DNA Damage Repair for Cancer

Subjects: Oncology

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DNA damage repair BRCA1 synthetic lethality targeted anticancer therapy

1. Introduction

Cancer is a major burden of disease and one of the leading barriers to improve life expectancy worldwide [1]. Despite many efforts to maximize cancer prevention, diagnosis and treatment, the incidence and mortality rates have been steadily increasing [1][2].

As a highly heterogeneous disease, cancer displays unique genomic and epigenetic variations among patients. As such, the success of conventional chemotherapeutics has been limited by the heterogeneity of patients' response, development of resistance and severity of side effects [3][4]. Conversely, targeted therapies take advantage of specific alterations in cancer cells, having emerged as a hopeful strategy to overcome these limitations [5][6][7][8].

There is a large amount of evidence that dysfunctional DNA damage repair (DDR) processes are frequently observed in cancer and are associated with genomic instability [5][7][9]. In fact, although cells own an equipped machinery to repair DNA toxic lesions, it may fail, predisposing them to accumulate DNA damage. The survival and proliferation of unrepaired DNA defective cells lead to the accumulation of mutations and genomic instability, strongly contributing to cancer development [5][10]. Indeed, DDR-deficient cells are frequently associated with hypersensitivity to DNA-damaging agents [11][12][13][14][15].

Breast cancer susceptibility gene 1 (BRCA1) is a tumour suppressor gene extensively involved in maintaining genomic integrity through multiple functions in DDR, transcriptional regulation, cell cycle checkpoint and protein ubiquitination [16][17][18]. BRCA1 is frequently dysfunctional in human breast, ovarian, pancreatic, among other cancers, contributing to the accumulation of genomic defects. Despite the increased risk conferred byBRCA1mutations to cancer onset, pre-clinical and clinical data have ascertained that BRCA1 impairment is commonly associated with chemosensitivity in cancer cells [13][16]. Other players involved in DDR can also be found

defective in cancer, including breast cancer susceptibility gene 2 (BRCA2),RAD51,RAD52, partner and localizer of BRCA2 (PALB2), ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and

This review aims to emphasize the potentiality of the DDR pathways, particularly of BRCA1 and interconnected molecules, in precision cancer therapy.

2. DNA Damage Repair Pathways

DDR is activated in response to different endogenous and exogenous stresses [5][19] (**Figure 1**). When aberrantly repaired, DNA damage might be associated with clinical outcomes such as neurodegeneration, infertility, and genomic instability, being a key contributing factor to neoplastic transformation and tumour development [8] Due to the complexity underneath detection and repair of DNA damage, cells evolved an intricate DDR network that, together with cell cycle regulation, promotes the maintenance of genomic stability and cellular viability [20]

