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REVIEW

RAD51C: a novel cancer susceptibility gene is linked to Fanconi anemia and breast cancer

Kumar Somyajit, Shreelakshmi Subramanya and Ganesh Nagaraju*

Department of Biochemistry, Indian Institute of Science, Bangalore-560012, India

*To whom correspondence should be addressed. Tel: +91 80 22933055; Fax: +91 80 23600814;
Email: nganesh@biochem.iisc.ernet.in

Germline mutations in many of the genes that are involved in homologous recombination (HR)-mediated DNA double-strand break repair (DSBR) are associated with various human genetic disorders and cancer. RAD51 and RAD51 paralogs are important for HR and in the maintenance of genome stability. Despite the identification of five RAD51 paralogs over a decade ago, the molecular mechanism(s) by which RAD51 paralogs regulate HR and genome maintenance remains obscure. In addition to the known roles of RAD51C in early and late stages of HR, it also contributes to activation of the checkpoint kinase CHK2. One recent study identifies biallelic mutation in *RAD51C* leading to Fanconi anemia-like disorder. Whereas a second study reports monoallelic mutation in *RAD51C* associated with increased risk of breast and ovarian cancer. These reports show *RAD51C* is a cancer susceptibility gene. In this review, we focus on describing the functions of RAD51C in HR, DNA damage signaling and as a tumor suppressor with an emphasis on the new roles of RAD51C unveiled by these reports.

Introduction

Homologous recombination (HR), a fundamental cellular process conserved in all organisms, maintains genome integrity by repairing endogenous and exogenous DNA double-strand breaks (DSBs). DSBs generated during DNA replication are preferentially repaired by sister chromatid recombination, an HR pathway that utilizes neighboring sister chromatid as a template (1–3). HR dysfunction may cause aberrant genetic rearrangements and genomic instability, resulting in chromosomal translocations, deletions, amplifications or loss of heterozygosity (LOH). Indeed, several cancer susceptibility genetic diseases such as ataxia telangiectasia, Nijmegen breakage syndrome, Bloom syndrome and Fanconi anemia (FA), which are due to lack of single genes show hyper- or hypo-recombination phenotype (4–10). Furthermore, germline mutations in *BRCA1* and *BRCA2*, which are known to regulate HR, cause hereditary breast and ovarian cancers, implying a crucial role of HR, and the genes that regulate HR in cancer prevention (11–13).

Replication of genome is hampered when the replication fork encounters lesions on DNA, resulting in stalling or collapsing of forks. HR plays a key role in the repair of double-strand break repair (DSBR) and daughter strand gaps that arise during replication and the restart of replication forks, ensuring duplication of the genome and cell division (6,14). Failure in the stabilization of stalled forks or in the processing of replication forks for restart could result in the accumulation of mutations and chromosomal aberrations (CAs) (15,16). Indeed, a variety of human genetic disorders associated with cancer are caused by mutations in the genes that protect genome integrity during DNA replication (6,17–19). Furthermore, HR has been known to play an important role in the repair of DNA interstrand cross-links (ICLs) in collaboration with nucleotide excision repair and translesion synthesis (20–22). HR deficiency may divert cells to alternative, error-prone repair pathways, including non-homologous end joining and single-strand annealing. These error-prone pathways significantly contribute to genome instability in the form of CAs, insertions or deletions and subtle mutations.

Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ATM and RAD3-related; CA, chromosomal aberration; DSB, double-strand break; DSBR, double-strand break repair; FA, Fanconi anemia; HJ, Holliday junction; HR, homologous recombination; ICL, interstrand cross-link; IR, ionizing radiation; LOH, loss of heterozygosity; MMC, mitomycin C; RPA, replication protein A; SDSA, synthesis-dependent strand annealing; ssDNA, single-stranded DNA.

RAD51 and RAD51 paralogs in HR

The central steps of HR involve processing of DSB ends, homology search and strand invasion. RAD51 protein, a eukaryotic ortholog of *Escherichia coli* RecA, polymerizes onto single-stranded DNA (ssDNA) to form a helical nucleoprotein filament (23,24). The presynaptic filament of RAD51 engages in homology search and invades homologous duplex DNA to generate D-loop structure (5,25). Synthesis initiated from the invading strand results in D-loop extension. This recombination intermediate is thought to be channeled into two subpathways, DSBR or synthesis-dependent strand annealing (SDSA) (Figure 1) (26–28). In DSBR, the extended D-loop can be captured by a second end of DSB to form a double Holliday junction (HJ). The double HJ can be resolved by a junction specific endonuclease to generate crossover or non-crossover products. Double HJ can also be resolved to non-crossover products by the concerted action of helicases and topoisomerases (29–31). Crossover-associated recombination is essential for proper chromosome disjunction in meiotic cells. In contrast, this pathway is highly suppressed in somatic cells as crossovers can lead to LOH and chromosome rearrangements (24,27,32–34). In SDSA, however, the nascent strand displaced from the D-loop pairs with second end of the DSB to resolve as a non-crossover product (Figure 1) (26,35,36).

