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17

BRCA2 Mutations and Consequences for DNA Repair

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1. Introduction

The *BRCA2* gene was the second gene discovered to be associated with early-onset, familial breast cancer. The BRCA2 protein is expressed in breast, ovarian, prostate, and pancreatic tissues and is associated with cancer predisposition in all four, with breast cancer being the most predominant (Goggins, Schutte et al. 1996). BRCA2 is functionally defined as a tumor suppressor and is most critical in maintenance of genomic integrity and DNA repair fidelity. The importance of BRCA2 in maintaining genomic integrity is based on its function to specifically repair double-strand DNA breaks (DSBs) via the process of homologous recombination (HR). However, BRCA2 resolves genomic lesions in concert with a number of DNA repair proteins, the most significant being RAD51 (Sharan, Morimatsu et al. 1997),(Yuan, Lee et al. 1999). RAD51 is a recombinase that is highly conserved, having homologues in *E. coli* and yeast, as well as in mammals. BRCA2 modulates the activity of RAD51 during DNA repair, and they both are found in nuclear DNA damage-induced foci, which are complexes of DNA repair proteins bound to DNA during the process of repair (Roth, Porter et al. 1985; Roth and Wilson 1986; Derbyshire, Epstein et al. 1994; Jackson and Jeggo 1995; Takata, Sasaki et al. 1998; Johnson and Jasin 2000). The relationship between BRCA2 and RAD51 has been determined to be a fundamental interaction in the repair of DSBs.

The role of BRCA2 as a tumor suppressor has been established by its importance in maintaining genomic integrity. The inability of the cell to repair DSBs can potentially cause small-scale lesions in regions of the DNA that encode single genes and incite large-scale lesions, such as chromosomal anomalies. The consequence of such damage can disrupt the normal expression of gene products that are required to regulate cell growth and arrest and induce apoptosis, thereby establishing a cellular environment that can foster malignant transformation.

Cancer cells that express mutated BRCA2 have been shown to have elevated sensitivity to the anti-cancer therapeutics called PARP (Poly [ADP-ribose] polymerase) inhibitors. PARP inhibitors prevent the binding of PARP to sites of damaged DNA, which serves as a signal to initiate DNA repair (Schreiber, Dantzer et al. 2006); (Ratnam and Low 2007). The effectiveness of PARP inhibitors in BRCA2-mutated cells is based on the premise of synthetic lethality, which is when two pathway defects alone are innocuous, but combined become lethal (Ratnam and Low 2007). The unresolved DSBs of BRCA2-mutated cells combined with the inhibition of PARP activity are effective in promoting DNA damage-

induced apoptosis. This finding has established mutated BRCA2 as a potential target in improving present anti-cancer therapeutic regimens.
The information that follows will provide a comprehensive understanding of BRCA2, starting from its functions at the molecular level in maintaining genomic integrity, to describing how deregulation can lead to disease predisposition and development, and concluding with the development of PARP inhibitors that use the DNA repair defects of BRCA2-mutations to improve the sensitivity of anti-cancer treatments towards BRCA2-tumors.

2. The role of BRCA2 in DNA repair

The BRCA2 protein specifically repairs double-strand DNA breaks (DSBs) via the process of homologous recombination (HR), thereby establishing its importance in maintaining genomic integrity. The *BRCA2* gene is found on chromosome 13q12.3 and encodes a protein of 3,418 amino acids, resulting in a molecular weight of approximately 340 kDa. BRCA2 resolves genomic lesions in a complex with several additional DNA repair proteins, the most significant being RAD51 (Sharan, Morimatsu et al. 1997; Yuan, Lee et al. 1999). RAD51 is a highly conserved recombinase, having homologues in *E. coli* and yeast, as well as in mammals. BRCA2 modulates the activity of RAD51 during DNA repair and this relationship is determined to be a fundamental interaction in repair of DSBs.