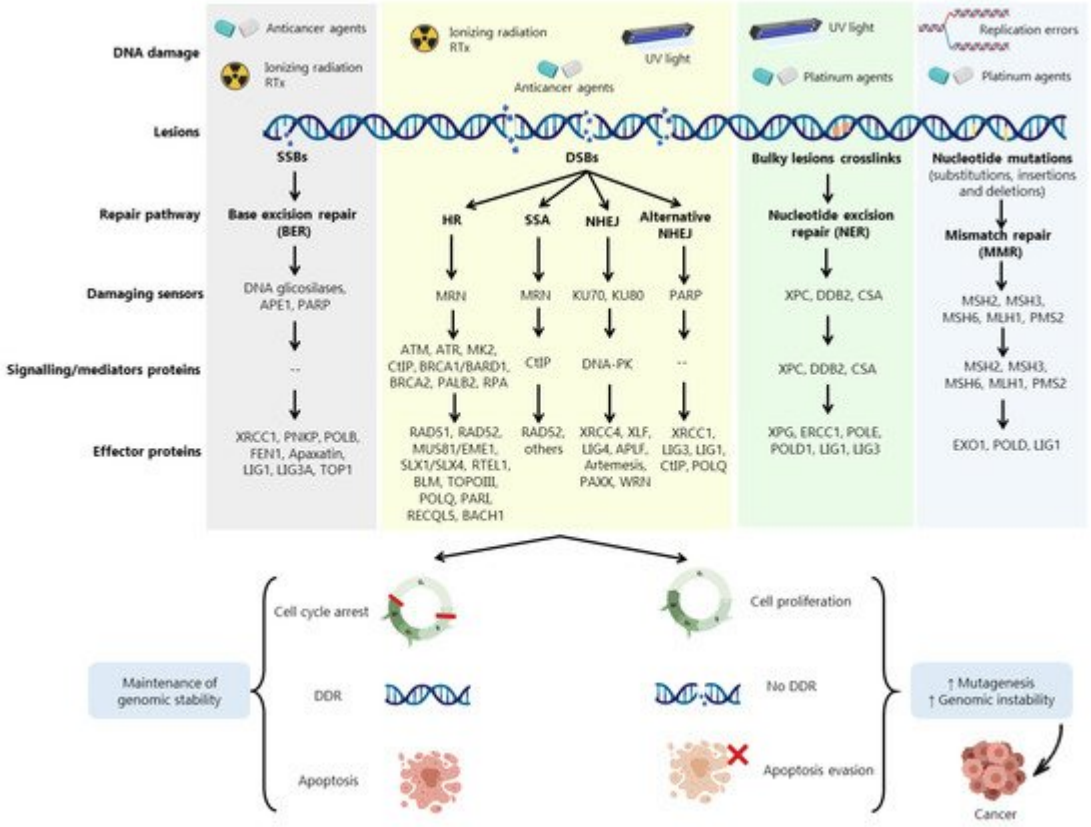

Figure 1. DNA damage agents and cellular repair pathways. DNA damage repair (DDR) pathways are activated in response to endogenous stresses (e.g., base depurination, deamination and reactive by-products of cellular metabolism) or exogenous exposure to different types of radiation or genotoxic agents. DDR comprises a network of proteins that are either DNA damage sensors, signalling mediators or effector proteins that execute DNA repair. The base excision repair (BER) pathway for single-strand breaks (SSBs), repairs minor DNA changes originated from oxidized or alkylated bases and small base adducts, with poly(ADP)-ribose polymerase (PARP) being the major player. The nucleotide base excision repair (NER) pathway deals with modified nucleotides that change the

double helix structure, such as those induced by ultraviolet (UV) light. The mismatch repair (MMR) pathway deals with DNA damage that disturb the DNA helical structure and replication errors as substitution, insertions and deletions. Four different DDR mechanisms are described for double-strand breaks (DSBs) repair: homologous recombination (HR), non-homologous end joining (NHEJ), alternative NHEJ and single-strand annealing (SSA) pathways. Loss or aberrations in DDR proteins allows cell cycle proliferation and evasion of apoptotic events, resulting in increased genomic instability and cancer development. Radiotherapy (Rtx); Apurinic/apyrimidinic endonuclease 1 (APE1); MRE11/RAD50/NSB1 (MRN); Xeroderma pigmentosum, complementation group C (XPC); DNA damage-binding protein 2 (DDB2); Cockayne syndrome group A (CSA); MutS homolog 2, 3 and 6 (MSH2, MSH3 and MSH6); MutL homolog 1 (MLH1); Ataxia-telangiectasia mutated (ATM); Ataxia telangiectasia and Rad3-related (ATR); Mitogen-activated protein kinase-2 (MK2); *C*-terminal-binding interacting protein (CtIP); Breast cancer susceptibility 1 and 2 (BRCA1 and BRCA2); BRCA1-associated RING domain (BARD1); Partner and localizer of BRCA2 (PALB2); Replication protein A (RPA); DNA-dependent protein kinase (DNA-PK); X-Ray repair cross complementing 1 and 4 (XRCC1 and XRCC4); Polynucleotide kinase 3'-phosphatase (PNKP); DNA polymerase beta (POLB); Flap structure-specific endonuclease 1 (FEN1); DNA ligase 1, 3A and 4 (LIG1, LIG3A and LIG4); DNA topoisomerase 1 and 3 (TOP1 and TOPOIII); Essential meiotic structure-specific endonuclease 1 (EME1); Regulator of telomere elongation helicase 1 (RTEL1); Bloom syndrome protein (BLM); DNA polymerase theta (POLQ); PCNA-associated recombination inhibitor protein (PARI); RecQ like helicase 5 (RECQL5); BRCA1-associated *C*-terminal helicase (BACH1); XRCC4-like factor (XLF); Aprataxin and PNKP like factor (APLF); Werner syndrome helicase (WRN); Xeroderma pigmentosum group G (XPG); Excision repair cross-complementation group 1 (ERCC1); DNA polymerase epsilon (POLE); DNA polymerase Delta 1 (POLD1); Exonuclease 1 (EXO1); DNA polymerase delta 1 (POLD).

