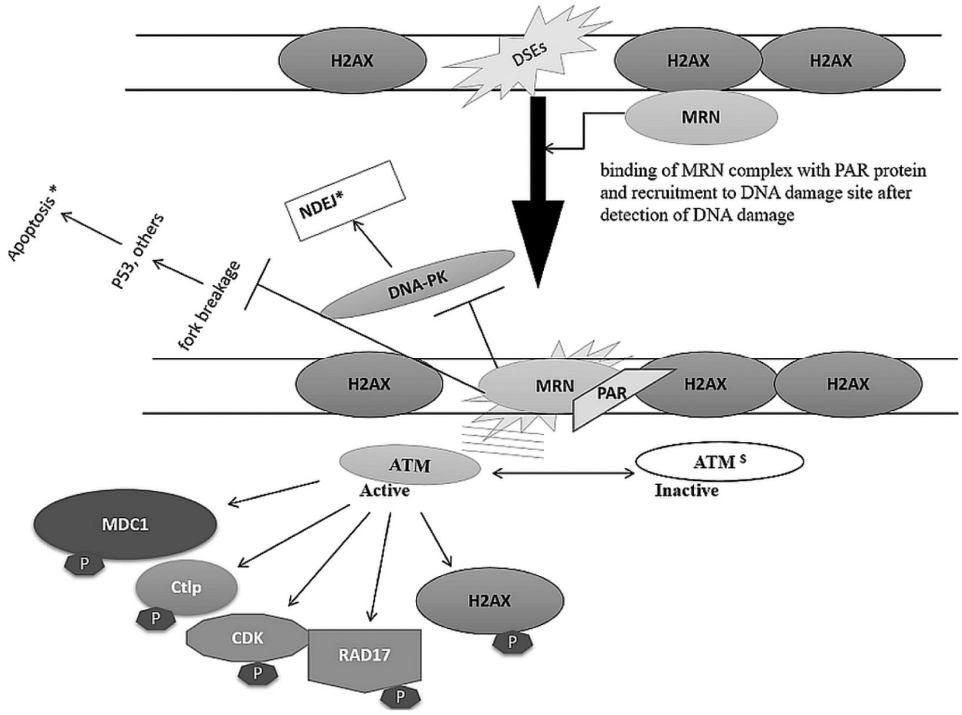

Figure 1. Detection of DNA damage and downstream signalling. *MRN complex suppresses p53 mediated apoptosis and NDEJ repair pathway $MRN complex mediated damage signalling results in activation of ATM which in turn amplifies phosophorylation cascade of other repair proteins.

arrest in S and G2 phases[25]. In addition, ATR-CHK1 structure promote phosphorylation of RPA proteins independent of RAD17 for strand recovery when damaged DNA is resected[26].

Association of BRCA1 with MRN complex and resection of damaged DNA ends

Following signal transmission in S/G2 phase, ctlp dissociates from BRCA1 and associates with MRN complex where it exclusively facilitates DNA end-resection and retention of MRN complex at DNA damage site[26–30]. However, BRCA1 (N-terminal, 341–748 amino acids) directly associates with MRN complex via RAD50 component. Earlier, it was only reported that BRCA1–MRN complex remain intact throughout HDR repair and very soon it was found that this process is partly dependent on ATM/ATR mediated hyper-phosphorylation of BRCA1 and CHK2 activation[26,28]. To initiate HDR repair, damaged double strand DNA ends are resected by MRN complex to expose ssDNA ends[5,22].

Strand recovery and invasion of ssDNA overhangs

To be the ultimate substrates of nucleases, ssDNA overhangs are promptly recovered by RPA proteins which are phosphorylated by ATR-CHK1 structure and RAD17 together[31]. Subsequently, RAD51-mediated strand invasion occurs, which is centrally required for HDR repair. RAD51 is a nucleoprotein that possesses DNA-dependent ATPase and recombinase activities for nuclear foci formation[16]. CDK-activated BRCA2 (breast cancer susceptibility gene 2), as recombination mediator, loads RAD51 at ssDNA overhangs. Once recruited at RPA recovered resected DNA sites, RAD51 catalyses strand invasion in which ssDNA overhangs invade homologous duplex DNA forming a displacement loop called D-loop (Figure 2).