2.1 The interaction between BRCA2 and the RAD51 recombinase

RAD51 catalyzes the strand exchange of DNA homologues to promote gene conversion and repair DSBs by HR (Ogawa, Yu et al. 1993) (Benson, Stasiak et al. 1994). HR is one of two pathways of repair of DSBs in mammals—the other being nonhomologous end-joining (NHEJ) (Derbyshire, Epstein et al. 1994), (Jackson and Jeggo 1995), (Roth, Porter et al. 1985), (Roth and Wilson 1986), (Takata, Sasaki et al. 1998) and (Johnson and Jasin 2000), (Figure 1). HR requires the damaged DNA molecule to use the undamaged homologue as a template in order to repair the DSB. NHEJ involves ligation of the DNA ends at the breakpoint junction regardless of whether the original genetic information is still present. As a result, HR confers greater accuracy in repair than NHEJ (Derbyshire, Epstein et al. 1994), (Jackson and Jeggo 1995), (Roth, Porter et al. 1985), (Roth and Wilson 1986), (Takata, Sasaki et al. 1998) and (Johnson and Jasin 2000). Studies performed in mice in which the *Rad51* gene was either mutated or completely knocked out have shown its importance in genomic stability and cell viability (Taki, Ohnishi et al. 1996) and (Sonoda, Sasaki et al. 1998). Nonfunctional RAD51 does not repair chromosome breaks and other DNA lesions, thereby leading to an accumulation of DSBs and stalled replication forks (Taki, Ohnishi et al. 1996) and (Sonoda, Sasaki et al. 1998). In addition, inactivation of the *RAD51* gene causes embryonic lethality (Tsuzuki, Fujii et al. 1996).

2.2 The structure of BRCA2

Yeast two-hybrid screening assays were used in the discovery of the interaction between RAD51 and BRCA2 (Mizuta, LaSalle et al. 1997), (Wong, Pero et al. 1997), (Chen, Chen et al. 1998) and (Marmorstein, Ouchi et al. 1998). And, studies examining the interaction between the two proteins have collectively shown that BRCA2 has two regions for RAD51 binding. The first region is in the mid-portion of BRCA2 and consists of eight highly conserved amino acid motifs called BRC repeats (Figure 2). The repeats have different binding affinities for RAD51:

repeats 1–4, 7, and 8 all interact with RAD51, with repeats 3 and 4 having the strongest affinity (Wong, Pero et al. 1997) (Bignell, Micklem et al. 1997). The second RAD51 binding site is located on the CTD (C-terminal domain) of BRCA2. This RAD51 binding site is described as playing a major role in the regulation of RAD51 recombination activity by displacing the single-strand DNA binding protein replication protein A (RPA) from the exonucleolytically processed 3′-single-strand overhangs of the DSBs, thus allowing RAD51 to bind and form nucleoprotein filaments (Yang, Jeffrey et al. 2002). The CTD portion of BRCA2 has been shown to be highly active in HR-mediated repair with RAD51 (Yang, Jeffrey et al. 2002). This region consists of five domains significant in DNA repair. The first is the α-helical domain, which interacts with the DMC1 protein—a meiosis specific paralog of RAD51 that forms nucleoprotein filaments and catalyzes strand exchange, and that BRCA2 requires for meiotic recombination (Thorslund, Esashi et al. 2007); (Jensen, Carreira et al.). The next three domains are the oligonucleotide–oligosaccharide binding domains (OB1, OB2, OB3) that have structural similarities with ssDNA binding proteins such as replication protein A (RPA). And, the fifth domain is the tower domain, which extends from OB2, and has structural similarities with the DNA binding domains of bacterial site-specific recombinases able to bind double-strand DNA (Yang, Jeffrey et al. 2002).