The nucleation of RAD51 protein onto ssDNA is impeded by the heterotrimeric replication protein A (RPA) protein, an abundant nuclear protein that binds to ssDNA with high affinity (5,24). Genetic and biochemical studies have identified recombination mediators in *Saccharomyces cerevisiae*. These recombination mediators counteract the inhibitory action of RPA and assist RAD51 polymerization onto ssDNA (5,27,32). Biochemical studies show that addition of catalytic amounts of RAD52 results in several fold stimulation of RAD51 recombinase activities by alleviating the inhibitory action of RPA (37,38), suggesting that mediator function of RAD52 is critical for RAD51-mediated HR (5,24). The *S.cerevisiae* RAD55 and RAD57 proteins are considered as RAD51 paralogs and these protein share 20–30% sequence identity with RAD51. These RAD51 paralogs possess a conserved nucleotide triphosphate-binding motif, form a stable heterodimer and bind ssDNA but exhibit weak adenosine triphosphatase activity (27,32,39). RAD55 and RAD57 proteins stimulate RAD51-mediated homologous pairing and strand exchange *in vitro* (40). Furthermore, overexpression of RAD51 can rescue ionizing

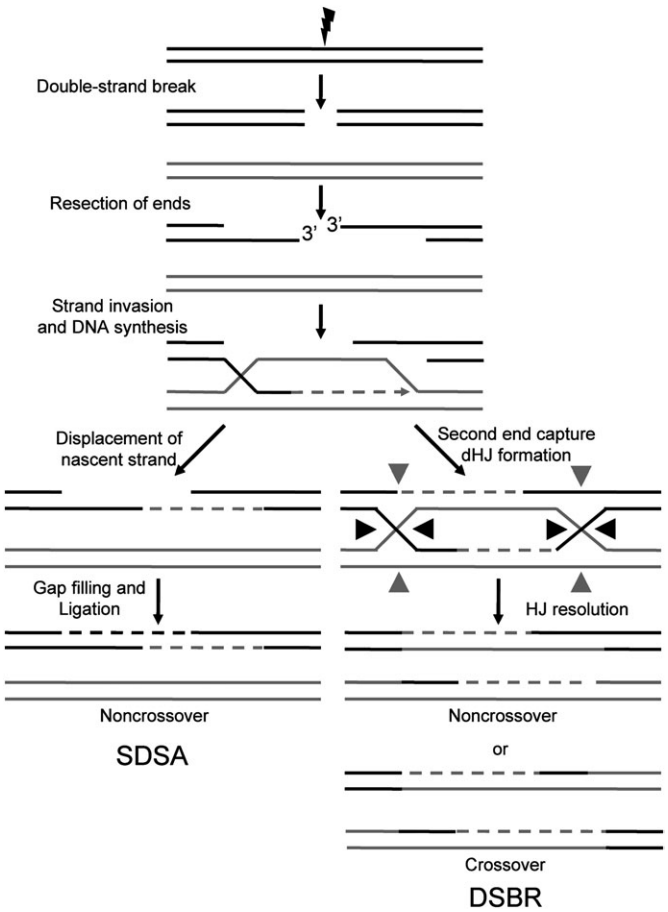

Fig. 1. Alternative mechanisms of DNA DSBR by HR. DSBs can be repaired by two alternative pathways of HR such as SDSA and DSBR. In both the pathways, DSB ends are processed by nucleolytic resection to generate 3′ ssDNA, which serves as a substrate for HR machinery to engage in homologous search and strand invasion. DNA synthesis will be initiated from the invaded strand in D-loop structures. In the SDSA pathway, the nascent strand will be displaced from the D-loop and anneals to the second end of the broken DNA, followed by gap filling and ligation DSBR is completed. In the DSBR pathway, extension of D-loop by nascent strand synthesis captures the second end to generate double-HJ structures. After the gap repair and ligation, HJs are resolved by resolvases to result in either non-crossover (black triangles) or crossover products (gray triangles).

radiation (IR) sensitivity of *rad55* or *rad57* mutants (41,42), indicating that RAD55–RAD57 complex plays an important role in formation or stabilization of the RAD51 filament (32).