Cells harbouring defects on a particular DDR pathway may compensate by becoming reliant on another repair pathway. In fact, despite showing partially overlapping functions, DDR pathways still exhibit different functionalities depending on multiple damage sensors, signalling factors (activators of cell cycle checkpoints) and effector DDR proteins Conversely, misrepaired DSBs may generate mutations or chromosome rearrangements that may lead to a malignant condition [21]. Three main mechanisms are required for DSBs repair: (i) damage detection, (ii) ability to control cell cycle and transcriptional programs, and (iii) mechanisms for catalysing the repair of the lesion [21].

Accumulated data have shown the involvement of DDR proteins in different stages of cancer development. Early stages of tumorigenesis have been associated with activation of DDR proteins due to the induction of replication stress and DNA damage, acting as a barrier to the proliferation of aberrant cells [20]. However, most of pre-malignant cells are able to escape this barrier by loss or aberrations in specific proteins associated with DDR and cell cycle regulation, such as BRCA1, BRCA2, ATM, RAD51, Fanconi anemia group A protein (FANCA) and p53, allowing these cells to evolve to malignant carcinomas (**Figure 1**). In more advanced stages, when the tumour is already established, (re)activation and overexpression of DDR factors support cells to evade the lethal effect of the therapeutic agents, eliciting resistance [20].

3. Role of BRCA1 in DSBs Repair

In 1990,BRCA1(located on chromosome 17) was identified as a classical tumour suppressor gene (TSG) due to the loss of a wild-type (wt) allele during tumorigenesis, being the first TSG associated with hereditary and sporadic cases of basal-like breast cancer [22][23].

Despite being a multifunctional protein, the BRCA1 tumour suppressive function is mainly ensured by its ability to maintain genomic integrity through regulation of diverse cellular processes, including DDR, cell cycle checkpoint, apoptosis, chromosome instability, among others [16][17]. Upon DNA damage, the opposite roles played by p53-binding protein 1 (53BP1) and BRCA1 seem to support cells in the switch between NHEJ and HR [24]. In fact, DNA repair pathways compete to select which mechanism should be employed. This choice is based on several factors, including cell cycle phase.

TheBRCA1gene has 24 exons, two of them untranslated, and encodes a large 1863-amino acid phosphoprotein that harbours multiple functional domains, including the highly conservedN-terminal zinc-finger Over 60% of theBRCA1gene is composed by a centrally located exon 11, which encodes two NLS and binding sites for several proteins [25][26] (**Figure 2**). This is one of the largest human exons (encoding 1142 amino acids) that partially contributes to BRCA1 nuclear localization and activity on cell cycle regulation and DNA repair, being highly required for a functional HR [27][28]. Together with exons 12 and 13, exon 11 encodes a coiled-coil domain that mediates interactions with PALB2 and a serine cluster domain (SCD) that is phosphorylated by ATM and ATR [25][26].