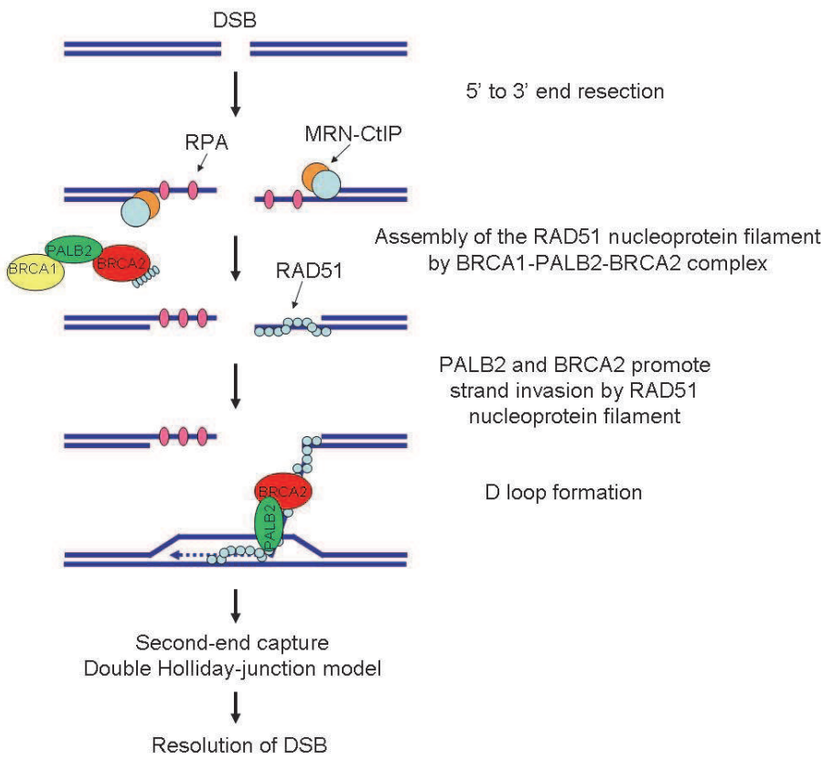

Fig. 1. Model of homologous recombination (HR)-mediated repair. After a DSB has occurred, the MRN-CtIP complex resects the 5′ends of the break. The 3′ssDNA overhangs are coated with replication protein A (RPA), which is displaced by RAD51. The BRCA1-PALB2-BRCA2 complex facilitates binding of RAD51 to form nucleoprotein filaments which invade the homologous strand, resulting in the D loop intermediate. This is followed by formation of the Holliday junction and resolution of the DSB.

Also located on the C-terminus of BRCA2 are its two nuclear localization signals (NLSs) (Spain, Larson et al. 1999) and (Yano, Morotomi et al. 2000). As a result, C-terminal mutations which disrupt, or truncations which remove, the NLSs are extremely detrimental to BRCA2 DNA repair functions, because they prevent nuclear localization. And, cell lines that have nonfunctional or absent BRCA2 NLSs primarily exhibit cytoplasmic localization of RAD51 after induction of DSBs by ionizing radiation (IR) (Spain, Larson et al. 1999). BRCA2 also interacts with RAD51 via a separate motif located at its C-terminus (Esashi, Christ et al. 2005). This interaction is regulated by cell cycle (CDK)-dependent phosphorylation of serine 3291 in exon 27 (and has been referred to, in some instances, as the "TR2" domain) and appears to function as a "switch" controlling recombinational repair activity during the transition from S/G2 to M phase in the cell cycle (Esashi, Christ et al. 2005). This phosphorylation site appears to be crucial in the checkpoint control mechanisms involved in the DNA repair pathway involving BRCA2.

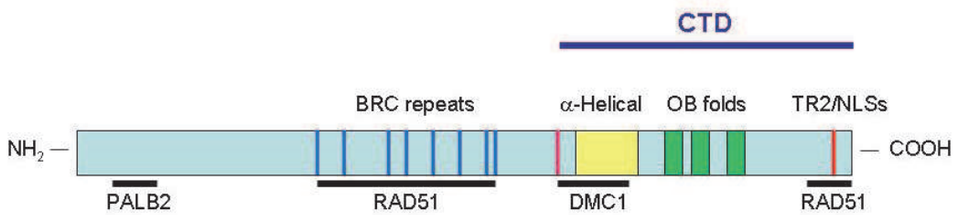

Fig. 2. Schematic of BRCA2. Starting at the N-terminus, the PALB2, the DMC1 and the two RAD51 binding sites on BRCA2 are indicated by black bars. The mid-portion contains eight highly-conserved BRC repeats. The CTD contains the α-helical domain, three OB-folds, the TR2 (location of S3291) domain, and putative nuclear localization signals (NLSs). The tower domain (not shown) extends from the second OB-fold (OB2).

The N-terminal region of BRCA2 does not bind RAD51; however, it does interact with a protein that is equally crucial to maintenance of genomic integrity, which is PALB2 (partner and localizer of BRCA2) (Xia, Sheng et al. 2006); (Rahman, Seal et al. 2007). PALB2 has been observed complexed with DNA damage-induced BRCA1/BRCA2 nuclear foci (Sy, Huen et al. 2009), (Zhang, Fan et al. 2009; Zhang, Ma et al. 2009). Subsequent studies have shown that PALB2 is crucial in the localization of BRCA2 to sites of DNA damage via associations with chromatin structures and in HR-mediated DNA repair ((Sy, Huen et al. 2009), (Zhang, Fan et al. 2009; Zhang, Ma et al. 2009). This indirectly influences the localization of RAD51 to sites of DNA damage, due to its reliance on BRCA2. During the process of HR-mediated repair, PALB2 appears to be crucial in "D-loop" formation, (Buisson, Dion-Cote et al.)2010) (figure 2). This is the step in which the 3′ overhangs of the dsDNA break, resulting from resection of the 5′ ends of the break, are coated with RAD51 protein to form nucleoprotein

filaments which invade the homologous template and form a Holliday junction. In the absence of PALB2, cells exhibit genomic instability and treatment with drugs that cause inter-strand crosslinks leads to increased chromosome breakage.

3. Mutations of BRCA2, DNA repair fidelity and disease predisposition

BRCA2 and its predecessor, *BRCA1*, were the first genes to be discovered that were associated with early-onset, familial breast cancer. Furthermore, germline mutations of *BRCA2* are also responsible for hereditary forms of ovarian, prostate and pancreatic cancer; however, the risk of acquiring breast cancer is most prevalent. Moreover, the risk for breast cancer is 50-80%, however, the degree of penetrance has been shown to vary (Tonin, Weber et al. 1996).