The genome-wide search for *S.cerevisiae RAD52* epistasis group genes in human and mouse resulted in the identification of *RAD51*, *RAD52*, *RAD54* and *RAD54B* (25,27,32,33). The human RAD51 (hRAD51) is highly conserved to that of ScRAD51 and display 68% amino acid identity. In contrast, human RAD52 and RAD54 are less conserved. Human RAD52 lacks a recombination mediator function (39), but human RAD54 physically interacts with RAD51 and stimulates its activity (43,44). Biochemical studies have shown that RAD54 functions at all three stages of HR; presynapsis, synapsis and postsynapsis (45–47). Notably, orthologs of *S.cerevisiae* RAD55 and RAD57 have not been identified in mammalian cells. An approach using human genes that complement IR sensitivity of mutant hamster cells was used to identify mammalian genes that are essential for HR. Using this approach, human *XRCC2* and *XRCC3*, which complements IR sensitivity of irs1 and irs1SF hamster cells, were discovered as members of a *RAD51*-like family of genes (48,49). By searching the human genome database for sequence similarity to RAD51, additional mammalian RAD51 paralogs; RAD51B, RAD51C and RAD51D were discovered (50–52). Sequence analysis reveals that mammalian RAD51 paralogs display 20–30% identity with hRAD51 and the homology is mainly restricted to adenosine triphosphate-binding motifs (53).

Early studies with Chinese hamster irs1 and irs1SF cells defective in XRCC2 and XRCC3, respectively, revealed that these cells were sensitive to DNA damaging agents such as IR, ultraviolet light, ethyl methanesulfonate and camptothecin. Interestingly, these mutants were extremely sensitive to ICL agent mitomycin C (MMC) (48,54–56). Both mutants exhibited high rates of spontaneous and IR-induced CAs, defective chromosome segregation (48,57), reduced RAD51 nuclear foci formation and decreased frequency of DSBR by HR (58–61). In addition, chicken cells deficient in RAD51B, C and D exhibited hypersensitivity to DNA damaging agents, high spontaneous CAs and reduced rate of sister chromatid exchange (62,63). Furthermore, Chinese hamster cells defective in RAD51C (irs3 and CL-V4B) and RAD51D also displayed a phenotype similar to that of irs1 and irs1SF cells (64–66). Genetic knockouts of *RAD51B*, *RAD51C*, *RAD51D* and *XRCC2* in mouse cause early embryonic lethality (67–70), implying a crucial role of RAD51 paralogs in the maintenance of genome integrity during development. Interestingly, DT40 cells defective in *xrcc3/rad51d*, in which both the BCDX2 and CX3 complexes are impaired, exhibit higher sensitivity to camptothecin and cisplatin than *rad51b/rad51d*-double mutants, in which only BCDX2 complex is dysfunctional (71). These observations clearly suggest that RAD51 paralogs have non-redundant functions in HR and genome maintenance.

RAD51C recombination functions

Biochemical and two-hybrid analyses indicate that RAD51 paralogs form two distinct complexes: the RAD51B/RAD51C/RAD51D/XRCC2 (BCDX2) complex and the RAD51C/XRCC3 (CX3) complex (56,72). Interestingly, RAD51C is part of both complexes. BCDX2 complex proteins have been shown to bind single- and double-stranded DNA and hydrolyze adenosine triphosphate (73–76). Homologous pairing activity has been reported for purified CX3 and XRCC2–RAD51D subcomplex (74,77). Furthermore, the BC heterodimer has been shown to function as a mediator in RAD51- and RPA-catalyzed strand exchange (73). These results suggest a role for RAD51 paralogs in the initial stages of recombination, assisting RAD51-mediated HR.

Recent genetic and biochemical studies indicate a possible role of RAD51 paralogs in the late stages of HR. RAD51B and BCDX2 complex have been independently shown to bind specifically to HJ and Y-junction substrates (78,79). Although a purified complex containing RAD51C and XRCC3 from HeLa cells has been reported to promote HJ branch migration and resolution, a similar activity was lacking for purified recombinant RAD51C (80). Interestingly, RAD51C sequences do not show any nuclease domain, suggesting that perhaps a nuclease associated with RAD51C could be involved in HJ resolution. Indeed, a recent study showed that purified GEN1 nuclease from HeLa cells possesses an HJ resolvase activity (81). It will be interesting to study whether RAD51C interacts with GEN1 or any other nuclease to promote HJ resolution. Using sister chromatid recombination reporter, gene conversions have been measured in hamster cells lacking RAD51 paralogs. Strikingly, cells defective with RAD51C, XRCC2 and XRCC3 exhibit increased frequency of long-tract gene conversion events, suggesting an important function of RAD51 paralogs in resolving recombination intermediates (34,82–84). It is possible that RAD51C might promote SDSA type of repair to suppress long-tract gene conversion by displacing the nascent strand from the sister chromatid (Figure 1). Helicases are implicated in this kind of a function (85,86). However, RAD51C lacks any helicase motif, suggesting that RAD51C in part with an unidentified helicase could be involved in the SDSA type of DSBR. Interestingly, RAD51D has been shown to physically interact with BLM helicase and, the purified RAD51D–XRCC2 complex stimulates BLM-mediated HJ resolution (87). It is probably that RAD51C may interact with BLM or any other helicase and facilitate SDSA to suppress long-tract gene conversion. However, further investigation is required to test this hypothesis.