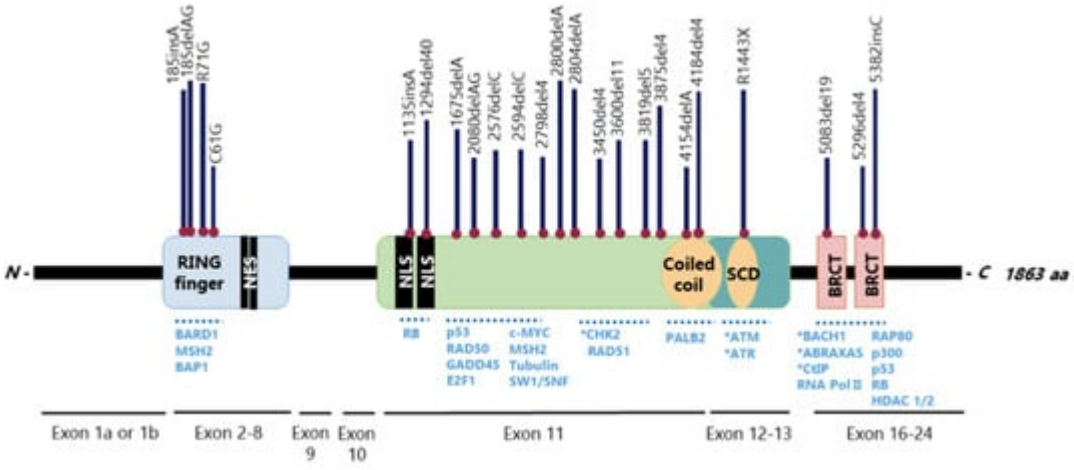

Figure 2. Structural organization of BRCA1 with respective interacting proteins and most prevalent mutations. Full length BRCA1 contains two conserved domains at its termini: *N*-terminus containing a really interesting new gene (RING) domain (exons 2–8) and tandem BRCA1 *C*-terminus (BRCT) repeats (exons 16–24). The BRCA1 RING domain interacts with BRCA1-associated RING domain (BARD1), mutS homolog 2 (MSH2) and the ubiquitin hydrolase BRCA1-associated protein 1 (BAP1). The BRCT domains form a phospho-binding module, recognizing a phospho-SPxF motif that allow BRCA1 A complex subunit (ABRAXAS), BRCA1-associated *C*-terminal helicase (BACH1) and *C*-terminal-binding interacting protein (CtIP) to physically interact with BRCA1. A number of other proteins may also bind to BRCA1 *C*-terminus, as p53, p300, receptor-associated protein 80 (RAP80), retinoblastoma (Rb), RNA polymerase II and histone deacetylases (HDAC1/2). Several proteins bind to exon 11, as

Rb, E2F transcription factor 1 (E2F1), growth arrest and DNA damage-inducible 45 (GADD45), p53, checkpoint kinase 2 (Chk2), RAD51, SWItch/Sucrose non-fermentable (SWI/SNF), among others. The interaction of BRCA1 with partner and localizer of BRCA2 (PALB2) and BRCA2 is mediated by the coiled-coil domain. The serine cluster domain (SCD) contains multiple ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) phosphorylation sites. BRCA1 contains two nuclear localization signals (NLS) and two nuclear export signals (NES). In the upper representation, the location and frequency of reported cases with BRCA1 pathogenic mutations are shown, including the most frequent C61G, 185delAG and 5382insC. (*) Phosphorylated proteins.

The BRCA1N-terminal RING domain dimerizes with BRCA1-associated RING domain (BARD1), forming stable heterodimers that enhance E3 ubiquitin-ligase activity and DDR [29][30][31][32][33][34] In addition, BRCA1 can undergo proteolytic degradation upon disruption of the BRCA1-BARD1 heterodimer [35][36] (**Figure 3**). The specific function of the BRCA1-BARD1 heterodimer, its dissociation and interaction with other proteins might be regulated by post-translational modifications as phosphorylation and ubiquitination [30] (**Figure 3**). Thus, the BRCA1-BARD1 heterodimer plays a crucial role in tumour suppression, interacting with proteins involved in cell cycle, DNA repair, chromosome stability, chromatin modulation, replication fork stability, transcription, among others [37][38]