3.1 Mutated BRCA2 in familial cancers

Most mutations in BRCA2 are the result of small deletions and insertions. In fact, a *BRCA2* mutation that has been of interest for almost two decades is the 6174delT mutation, in which the thymine at position 6174 is deleted. This mutation disrupts BRC repeats 5 and 6, and introduces a premature stop codon that abruptly truncates the protein (Neuhausen, Gilewski et al. 1996; Oddoux, Struewing et al. 1996; Roa, Boyd et al. 1996; Abeliovich, Kaduri et al. 1997; Levy-Lahad, Catane et al. 1997). The truncated form no longer possesses the CTD region, which comprises the domains required for DNA repair and recombination, the second RAD51 binding site, TR2/S3291, and the putative nuclear localization signals. As a consequence, cells with this mutation exhibit inefficient repair of DSBs, loss of genomic stability, and sensitivity to radiation and DNA crosslinking agents (Goggins, Schutte et al. 1996),(Ozcelik, Schmocker et al. 1997). The 6174delT is a founder mutation in the Ashkenazi Jewish population at a frequency of 1.36% ((Tonin, Weber et al. 1996)). And, it is the only BRCA2 mutation, along with three BRCA1 mutations, that is carried in 78-96% of Ashkenazi Jews with detectable mutations (Oddoux, Guillen-Navarro et al. 1999) (Mangold, Wang et al.)

Another *BRCA2* mutation that was also discovered to have a founder's effect is the 999del5 mutation, which was discovered in an Icelandic population (Thorlacius, Olafsdottir et al. 1996). It is a five base-pair deletion that starts at nucleotide 999, codon 257 in exon 9. The mutation introduces a frame-shift that prematurely truncates the protein, and renders it nonfunctional, similar to the effect of the 6174delT founder mutation in the Ashkenazi Jewish population. Carriers of the mutation exhibit familial forms of male or female breast, prostate or pancreatic cancer. However, there are varying forms of penetrance, in which some carriers have never been diagnosed with cancer. In fact, there is either absolutely no phenotypic expression or diagnosis of varying forms of cancer (Thorlacius, Olafsdottir et al. 1996).

In a study of BRCA1/2 mutations performed in a Serbian population, one family was shown to carry a BRCA2 mutation that was an insertion of two nucleotides, c.4367_4368dupTT (Dobricic, Brankovic-Magic et al.). The mutation causes a frame-shift that alters codons 1381-1387 and introduces a premature stop codon at position 1388, resulting in a loss of > 2,000 amino acids at the C-terminus. The protein product lacks BRC repeats 3-8, as well as the crucial CTD and TR2 domains, rendering BRCA2 completely non-functional in regulating RAD51 activity, as well as in promoting HR-mediated repair of DSBs (Dobricic, Brankovic-Magic et al.)

3.2 Mutated BRCA2 in the development of Fanconi Anemia

Another inheritable condition resulting from mutated BRCA2 is the disorder Fanconi Anemia (FA). The disorder is rare and is characterized by aplastic anemia in childhood, susceptibility to leukemia and cancer, and hypersensitivity of FA cells to interstrand crosslinking agents, such as cisplatin (D'Andrea, 2010). The FA proteins are the products of 13 genes that comprise the following subtypes, FA-A, B, C, D1, D2, E, F, G, I, J, L, M, and N. And, eight of those gene products encoding proteins FANCA-C, FANCE-G, FANCL, and FANCM form a nuclear multi-protein core complex (the FA complex) that functions in the DNA repair pathway. Furthermore, it was discovered that genes underlying the FA-D1 (*FANCD1*) and FA-N (*FANCN*) subtypes were *BRCA2* and *PALB2*, respectively. Ultimately, the multi-protein core complex is responsible for monoubiquitylating FANCD2 on lysine 561 in order to activate the Fanconi Anemia pathway in response to S-phase progression or DNA damage (Zhi, Wilson et al. 2009), (Figure 3).