RAD51 paralogs have been reported to have specialized roles: RAD51C in checkpoint activation by checkpoint kinase CHK2 (88) and in maintaining centrosome integrity (89), RAD51D in telomere maintenance (90), XRCC3 in the restart of stalled replication forks (91), in replication fork progression (92) and telomere maintenance by T-loop deletion (93). Interestingly, recent studies show that RAD51C has novel functions; in the DNA damage response, in the repair of ICLs possibly in collaboration with FA proteins and finally as a cancer susceptibility gene. We discuss these novel and important functions of RAD51C in the following section.

Role of RAD51C in DNA damage responses

In response to DSBs and other types of DNA damage, eukaryotic cells have evolved to induce a complex network of DNA damage signaling, which coordinate cell cycle checkpoint with DNA repair to maintain genome stability. Ataxia-telangiectasia mutated (ATM) and ATM and RAD3-related (ATR) kinases are key players in the DNA damage signaling mechanism. ATM and ATR mutation causes defective response to DNA damage, resulting in disorders, such as ataxia-telangiectasia and ATR-Seckel syndrome, respectively, which are characterized by developmental defects, DNA damage sensitivity and cancer predisposition (8,94–96).

In a recent study, Badie *et al.* (88) show that RAD51C localizes to the sites of DNA damage before and independently of RAD51, supporting that RAD51C has a role in the early stages of HR. Interestingly, RAD51C accumulation at the sites of DSBs was dependent on ATM, NBS1 and RPA, suggesting that processing of DSB ends is a prerequisite for RAD51C localization and downstream activation of HR through RAD51 assembly. Strikingly, their studies show that RAD51C is required for phosphorylation of CHK2 by ATM, which is required for checkpoint activation. Defective CHK2 activation causes progression of cells into G_2/M phase in response to DNA damage (88). These results reveal a novel function of RAD51C in signaling and checkpoint function. However, whether RAD51C is a target of ATM phosphorylation remains to be tested. ATM/ATR target substrates possess conserved SQ/TQ motifs and RAD51C sequence contains TQ motifs, indicating that RAD51C is a possible substrate for ATM/ATR kinase (Figure 2). The RAD51C-interacting partner XRCC3 is also required for CHK2 activation (88). However, whether dynamic interaction of RAD51C and XRCC3 is essential for CHK2 phosphorylation and checkpoint activation remains to be studied.

The checkpoint kinase CHK2 transduces DNA damage signaling from ATM and regulates cell cycle progression, DNA repair and apoptosis or senescence (97). There is evidence that CHK2 controls DNA repair; following activation by ATM, CHK2 phosphorylates various downstream effectors including BRCA1 (98). CHK2-dependent BRCA1 S988 phosphorylation regulates DSBR by HR (99), suggesting a direct role of CHK2 in DNA repair. The expression of BRCA2 and XRCC1 that are required for HR and base excision repair, respectively, is controlled by FOXM1 transcription factor, and the stability of FOXM1 transcription factor increases upon phosphorylation by CHK2, suggesting that DNA damage-induced CHK2 activation controls BRCA2 and XRCC1 expression (100). It will be interesting to test whether any of these CHK2-dependent functions are affected by RAD51C deficiency. In addition, whether RAD51C has any role in CHK2-dependent apoptosis or senescence remains to be investigated.

Germline mutations in p*53*, *ATM*, *ATR*, *BRCA1* and *BRCA2* whose products are known to regulate DNA repair and checkpoint functions leads to genetic instability disorders and various types of cancer (101). Similarly, mutations in *CHK2* have been identified in familial breast and prostate cancer (95,102). There are also reports which indicate that germline mutation in *CHK2* increases the risk of developing ovarian, colorectal, kidney, thyroid and bladder cancer, suggesting that *CHK2* is a multiorgan cancer susceptibility gene (97). Interestingly, germline mutations in *RAD51C* have been identified recently in FA-like disorder and breast and ovarian cancer, indicating that *RAD51C* is a cancer susceptibility gene (103,104). Since CHK2 activation is dependent on RAD51C, it will be interesting to study whether *RAD51C* is also mutated in various types of cancer associated with *CHK2* mutation.