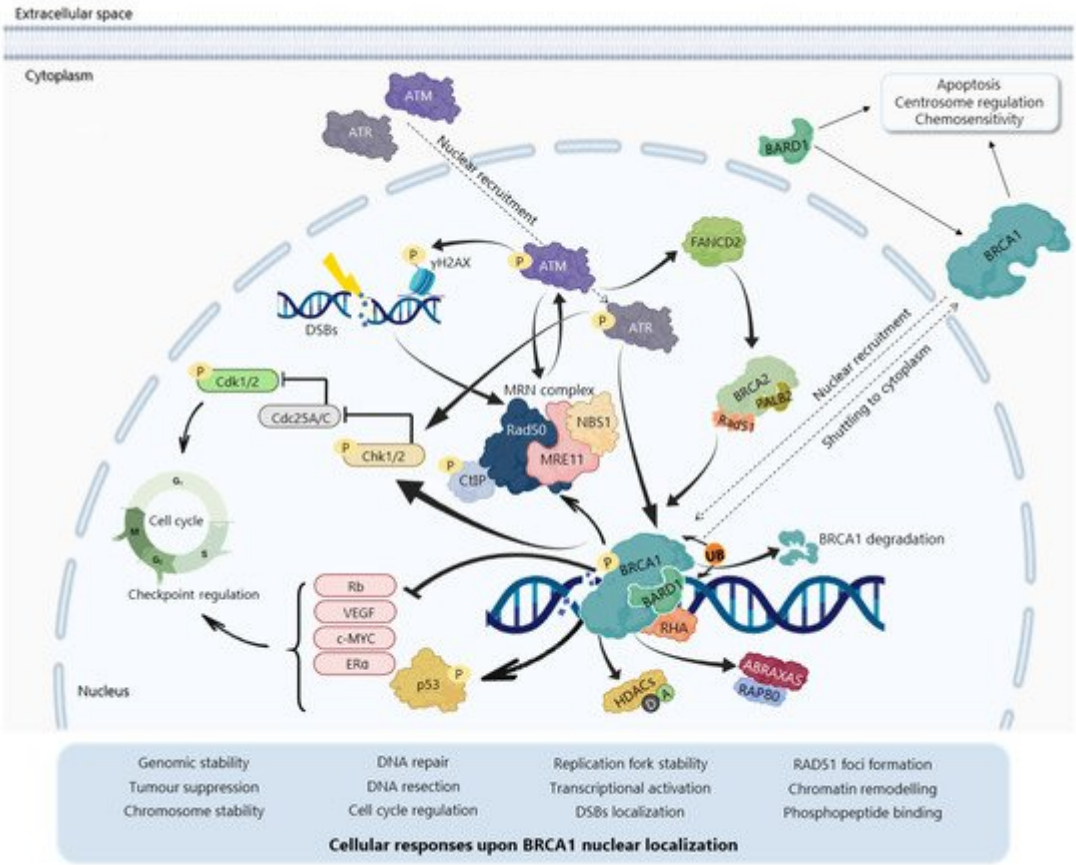

Figure 3. BRCA1 localization and molecular functions upon DNA damage. DNA damage activates the MRN complex (consisting of MRE11, meiotic recombination 11 homolog A; NBS1, Nijmegen breakage syndrome 1; and RAD50), which phosphorylates and recruits ataxia-telangiectasia mutated (ATM). Ataxia telangiectasia and Rad3-related (ATR) is also recruited to damaged sites during replication stress. DNA damage can also directly activate

ATM/ATR, which can phosphorylate/activate several proteins as checkpoint kinases 1 and 2 (Chk1/2), histone H2AX (γH2AX) and BRCA1. Phosphorylated BRCA1 concentrates in focal areas of DNA damage. At nuclear foci, the BRCA1/BRCA1-associated RING domain (BARD1) heterodimer participates in several molecular mechanisms as DNA repair, cell cycle regulation and transcriptional activation, in association with protein binding partners as MRN complex proteins, *C*-terminal-binding interacting protein (CtIP), BRCA2/Partner and localizer of BRCA2 (PALB2), RAD51, BRCA1-associated *C*-terminal helicase (BACH1), BRCA1 A complex subunit (ABRAXAS), receptor-associated protein 80 (RAP80), histone deacetylases (HDACs), RNA helicase A (RHA), among others. The BRCA1-BARD1 heterodimer ubiquitinates several proteins, including BRCA1 and BARD1 although with no degradation by auto-ubiquitination, resulting in increased BRCA1 E3 ubiquitin ligase activity. BARD1 phosphorylation abolishes the heterodimer E3 ligase activity. BRCA1-BARD1 hetero-dimerization results in BRCA1 nuclear translocation and retention, while disruption of this complex leads to BRCA1 shuttling to cytoplasm, where BRCA1 influences apoptosis, chemosensitivity and centrosome regulation. Phosphorylation (P); Ubiquitination (Ub); Acetylation (A); Deacetylation (D); Cell division control protein 25 A/C (Cdc25A/C); Estrogen receptor (ER); Vascular endothelial growth factor (VEGF