Surprisingly, to be the double-edged sword, RPA poses its inhibitory effect on RAD51-mediated strand invasion. Therefore, this interplay between RAD51 and RPA is critically supervised by PALB2 (Partner and Localizer of BRCA2) which not only suppresses inhibitory effect of RPA, but also promotes RAD51 recruitment to resected DNA site stimulating filament formation[1]. On the other hand, PALB2 has a self-associating property for PALB2-PALB2 interaction which halts HDR repair by impeding RAD51 recruitment for foci formation[32] (Figure 2). This self-association is totally abolished when BRCA1 interacts with PALB2 competitively thus indirectly facilitating RAD51 function in HDR repair. In short, these two ultimate steps of HDR-mediated DNA repair necessitate interactions among certain mediator proteins.

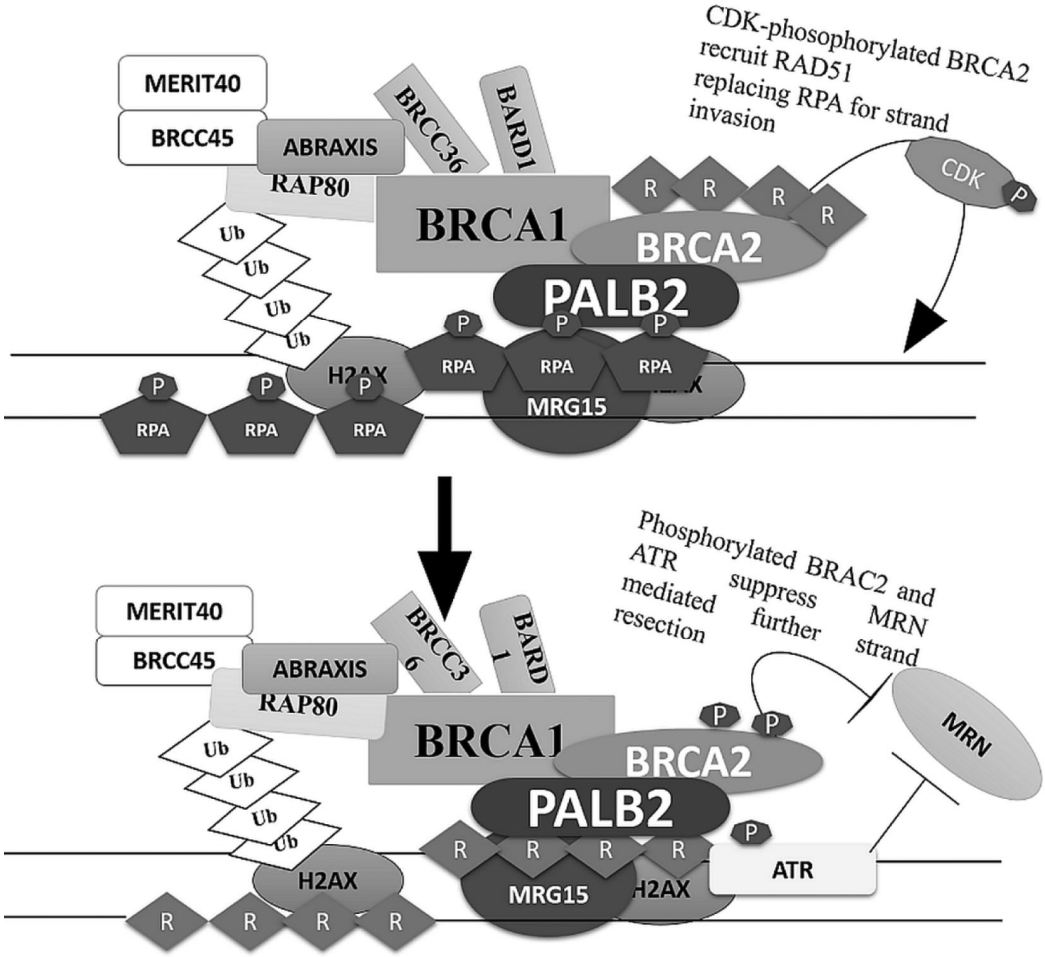

Figure 2. Following detection and end resection by MRN complex, BRCC complex is formed to promote damage repair by facilitating RAD51-mediated strand invasion and inhibiting further resection of damaged site. In this complex, BRCA1 is functionally linked to BRCA2 by physical association with PALB2. This complex is stabilized when histone bound ABRAXIS-RAP80-MERIT40-BRCC45 associates with it and regulates its activity at DNA repair site.