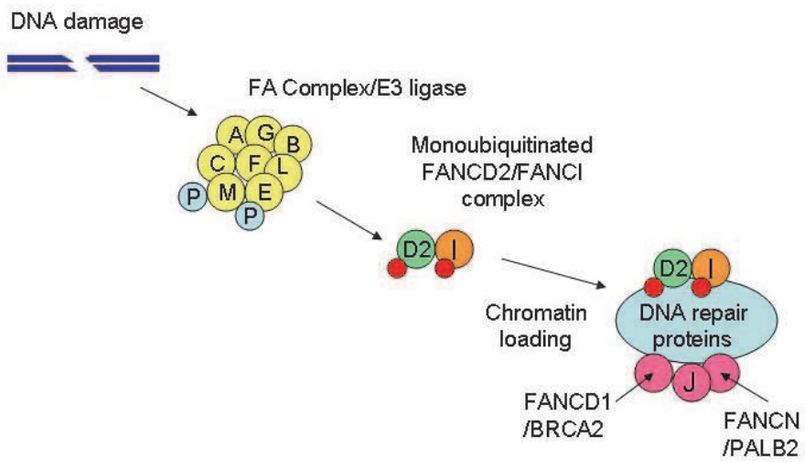

Fig. 3. Schematic of the Fanconi Anemia Pathway. After DNA damage, the ATR kinase phosphorylates and activates the FA core complex, comprised of FANCA, -B, -C, -E, -F, -G, -L, and –M. The complex functions as an E3 ligase and monoubiquitinates the FANCD2/FANCI complex, which then targets chromatin, where it assembles with other DNA repair proteins and FANCD1/BRCA2 and FANCN/PALB2 to repair damaged DNA.

The discovery of the FANCD1 protein being identified as BRCA2 was surprising, yet quite rational given the similarities between FANCD1 and BRCA2 mutated cells. They both exhibit chromosomal instabilities, sensitivity to ionizing radiation and crosslinking agents, and inefficient HR-mediated repair of DSBs. The role of FANCD1/BRCA2 in this protein complex is to act downstream in concert with the FA complex, additional FA members, and DNA repair proteins. However, Fanconi Anemia, subtype D1 is caused by biallelic inactivation of BRCA2; however, risk of breast, ovarian, prostate, and pancreatic cancers are associated with heterozygous BRCA2 mutations (Howlett, Taniguchi et al. 2002).

The FA proteins function in a DNA damage repair pathway, with the multi-protein core complex ultimately being responsible for monoubiquitylating the FANCD2 and FANC1 proteins, in response to DNA damage or entry into S phase of the cell cycle. Activation of the core complex is initiated by phosphorylation of FA proteins by the DNA damage sensing kinases ATM and ATR. After phosphorylation, the core complex assembles to form

a nuclear ubiquitin E3 ligase complex. The complex proceeds to monoubiquitylate the FANCD2 and FANC1 proteins thereby causing them to move to chromatin structures and form nuclear foci at sites of DNA damage. The FA complex interacts with FA members, FANCD1/BRCA2, FANCN/PALB2 and FANCJ, along with other DNA repair proteins to promote HR-mediated resolution of DSBs. Given that this pathway is involved in HR-mediated repair, it was not surprising to discover the involvement of RAD51 and BRCA1 downstream in the FA DNA repair pathway. And, because of the involvement of BRCA2, along with BRCA1, this pathway is now referred to as the Fanconi Anemia/BRCA pathway or network (D'Andrea).

3.3 BRCA2 in cell cycle signaling and the DNA damage response

To activate BRCA2, a sequence of cell signaling events is initiated in response to DNA damage, called the DNA damage response (or DDR). When the cell has experienced DSBs, either by exogenous sources such as ionizing radiation or exposure to crosslinking agents such as cisplatin, or endogenous sources such as free radicals and stalled replication forks, the goal is to immediately arrest cell division and repair the damage. When efficient DNA repair does not occur, apoptosis is induced to prevent propagation of genetic mutations. The phosphoinositide 3-kinase related kinases (PIKKs), ataxia-telangiectasia mutated kinase (ATM), and ATM and Rad 3-related kinase (ATR), are crucial in the detection and subsequent resolution of DNA damage. Furthermore, they are also involved in the Fanconi Anemia pathway, as previously described. ATM and ATR "cross-talk" with each other, given that ATM activates ATR in response to ionizing radiation, and ATR activates ATM in response to ultraviolet light. With respect to the DDR pathway that involves BRCA2, resolution of DSBs is initiated by activation of ATR, after phosphorylation by ATM. ATR proceeds to phosphorylate and active Chk1, which then phosphorylates RAD51. Chk1 arrests the cells in S and G2 phases to ensure DNA is repaired before synthesis and cell division. At this point, RAD51 is now able to engage in HR-mediated repair of DSBs under the regulation of BRCA2 (McNeely, Conti et al.), (Connell, Shibata et al.).