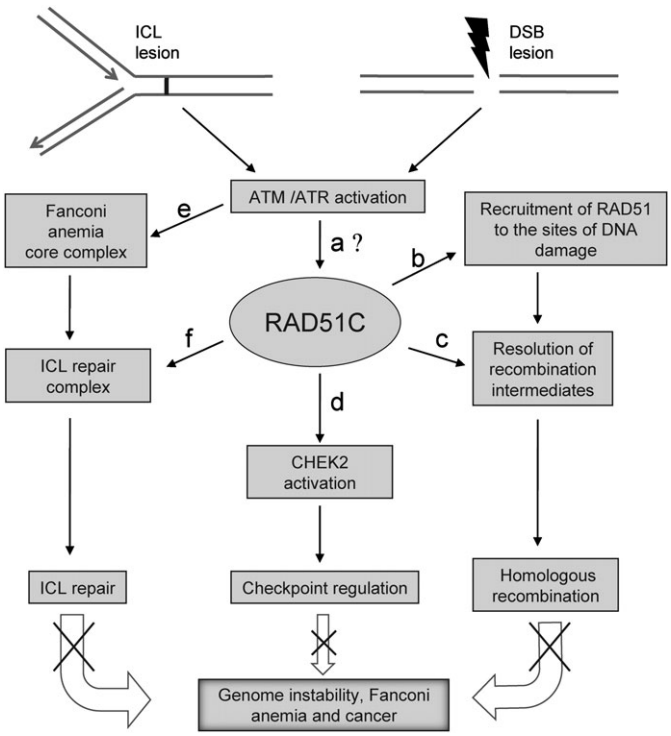

Fig. 2. A model for the role of RAD51C in DSBR by HR, ICL repair and checkpoint activation. (a) In response to DSBs, RAD51C localizes to DSBs in an ATM/ATR-dependent manner. (b) RAD51C controls RAD51 recruitment to the processed DSB ends in the initial stages of HR. (c) RAD51C also regulates resolution of recombination intermediates at the later stages. (d) RAD51C is required for CHK2 activation and checkpoint function. (e) FA core complex is activated and recruited by ATR kinase in response to ICL lesion. (f) RAD51C appears to be part of the ICL repair complex contributing to the ICL repair. Defect in the RAD51C HR function and checkpoint regulation may cause FA-like disorder and cancer.

RAD51C as a novel gene in the FA pathway

FA is a rare autosomal and X-linked recessive chromosome instability syndrome characterized by congenital abnormalities, progressive bone marrow failure, predisposition to acute myelogenous leukemia and malignancies (105–107). The hallmarks of FA cells are genome instability and extreme sensitivity to DNA ICL agents, such as MMC, diepoxybutane and cisplatin (107). FA is a genetically heterogeneous disorder, and complementation analyses by cell fusion experiments revealed at least 13 complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M and N) (108,109). Many FA genes code for subunits of FA core complex (FANCA, B, C, E, F, G, L and M), and one of the main functions of this complex is to monoubiquitinate FANCD2 and FANCI, which is essential for DNA repair (110,111). FANCL has been shown to be ubiquitin ligase responsible for monoubiquitination of FANCD2 and FANCI (108,110,112,113). Interestingly, three of the FA genes, *FANCD1*, *FANCN* and *FANCJ*, have been found to be breast cancer susceptibility (*BRCA*) genes *BRCA2*, *PALB2* and *BACH1*, respectively (109,114–119). FA proteins and another well-known breast cancer susceptibility protein BRCA1 cooperate in a common pathway of DNA damage response and repair of ICLs (111). FA proteins are also known to have molecular and functional interaction with proteins such as ATM, ATR, NBS1 and BLM, responsible for other rare genetic instability syndromes ataxia telenagiectasia, Seckel syndrome, Nijmegen breakage syndrome and Bloom syndrome, respectively (6,108).