Formation of BRCC complex for HDR

Strikingly, BRCA1 was also shown to associate with BARD1 (BRCA1 Associated RING Domain protein 1) forming a heterodimer that causes a dramatic alleviation in ubiquitin ligase activity of BRCA1. Critically, it is interesting to review that this interaction is partly dependant on RING domains but minimally involve 1–109 sequence of amino acids of BRCA1 and 26–119 residues of BARD1[12]. Very soon, it was revealed that BRCA1 also co-colonizes with BRCA2, RAD51, BRCC36 and BRCC45 proteins in an assembly called BRCC complex. The BRCA1-BARD1 heterodimer was confirmed to be a part of a larger BRCC complex of proteins[33]. Moreover, BRCC36, as a scaffold protein, attracts protein repository to the BRCC complex and controls BRAC1 E3 ligase activity by de-ubiquitination (Figure 2).

Importantly, in S-phase synchronized cells, BRCA1 does not simultaneously interact with MRN and BRCC complexes at the damaged DNA sites. Conversely, mutually exclusive BRCA1-MRN heterodimer, as sensor of DNA damage, signals to recruit downstream machinery of damage repair including the BRCC complex[33] at DNA damage sites whilst BRCC directly functions in HDR repair pathway[15,28,34]. In fact, it is still unclear what is exactly the BRCC complex needed for in HR, however it was found that its E3 ligase activity stimulates this BRCC complex to mobilize and stabilize at resected DNA sites. Thus, the BRCC complex promotes formation of nuclear foci of RAD51 hence facilitating the repair pathway[29] (Figure 2).

Stabilization of BRCC complex at damaged sites

Additionally, recruitment of Rap80 fuels BRCA1 mobilization to DSEs to promote error-free HDR repair. Interestingly, in BRCA1 independent Abraxas-Rap80 foci, Abraxas act as adaptor protein whilst for efficient repair activity, BRAC1 is wholly dependent on Rap80 (refs 35, 36).

Recently, it was confirmed that Rap80 tightly associates with abundantly occurring BRCC complex following DNA damage and stabilizes it at damaged site for error-free repair. Abraxas-Rap80–BRCC36 complex is in turn stabilized by BRCC45-MERIT40 heterodimer. Importantly, in this assembly, association of Abraxas with BRCC36 and BRCC45 occurs through distinct regions. Other investigations unveil the fact that following irradiations, whole assembly of BRCA1-BARD1-Abraxas-Rap80-BRCC45-BRCC36-MERIT40 complex was co-localized with the nuclear foci of BRCA1 (Figure 2)[35,37].

Collaborative interaction of BRCA1–BRCA2–PALB2–RAD51 complex

BRCA2 and BRCA1, the central mediators of HDR repair, were identified to be co-localized in BRCC complex. To link these tumour suppressors, PALB2 directly binds to BRCA1(1393-1475 a. a.) and functionally associates it with BRCA2 via its amino and carboxyl (6–90 a. a specifically Leu21 and 24) terminus respectively[13,15,17,38–40]. Thus, the functional partnership of BRCA1 and BRCA2 in the DDR further elaborates the genetic similarity of either protein (Figure 2)[17,34].

To form a stable nuclear foci, BRCA1 and BRCA2 are potentially reliant on cooperative inter-linkage of BRCA1/PALB2/BRCA2/RAD51 complex which occur constitutively to be a crucial determinant of DNA damage responsiveness[15,31]. From recent analysis of purified MRG15 containing protein complexes, it was revealed that MRG15 directly mediates interaction with whole BRCA complex (BRCA1/PALB2/BRCA2 /RAD51) required for HDR repair[41]. Importantly, BRCA1 is required to position PALB2 and another protein[42], H2AX bound MRG15 which determines the accessibility or affinity of PALB2 for chromatin (Figure 2)[43].

Conclusion

In short, as a large scaffolding protein, BRCA1 appears not only to mediate DNA damage repair but ensures effective regulation of the BRCA1/PALB2/BRCA2/RAD51 assembly of nuclear foci by controlling PALB2 as a molecular switch of HDR repair and BRCA2 as a caretaker of genome integrity[44]. In summary, a defect anywhere in this BRCA1/PALB2/BRCA2/RAD51 assembly halts formation and stabilization of nuclear foci of BRCA2 and ultimately RAD51 at DNA damage sites compromising HDR repair progression[15,45,46].

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