The BRCA1 BRCT domain functions as phosphopeptide recognition modules that enables the BRCA1 binding to phosphorylated partners as BRCA1 Upon DNA damage, BRCA1 phosphorylation by ATM and ATR leads to post-translational modifications of BRCA1-binding proteins and to the subsequent activation of several associated proteins, including checkpoint kinases 1/2 (Chk1/2) and p53, which regulate cell cycle checkpoints [39] BRCA1 also regulates chromatin structure through acetylation and deacetylation of histone proteins by interaction with multiple histone deacetylases (HDAC1 and HDAC2) Thus, due to its rich functional domains, BRCA1 interacts with several transcriptional factors and numerous proteins encoded by tumour suppressors, oncogenes, DNA repair genes, cell cycle regulators, ubiquitin hydrolases and ligases, signalling transducers and chromatin modifying proteins (**Figure 2**), supporting the complex network involving BRCA1

In DSBs repair by HR, BRCA1 participates as a central component of macromolecular protein complexes, each one composed of unique protein binding partners, as phosphorylated ABRAXAS, BACH1 and CtIP [8][24][40][41] (**Figure 4**). These complexes, called BRCA1 A, B, C and D helped to recognize the multiple functions of BRCA1 not only in DDR, but also in the transcriptional regulation of genes involved in other cellular processes [40][42]. (**Figure 4**), with distinct and sometimes overlapping roles for maintenance of genomic stability [40].