Recent studies indicate that the primary function of FA pathway is to activate DNA damage signaling and ICL repair in the S-phase of the cell cycle. First, the activation of the FA pathway through monoubiquitination of FANCD2 and FANCI occurs in S-phase. Second, during S-phase, monoubiquitinated FANCD2 and FANCI specifically localizes with replication and repair foci containing BRCA1, BRCA2, RAD51 and PCNA proteins (111,120,121). These proteins are required for the error-free replication of DNA and repair of damaged DNA during replication by HR (106,108,122–124). Current studies indicate that activation of the FA pathway occurs by ATR-dependent phosphorylation of FANCI (Figure 2) (125–128). Following activation, the molecular mechanism by which FA pathway regulates DNA repair process is poorly understood. However, there is evidence that FANCD2 and FANCI (ID complex) facilitate translesion synthesis and HR at the site of stalled/collapsed replication forks (124,127,129).

Although mammalian RAD51 paralogs were known to have a critical function in HR, genome maintenance and in the survival of the organism, none of the RAD51 paralogs were known to be associated with any human disorder. In a recent study, Vaz *et al.* (103) show biallelic mutation in *RAD51C* leading to FA-like disorder. They identified homozygous missense mutation in the *RAD51C* gene in a consanguineous family with multiple severe abnormalities characteristic of FA. Children in the family showed congenital developmental abnormalities and, lymphocytes from patient showed increased sensitivity to MMC, elevated chromosome breakage and pronounced G_2 arrest in response to ICL damage, which are hallmarks of FA cells. However, no hematological abnormalities and cancer were diagnosed in these patients. Thus, authors classify it as an FA-like disorder and assign *RAD51C* as FA-O, a 14th in the FA family of genes (103,130). Interestingly, the levels of FANCD2 and its monoubiquitinated form were not affected in these patients, suggesting a downstream defect in the FA pathway (Figure 2). Sequencing of *RAD51C* exon 5 revealed a homozygous missense mutation (G773A → R258H). Notably, RAD51C arginine 258 residue is highly conserved in various organisms including mice, hamster and chicken, implicating a crucial role of this residue in HR.

The molecular mechanism by which RAD51C regulates HR and ICL repair is poorly understood. Nonetheless, the Chinese hamster RAD51C-deficient irs3 and CL-V4B cells and *RAD51C*-knockout chicken DT40 cells have served as excellent models to understand the biology of RAD51C (56). Using these cells, it will be interesting to study the role of RAD51C R258H in HR-mediated DSBR, in the repair of ICLs, checkpoint function, chromosome segregation and genome maintenance. These studies will provide new insights into the development of an FA-like disorder due to a germline mutation in *RAD51C*. Preliminary studies from our group indicate that RAD51C R258H partially rescues the MMC hypersensitivity of CL-V4B cells compared with WT RAD51C (K.S and G. N., unpublished results). Indeed, Vaz *et al.* (103) found a similar phenotype with irs3 cells, suggesting that RAD51C R258H is a hypomorphic mutant.

How RAD51C contributes to ICL repair, perhaps in collaboration with FA proteins is not clear yet. Given the known functions of RAD51C in HR and its ability to bind branched DNA and Y-junctions (79), it is possible that RAD51C may be required for binding to ICLs to activate the FA pathway. It is probably that the RAD51C-interacting protein XRCC3 also may be involved in ICL recognition. Interestingly, XRCC3 has been shown to interact with FANCG, FANCD1/BRCA2 and FANCD2 in a newly identified complex (131). However, it is not known whether RAD51C is also part of this complex. Furthermore, an epistatic interaction between FA proteins and XRCC2 and XRCC3 has been reported, suggesting the involvement of additional RAD51 paralogs in FA pathway of ICL repair (131,132). Vaz *et al.* (103) found that cellular levels of FANCD2 and its monoubiquitinated form were not affected in primary fibroblasts from a patient with *RAD51C* mutation, suggesting that RAD51C functions outside the FA core complex (Figure 2). Conceivably, RAD51C may be essential for recruiting RAD51 and other repair factors to initiate HR during ICL repair. It is possible that the RAD51C hypomorphic mutant found in FA-like disorder may be able to recognize ICLs to activate FA pathway but is defective in HR during ICL repair. Further investigations are required to test these hypotheses.