BRCA2 appears to play a crucial role during S and G2 phases of the cell cycle. First, during S phase, replication forks can stall and collapse due to exogenous or endogenous sources of damage. A DNA strand break at a replication fork can mimic a DSB as a result of the nascent DNA chain that is being synthesized at the fork. At this point, activated RAD51 is required to repair the break and subsequently stalled fork. It has been proposed that deficient BRCA2, which functions to regulate RAD51 during HR-mediated repair, may be a major cause of diseases resulting from an accumulation of stalled replication forks and consequential DNA breaks that remain unrepaired (Lomonosov, Anand et al. 2003). And, with respect to G2 phase, in a study where the binding of BRCA2 with RAD51 was inhibited in cells expressing the BRC4 repeat, which competed against endogenous full-length BRCA2, there was a failure to initiate radiation-induced G2/M checkpoint arrest. These results implied that the interaction between BRCA2 and RAD51 was imperative for G2/M checkpoint control (Chen, Chen et al. 1999).

The majority of *BRCA2* mutations that are associated with cancer predisposition tend be truncations that remove substantial portions of the CTD, which is where the domains required for DNA repair are located. This region of the protein also appears to be significant in cell cycle changes due to the DNA damage response, via the TR2 domain. The TR2 domain contains a serine at 3291 that is CDK phosphorylated and appears to be one method

in which binding between BRCA2 and RAD51 is regulated (Esashi, Christ et al. 2005). There is reduced phosphorylation at this site during S phase, which allows BRCA2 and RAD51 to interact, and engage in HR-mediated repair resulting from replication-induced DNA breaks. In addition, phosphorylation is reduced in response to ionizing radiation. However, phosphorylation of S3291 increases during G2/M to inactivate HR from occurring during mitosis. Further support for this region of the protein being a cancer-related mutation site is evidenced by the association of the P3292L mutation with breast cancer incidence (Esashi, Christ et al. 2005). The TR2 domain also only interacts with multimeric forms of RAD51, both in the presence and absence of DNA (Esashi, Galkin et al. 2007). And, RAD51 monomers bearing mutations that prevent self-association do not interact with the TR2 domain. The impact that this has on BRCA2 function is quite remarkable and has been elegantly summarized (Esashi, Galkin et al. 2007). In the absence of DNA damage, the TR2 domain is phosphorylated at S3291, preventing association of the C-terminus of BRCA2 with RAD51, as well as keeping BRCA2 inactive. However, concurrently, RAD51 is associated with BRCA2 via the BRC repeats in monomeric form. And, it has been noted that the BRC repeats may serve as a negative regulator of RAD51 by preventing it from forming nucleoprotein filaments with ssDNA until after damage has been detected and the DNA has been prepped for HR-mediated repair. After DNA damage has been detected, S3291 is dephosphorylated, now allowing BRCA2 to become activated. The C-terminus can now bind with RAD51 in multimeric form, and the OB folds which possess ssDNA binding activity, deliver RAD51 to sites of DNA damage. This change in BRCA2 function from negatively regulating RAD51 to mobilizing it to sites of damage may be driven by the self-assembly of RAD51 from a monomeric to a multimeric state in response to DNA damage (Esashi, Galkin et al. 2007). This detrimental function of the C-terminus of BRCA2 further substantiates how truncations of this region of the protein, which are commonly seen in BRCA2-cancers, incite genomic instability and subsequent malignant transformation.

The role of the BRC repeat region has been somewhat controversial. It has been described as the region of BRCA2 that is responsible for delivering RAD51 to ssDNA at sites of DNA damage (Carreira, Hilario et al. 2009), (Shivji, Davies et al. 2006) but, conversely, as a negative regulator of RAD51, which was described in the previous section (Nomme, Takizawa et al. 2008), (Davies and Pellegrini 2007). Results of a study investigating cancer-associated mutations of BRC repeats supported their role as a negative regulator that binds and inhibits RAD51 from engaging in HR. But, then releases RAD51 monomers upon detection of DNA damage, thus allowing RAD51 to multimerize and interact with the BRCA2 TR2 region for mobilization to sites of damage. At this point, RAD51 is ready to form nucleoprotein filaments on the 3′ ssDNA overhangs at the breakpoint junction, which will invade the DNA homologue to be used as the template for repair. Considering that the BRC repeats are important for modulating RAD51 activity, several cancer-associated mutations, primarily point mutations, have been identified in this highly conserved region of BRCA2. Cancer-associated mutations have been identified in BRC motifs 1(T1011R), 2(F1219L, S1221P), 4(G1529R), and 7(T1980I) (Esashi, Galkin et al. 2007). The effect of mutations in BRC motifs 2 and 4 on RAD51-mediated HR repair was assessed. The results determined that such mutations prevent binding of monomeric RAD51 to the BRC repeats, which prevents recruitment of RAD51 to DSBs, thereby inhibiting nucleoprotein filament formation and impairing HR-mediated repair (Tal, Arbel-Goren et al. 2009).