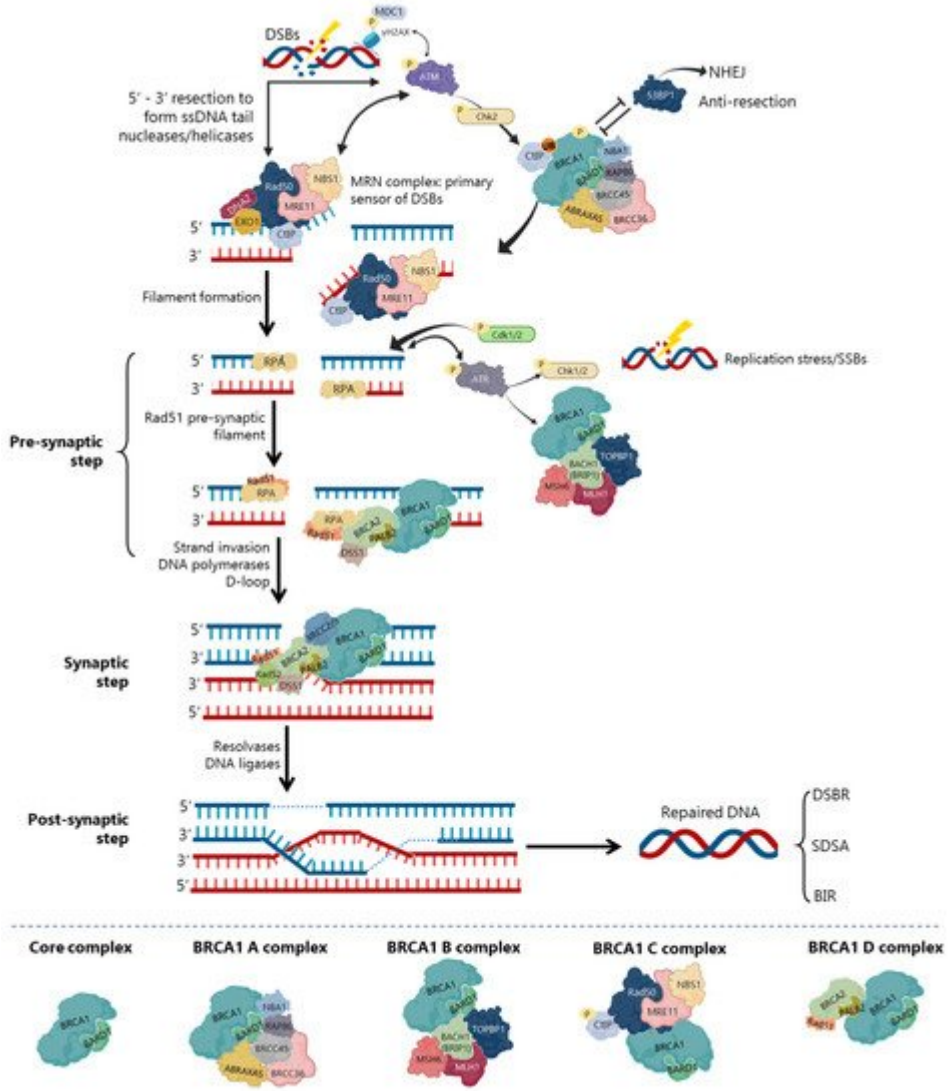

Figure 4. Double-strand breaks (DSBs) repair by homologous recombination (HR). DNA end resection occurs in the primary steps, a process that leads to nucleolytic degradation of DSBs 5′-ending strands to generate a 3′-end single stranded DNA (ssDNA). MRE11/RAD50/NSB1 (MRN) complex is the first to be recruited to DSBs sites, competing with Ku70/80 from non-homologous end joining (NHEJ) pathway. MRN complex phosphorylates ataxia-telangiectasia mutated (ATM) and recruits it to DSBs sites, leading to its auto-phosphorylation and phosphorylation of MRN complex. ATM phosphorylates checkpoint kinase 2 (Chk2) and the histone H2AX (γH2AX), recruiting the mediator of DNA damage checkpoint 1 (MDC1), which enhances ATM phosphorylation and promotes MRN and BRCA1 A complexes recruitment to damage sites. p53-binding protein 1 (53BP1) antagonizes BRCA1 in DSBs resection. Together with *C*-terminal-binding interacting protein (CtIP) (phosphorylated by MRN, ATM, cyclin-dependent kinase 2 (CDK2) and ubiquitinated by BRCA1), the MRN complex initiates DSBs resection to expose ssDNA with 3′ ends that undergo strand invasion into a homologous duplex (red), promoting HR. Ataxia telangiectasia and Rad3-related (ATR) is the primary sensor of replication stress (stalling of replication forks or formation of SSBs), which phosphorylation activates Chk1 and Chk2 and it is recruited to ssDNA sites. ssDNA tails are coated by replication protein A (RPA) followed by the formation of a D-loop structure through RAD51 load on the ssDNA. This is mediated by several proteins as BRCA2/Partner and localizer of BRCA2 (PALB2)/DSS1, BRCA1/BRCA1-associated RING domain (BARD1) and RAD51 cofactors, which allows RAD51 microfilaments

formation and subsequent 3′-end strand invasion into the homologous DNA template and D-loop formation. The strand displaced by synthesis (red) anneals to the other resected end of the DSB (blue). To complete the HR process, the newly synthesized strand can dissociate to anneal to the other end. Different outcomes are possible, namely formation of Holliday junctions through DSBs repair (DSBR), synthesis-dependent strand annealing (SDSA) and break-induced DNA replication (BIR). Ubiquitination (UB); Phosphorylation (P); Exonuclease 1 (Exo1); DNA helicase/endonuclease 2 (DNA2); DNA topoisomerase 2-binding protein 1 (TOPBP1); BRCA1 A complex subunit (ABRAXAS); BRCA1-associated *C*-terminal helicase (BACH1); MutL homolog 1 (MLH1); MutS homolog 6 (MSH6); BRCA1/BRCA2-Containing Complex Subunit 36 (BRCC36) and BRCC45; Receptor-associated protein 80 (RAP80); X-Ray repair cross complementing 2 (XRCC2) and XRCC3.

The repair of replication forks or DSBs is initiated by DNA strand resection to generate a 3′-tailed single stranded DNA (ssDNA) In HR, the MRN complex forms a physical bridge, spanning the DSBs end to recruit/retain ATM at DSBs sites. ATM phosphorylates histone H2AX (γH2AX), which recruits the mediator of DNA damage checkpoint 1 (MDC1), enhancing ATM phosphorylation and promoting recruitment of MRN and BRCA1 In the initial phase of HR, CtIP physically interacts with MRN complex (**Figure 4**), facilitating 5′ end-resection of DSBs.

Precluding the formation of secondary structures at the ssDNA, RPA occupies the 3′-tailed ssDNA derived from DNA end resection, protecting DNA tail from nucleolytic attack and removing the secondary structure [43]. To exchange RPA and facilitate RAD51 loading onto DNA, some mediators, like BRCA2 and PALB2, participate in RAD51-mediated pre-synaptic filament formation, a key intermediate that catalyses homologous pairing and initiates DNA strand invasion [44][26][37][43]. The BRCA1-BARD1 heterodimer helps RAD51-coated ssDNA to invade double stranded DNA (dsDNA) with homologous sequences, enhancing its ability to form a displacement loop structure (D-loop) [24][37]. Accordingly, BARD1 mutations or deletions at residues 758–1,064 of BRCA1 (harbouring the RAD51-interaction domain) abolish the BRCA1-BARD1 ability to promote D-loop and synaptic complex formation [26][37], compromising HR activity and RAD51 nuclear localization [30][45].