RAD51C as a novel cancer susceptibility gene

Breast cancer is the most predominant cancer among females and ∼5% of all the reported breast cancer cases are due to inherited mutations in *BRCA1* and *BRCA2* genes. Individuals affected with germline mutation in *BRCA1* and *BRCA2* tumor suppressor genes also develop ovarian cancer. Interestingly, analyses of tumors from these patients reveal LOH at *BRCA1* and *BRCA2* gene loci with a loss of WT allele and retention of disease predisposition allele (12,133). Genetic and biochemical studies identify a role of BRCA1 and BRCA2 in HR-mediated DSBR in mitotic and meiotic cells. BRCA1 localizes with RAD51 in discrete nuclear foci in S and G_2 phase mitotic cells, and BRCA2 interacts with RAD51 stochiometrically (134,135). Further studies show that BRCA1 and BRCA2 exist in a biochemical complex and colocalize in somatic and meiotic cells (136). Like RAD51 and BRCA1, BRCA2 also localizes to the sites of stalled replication forks in response to hydroxyurea and ultraviolet irradiation (136). *BRCA1* and *BRCA2* null mice die early in development, and cells lacking *BRCA1* or *BRCA2* reveal genome instability in the form of spontaneous chromosome breakage, severe aneuploidy and centrosome amplification, suggesting a role of BRCA1 and BRCA2 in HR and in the maintenance of genome integrity (11,137–140). More direct evidence for the role of BRCA1 and BRCA2 in HR came from studies using a reporter to quantify HR in response to DSBs induced by I-SceI expression. Cells lacking *BRCA1* and *BRCA2* display reduced HR (∼5 fold) compared with WT cells (141,142).

BRCA1 has been shown to undergo phosphorylation at multiple sites by ATM and ATR kinases. Mutation in two of these phosphorylation sites, Ser1423 and Ser1524 fails to correct the radiation hypersensitivity of cells lacking BRCA1, suggesting that these phosphorylations are crucial for DSBR (143). In addition, BRCA1 and ATR colocalizes in response to replication stress induced by hydroxyurea and ultraviolet treatment. Consistent with this observation, BRCA1 has been shown to be phosphorylated by ATR at several sites including Ser1423 (144). BRCA1-mutant cells exhibit an intact G_1–S checkpoint but are defective in IR-induced G_2–M checkpoint. In addition, BRCA1 phosphorylation on Ser1387 by ATM is required for activation of an intra-S-phase checkpoint (145–147). Recent studies show that CHK2-mediated BRCA1 Ser988 phosphorylation is important for HR-mediated DSB repair by suppressing microhomology-mediated non-homologous end joining (148,149). Together these observations support the role of BRCA1 in the coordination of checkpoint activation and HR in response to DNA damage and in maintaining genome stability.

Genetic and biochemical studies indicate that the role of BRCA1 and BRCA2 in HR appears to be in the regulation of RAD51 function. RAD51 was identified as the first protein to interact with BRCA2 (150). The well-conserved BRC repeats in the central part of BRCA2 are essential for direct interaction with RAD51, and this association is critical for HR (11,139,140,151). BRCA2 has been thought to be required for the mobilization of RAD51 into the nucleus and to sites of DNA damage. Consistently, BRCA2-deficient cells fail to show RAD51 nuclear foci formation in response to DNA damage (152). BRCA2 C-terminal domain interacts with another highly conserved protein DSS1 (153), which is required for efficient HR *in vivo* (154,155). The *Ustilago maydis* BRCA2 homolog, Brh2 contains only one BRC domain, and Brh2 together with DSS1 efficiently catalyzes RAD51 polymerization onto RPA-coated double-stranded DNA–ssDNA junction (156). Consistent with this observation, BRC3 and BRC4 repeats and DNA-binding domain of human BRCA2 (BRC3/4-DBD) has been shown to stimulate presynaptic assembly of RAD51 onto ssDNA (157). Furthermore, recent studies demonstrate that full-length BRCA2 facilitates RAD51 filament assembly onto RPA-coated ssDNA (158–160). These results clearly suggest recombination mediator function of BRCA2 in RAD51-promoted HR.

BRCA1 and *BRCA2* are high penetrance breast cancer susceptibility genes that show >10-fold increase in the risk of breast cancer.

Screening of mutations identified additional breast cancer susceptibility genes such as *ATM*, *CHEK2*, *BACHI* (*BRIP1*) and *PALB2* (161). These genes are known to have role in genome maintenance and HR, and mutation in these genes are rare and, confer intermediate risk of breast cancer. In a striking recent study, Meindl *et al.* report an increase in the risk of breast and ovarian cancer for monoallelic germline mutations in *RAD51C* (104,162). Their study provides the first evidence to show that *RAD51C* is a cancer susceptibility gene. They identified a total of 14 mutations in *RAD51C*, which include base insertions, splice site mutations and missense mutations. Among 10 missense mutations, retroviral expression of human *RAD51C* G125V and L138F was unable to rescue the hypersensitivity of Δ*RAD51C* chicken DT40 cells to MMC. Consistent with their observation, expression of *RAD51C* G125V and L138F missense mutants did not restore normal RAD51 foci formation in the *RAD51C* mutant fibroblasts, suggesting an obvious HR defect in these *RAD51C* missense mutants. Screening of the remaining *RAD51C* missense mutants in cell survival studies revealed that, four of the missense mutations, *RAD51C* D159N, G264S, T287A and R366Q partially rescued the MMC sensitivity of DT40 cells that lack *RAD51C*. In contrast, the remaining missense mutants behaved similar to WT RAD51C in MMC sensitivity assay.