A great deal of attention has been focused on the role of the C-terminus and BRC repeat region of BRCA2 in HR-mediated repair. However, mutations of the N-terminus also have detrimental effects on protein function. The N-terminus of BRCA2 binds the protein PALB2 (partner and localizer of BRCA2) (Xia, Sheng et al. 2006); (Rahman, Seal et al. 2007), (Figure 2). PALB2 is also a member of the Fanconi Anemia pathway, denoted as FANCN, in the same manner in which BRCA2/FANCD1 is involved, as well (Figure 3). And, just as biallelic mutations of BRCA2/FANCD1 cause a subtype of Fanconi Anemia, and susceptibility to childhood cancers, biallelic mutations of PALB2/FANCN have a similar phenotype (D'Andrea). With respect to the interaction with BRCA2, PALB2 is responsible for localizing BRCA2 to the sites of DNA damage in order to promote repair (Xia, Sheng et al. 2006). Mutations of the PALB2 binding site on BRCA2 prevent this interaction, causing impaired formation of RAD51 damage-induced foci, and unresolved DSBs (Xia, Sheng et al. 2006). Furthermore, PALB2 is also able to bind DNA and enhance the recombination activity of RAD51 (Dray, Etchin et al.).

4. Therapeutic regimens designed to target BRCA2 defects

Cells that are defective in BRCA1 and BRCA2 retain unresolved DSBs. This attribute, which is detrimental in terms of genomic instability and risk for cancer, is actually a potent target for inhibitors of Poly(ADP-ribose) polymerase, or PARP, in the eradication of transformed cells.

4.1 Efficacy of PARP inhibitors in treating BRCA2-tumors

PARPs are a family of 17 enzymes, with PARP-1 and -2 having been shown to be involved in DNA repair. PARP-1 is a nuclear protein with a zinc-finger DNA binding domain (Amir, Seruga et al.). It is responsible for binding to the sites of single-strand breaks, signaling damage at the site, and the initiating repair. The zinc finger domain binds to ssDNA breaks, cleaves NAD+, and attaches multiple ADP-ribose units to the protein. This results in an extremely negatively charged target which causes unwinding of the damaged DNA, followed by repair by the Base-Excision Repair (BER) pathway (Schreiber, Dantzer et al. 2006); (Ratnam and Low 2007). However, PARP-1 has also been shown to serve as an anti-recombinogenic factor at sites of damage where it has bound, thereby having implications on inhibiting HR-mediated repair (Amir, Seruga et al.), (Sandhu, Yap et al.). BRCA1 and -2 mutant cells are defective in repair of DSBs, and as a consequence, are sensitive to agents that induce DSBs. PARP-1 inhibitors have been shown to be effective in selectively targeting BRCA1 and -2 defective cells by converting SSBs, which have been induced by the use of chemotherapeutic agents, ionizing radiation, or occurring in normal cellular processes, such as stalled replication forks, to DSBs. The SSBs would have normally been identified and resolved by PARP-1 binding and the BER pathway; however, PARP-1 inhibitors prevent such resolution, and during DNA synthesis, the SSBs are converted to DSBs. The DSBs are normally resolved by HR-mediated repair involving BRCA1, and most important BRCA2, with the recombinase RAD51. However, this is deficient in BRCA-mutant cells and the addition of PARP inhibition enhances DNA-damage induced cell cycle arrest and apoptosis. This process eradicates the tumor cells.

4.2 Development of PARP inhibitors

The first PARP-1 inhibitor created was 3-aminobenzamide (3-AB). It causes inhibition of PARP-1 by competing with NAD+ as a substrate. However, 3-AB showed poor specificity

and inhibited de-novo purine synthesis (Purnell, Stone et al. 1980); (Drew and Plummer). Approximately, twenty years have passed since the synthesis of 3-AB, and the focus has been to create PARP-1 inhibitors with greater specificity for PARP-1 inhibition, only. In 2003, the PARP-1 inhibitor AG014699 was the first to enter clinical trials (Plummer and Calvert 2007), (Drew and Plummer). Xenograft studies showed significant delay of tumor growth when AG014699 was combined with irinotecan and irradiation and tumor regression when combined with temozolomide (Ratnam and Low 2007). There are presently at least eight PARP inhibitors in clinical trials (Drew and Plummer), (Amir, Seruga et al.), (Table 1). PARP inhibitors are effective at sensitizing tumor cells to other chemotherapeutic agents, and can be used as a combination therapy with platinums, temozolomide, topopisomerase I inhibitors, and γ-/X-radiation (Ratnam and Low 2007), (Curtin, Wang et al. 2004), (Miknyoczki, Jones-Bolin et al. 2003), (Nguewa, Fuertes et al. 2006), (Chalmers, Johnston et al. 2004), (Fernet, Ponette et al. 2000), (Veuger, Curtin et al. 2003). Due to PARP inhibitors effectively promoting cell cycle arrest and subsequent apoptosis, clinical trials are testing their efficacy as single-agents in the treatment of BRCA1- and BRCA2-tumors (Ratnam and Low 2007).