Finally, with the support of DNA polymerases, the second end of the damaged chromosome is captured, and anneals to the complementary strand of the intact homologous DNA template [44][24]. In the DSBs repair (DSBR), after priming DNA synthesis and sealing the break, the second end is captured, and a double Holliday junction intermediate is formed. After DNA synthesis and strands ligation, the two Holliday junctions can be resolved by the catalytic function of resolvases to generate crossover products, or dissolved to generate non-crossover products and complete the repair [44][46]. In the synthesis-dependent strand annealing (SDSA) model, the invading strand is displaced from a D-loop by helicase activity and annealed with the 3’ single-stranded tail to complete DNA synthesis and repair.

BRCA1Mutcarriers are at high risk for developing different types of cancer, including breast, ovarian, pancreatic, prostate, laryngeal and fallopian tube cancers [25][47][48]. Since its discovery, more than 1600 mutations have been identified inBRCA1[49], such as frameshift insertions/deletions, nonsense truncation mutations that lead to premature chain termination, and many single nucleotide polymorphisms in the coding or noncoding sequences. Also, a number of missense BRCA1 mutations present clinical relevance, being associated with increased risk of

both hereditary and sporadic cancers [47][50]. Heterozygous BRCA1Mutare commonly related to genetic deficiencies in other TSGs and DDR factors, such as phosphatase and tensin homolog (PTEN),ATM/ATR,CHEK2andTP53[16][42][47].

Some highly prevalent pathogenic BRCA1 mutations are more frequent in isolated groups (founder mutations), supporting the existence of distinct incidences among the world population. Although initially described with complete loss of BRCA1 expression, BRCA1Mut:185delAG alleles escape degradation, being translated from an alternative site downstream of the stop codon, which results in a RING-less protein [51]. Besides the loss of BRCA1-BARD1 interaction and subsequent E3 ligase activity, this alternative translation produces a stable and HR-proficient protein that retains the capability to interact with DNA and HR proteins [42][51]. Conversely, BRCA1Mut:185delAG presents a remarkable decrease in BARD1 binding ability [18].

Mutations at BRCT domains commonly present loss of protein expression associated with reduced transactivation activity, growth suppression [52] and aberrant cellular localization [53]. However, recent studies have shown that the truncated mRNA seems to encode a stable protein with potentially new cellular functions due to distinct protein-protein interactions [53]. homozygous BRCA1Mut:5382insC are associated with deficient HR activity and chemosensitivity [54]. Indeed, although BRCA1Mutat BRCT domain has been described as associated with chemosensitivity, the breast HCC1937 cancer cells displayed resistance to PARPi related to residual HR activity by retaining the integrity of RAD51 binding region [55].

Besides somatic mutations,BRCA1promoter hypermethylation and decreasedBRCA1expression, by epigenetic silencing or gene depletion, can also render a dysfunctional BRCA1 pathway in non-hereditary cancers [16][56]. These mechanisms are described to likely contribute to the “BRCAness genotype”, associated with similar biological and clinical phenotypes to that of tumours harbouring BRCA1Mut. However, whether BRCAness mechanisms confer the same functional deficiency is still unclear [56].

A strong connection between triple-negative breast cancer (TNBC) In fact, over 80% of BRCA1Mutbreast cancers are TNBC [57], which is an aggressive form of the disease. Although initially responsive to chemotherapy, most TNBC patients quickly relapse and acquire therapeutic resistance [58][59]. TNBC with pathogenic BRCA1Mutalso demonstrates to be particularly sensitive to platinum and PARPi agents, in both neoadjuvant and adjuvant settings [59].

Despite the lower prevalence, ovarian cancer is three-fold more deadly than breast cancer, with over 70% of patients having late-stage disease [60][61]. In fact, over 10–15% of ovarian cancers are related to germlineBRCA1andBRCA2mutations. Also, these tumours have a dysfunctional DDR pathway that initially promotes sensitivity of cancer cells to chemo- and radiotherapy, although the cell population ultimately ends up developing therapeutic resistance [8]. Indeed, most BRCA1Mut-related ovarian cancer patients experience relapse associated with platinum resistance [62].

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