The RAD51C amino acid residues that were found mutated in patients susceptible to breast and ovarian cancer are highly conserved in chicken, mouse and Chinese hamster cells, suggesting a critical role of these residues in HR and tumor suppressor function. Using RAD51C-deficient hamster and chicken DT40 cells, it will be interesting to test whether these hypomorphic mutants are defective in HR, DNA damage signaling, centrosome maintenance, and chromosome segregation to gain insights into breast and ovarian cancer susceptibility.

The pedigree analyses by Meindl *et al.* (104) clearly indicate that, like *BRCA1* and *BRCA2*, *RAD51C* is a high penetrance breast cancer susceptibility gene. The gross chromosomal rearrangements observed with *BRCA* gene mutation is probably due to defect in the repair of DSBs and daughter strand gaps by HR mechanism (163,164). The chromosomal abnormality with *BRCA* gene mutation is thought to trigger accumulation of mutations and altered expression of oncogenes leading to tumor progression. Indeed, gross chromosomal rearrangements are the most frequent type of lesions found in human tumors that lead to the loss of tumor suppressor loci (139). The RAD51C HR dysfunction may similarly cause gross chromosomal rearrangements leading to breast and ovarian cancer susceptibility (Figure 2). In agreement with this, chicken DT40 and hamster cells with a *RAD51C* mutation exhibit spontaneous chromosomal aberrations similar to *BRCA* genes (56,63–65). Together, these observations support that *RAD51C* is a caretaker and tumor suppressor gene.

Conclusion and future direction

Despite our understanding of the role of RAD51C in initial and late stages of HR, the underlying mechanism(s) remains obscure. The fact that accumulation of RAD51C at the sites of DSBs is dependent on ATM suggests that phosphorylation of RAD51C by ATM may be one mechanism by which RAD51C assists RAD51 function (Figure 2). Further investigation is required to test this hypothesis. Helicases and nucleases are required for the resolution of HJ structures or other type of recombination/repair intermediates. The lack of any known nuclease or helicase motifs in the RAD51C suggests that the role of RAD51C in resolving HJ structures in the late stages of HR may be indirect. It will be interesting to test whether RAD51C interacts with any of the helicases or nucleases and stimulates its activity.

The RAD51C-dependent CHK2 activation reveals a novel role of RAD51C in DNA damage response and checkpoint function (88). The recent reports of Vaz *et al.* (103) and Meindl *et al.* (104) provide the first evidence of *RAD51C* mutation leading to rare human genetic disorder and cancer . These observations clearly indicate that RAD51C is multifunctional (Figure 2). *RAD51C* mutations that were identified in FA and, breast and ovarian cancer patients reveal that there are independent HR functions of RAD51C in FA and BRCA pathway (130,162). Consistent with this hypothesis, partial loss of RAD51 foci formation was observed in FA cells with *RAD51C* mutation (103), suggesting that RAD51C-regulated HR during ICL repair may be compromised. RAD51C may also regulate HR outside the ICL repair, which is further supported by the fact that RAD51C deficiency shows severe HR defect (∼10-fold), measured by HR reporter and RAD51 foci formation (34,63–65,165,166). In addition, genetic knockout of *RAD51C* is embryonically lethal (70). Further investigation is necessary to understand the independent roles of RAD51C in FA and BRCA pathway.

As discussed above, mutation in *BRCA1* and *BRCA2* causes familial breast and ovarian cancer. These genes are essential for HR and in particular BRCA2 is known to act as recombination mediator helping RAD51 polymerization onto ssDNA. RAD51C also has been implicated as a recombination mediator despite the lack of molecular mechanism. It will be interesting to study whether RAD51C and BRCA2 act in a common pathway or independently to regulate RAD51-mediated HR. The LOH that was observed at *RAD51C* loci in breast cancer patients suggest a critical role of RAD51C in tumor suppressor function. This is further supported by the fact that mice knockout of *RAD51C* is embryonically lethal and show suppression of tumor formation similar to TP53 (167). The identification of *RAD51C* as a high penetrance breast and ovarian cancer susceptibility gene provides a clue that perhaps mutation in other *RAD51* paralogs may cause cancer susceptibility. Indeed, a multistage genome-wide association study identifies that *RAD51B* mutation can lead to breast cancer risk (168).

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