Agent	Single/combination therapy	Disease
Olaparib (AZD2281)	Single agent Combination trials	BRCA-related tumors Solid tumors
BSI-201	Single agent Combination trials (gemcitabine/carboplatin)	Triple negative breast cancer Advanced solid tumors
AG014699	Single agent Combination trials (temozolomide [TMZ]	Solid tumors Melanoma
ABT-888	Single agent	Solid tumors and lymphoid malignancies
INO-1001	Single agent Combination with TMZ	Melanoma Glioblastoma mutiforme
MK4827	Single agent	Solid tumors BRCA ovarian
GPI21016	Combination with TMZ	Solid tumors
CEP-9722	Combination with TMZ	Solid tumors

Table 1. PARP inhibitors presently in clinical trials

4.3 Clinical implications of PARP inhibitor use

In general, there is very high enthusiasm for the use of PARP inhibitors in the treatment of *BRCA2*-cancers. The requirement for specificity is met because the *BRCA1/2*-mutated cells are most sensitive to the inhibitors, due to their DNA repair defects, and the premise of "synthetic lethality", which is when two pathway defects alone are innocuous, but combined become lethal (Ratnam and Low 2007). The combination of impaired HR-mediated repair due to the *BRCA1/2*-mutation and the inhibition of PARP-1 to signal the DNA breaks provides the "synthetic lethality" that is necessary for the efficacy of PARP inhibitors in the treatment of *BRCA*-tumors. Furthermore, the therapeutic benefit of PARP inhibitors appears to greatly outweigh the undesirable side effects; however, there are areas

of concern. First and foremost, PARP inhibitors are still in the early stages of clinical testing. Therefore, the optimal dosage and duration of treatment have not been definitively determined. And, although PARP inhibitors are effective against BRCA-tumors, there is the potential for possible toxicity in normal tissues. In the Olaparib phase I study, DSB accumulations were observed in normal tissues (eyebrow hair follicles), (Drew and Plummer). In addition to toxicity, the inhibitors may disrupt DNA repair pathways in normal tissue from DNA damage acquired through sun exposure or other environmental agents (Ratnam and Low 2007). And, the potential for secondary cancers to occur through genomic instability from inhibition of PARP-1 is possible. In an in vivo study of PARP-1 deficiency, female mice developed mammary carcinoma (Tong, Yang et al. 2007); (Drew and Plummer). Furthermore, secondary mutations after PARP inhibitor treatment may lead to drug resistance. Previous reports have observed intragenic secondary mutations/deletions of BRCA2 occurring after treatment with PARP-1, and the anti-cancer agent cisplatin, which restored the open-reading frame and led to the expression of new BRCA2 isoforms. This resulted in reversal of the original BRCA2 mutation and resistance to PARP inhibitors (Edwards, Brough et al. 2008), (Sakai, Swisher et al. 2008), (Drew and Plummer).

5. Conclusion

Overall, the use of PARP inhibitors appears to be very promising in the treatment of BRCA-tumors as a single agent, and as a chemotherapeutic/radiation sensitizer when used in combination with anticancer therapeutics or γ-radiation. The on-going clinical trials will provide more information about the aspects of PARP inhibitor usage that are presently vague, such as proper dosage and duration of treatment, possible effects on DNA repair mechanisms in normal cells, possible induction of secondary mutations, and acquired resistance of tumors over the course of treatment.

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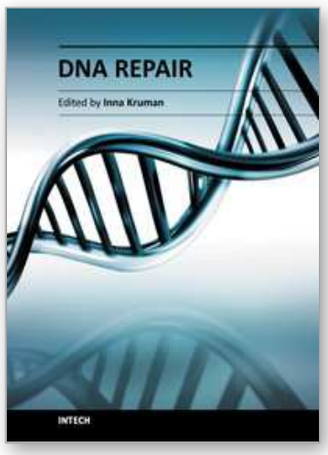

DNA Repair

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The book consists of 31 chapters, divided into six parts. Each chapter is written by one or several experts in the corresponding area. The scope of the book varies from the DNA damage response and DNA repair mechanisms to evolutionary aspects of DNA repair, providing a snapshot of current understanding of the DNA repair processes. A collection of articles presented by active and laboratory-based investigators provides a clear understanding of the recent advances in the field of DNA repair.

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