Defects in homologous recombination repair behind the human diseases: FA and HBOC

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Defects in homologous recombination repair behind the human diseases: Fanconi anemia and hereditary breast and ovarian cancer.

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Abstract (100 - 200words)

Hereditary breast and ovarian cancer (HBOC) syndrome and a rare childhood disorder Fanconi anemia (FA) are caused by homologous recombination (HR) defects, and some of the causative genes overlap. Recent studies in this field have led to the exciting development of PARP inhibitors as novel cancer therapeutics, and have clarified important mechanisms underlying genome instability and tumor suppression in HR defective disorders. In this review, we provide an overview of the basic molecular mechanisms governing HR and DNA crosslink repair, highlighting BRCA2, and the intriguing relationship between HBOC and FA.

Introduction

Breast or ovarian cancer is common, and one of the leading causes of mortality in woman in developed countries (Permuth-Wey & Sellers 2009; Toss *et al.* 2015; Pederson *et al.* 2016). While hereditary forms of breast and ovarian cancer (HBOC) are estimated to account for only 5 to 20 % of all cases of breast or ovarian cancer (Permuth-Wey & Sellers 2009; Toss *et al.* 2015; Pederson *et al.* 2016), HBOC has been recently attracting considerable public attention in many parts of the world, including Japan. A large fraction of HBOC cases (Aloraifi *et al.* 2015; Caminsky *et al.* 2016) are caused by monoallelic, i.e. dominant, mutations in either the *BRCA1* or *BRCA2* genes, the well-known tumor suppressors that were identified by linkage analysis in the mid-1990s (Miki *et al.* 1994; Wooster *et al.* 1995) . The lifetime risk of developing breast or ovarian cancer can be up to ~80% in these affected individuals. Mutations in *BRCA1/2* also predispose individuals to cancer in other organs such as prostate or pancreas. The *BRCA* genes are considered to be "caretakers", and they function in homologous recombination (HR) repair, thereby protecting our genome from carcinogenic alterations. Furthermore, cancer genome sequences have revealed an unexpectedly high frequency of HR gene mutations in sporadic cases of high grade serous ovarian cancer (Cancer Genome Atlas Research Network 2011), highlighting an important role of HR in cancer prevention via genome maintenance.

Since the loss of HR activities by the biallelic disruption of either the BRCA1 or BRCA2 genes in mice results in early embryonic lethality (for example see (Ludwig *et al.* 1997)), it was a real surprise to the scientific community that the D'Andrea lab at Harvard University discovered biallelic mutations in *BRCA2* in a subset of patients with

the rare childhood hematological disorder, Fanconi anemia (FA)(FA-D1 subgroup) in 2002 (Howlett *et al.* 2002). These *BRCA2/FANCD1* mutations turned out to be hypomorphic and somehow compatible with life.

FA is very rare, but still the most prevalent among the inherited forms of bone marrow failure syndrome (Auerbach 2009). It is primarily an autosomal recessive disorder that is clinically characterized by congenital malformations, progressive development of hypoplastic anemia and cancer predisposition that often results in hematological malignancies such as acute myelogenous leukemia (AML) or myelodysplasia (MDS) as well as various solid tumors, especially head and neck squamous carcinoma (Alter 2014). FA was first described in 1927 by the Swiss pediatrician Guido Fanconi (Lobitz & Velleuer 2006). Traute Schroeder and colleagues reported spontaneous chromosomal breakage in FA in 1964 (Schroeder *et al.* 1964), and then Sasaki and Tonomura discovered ten years later that FA cells are extremely sensitive to ICL inducing agents such as MMC (Sasaki & Tonomura 1973), resulting from defective interstrand crosslink (ICL) repair. It is now generally accepted that an ICL is repaired through consecutive steps of multiple DNA repair activities including the HR mechanism (Figure 1A)(Duxin & Walter 2015).

More recently, it has been recognized that mutation carriers of some of the FA genes (e.g., parents of the FA patients) may actually develop HBOC, and that the other HBOC genes (i.e., BRCA1) can cause an FA-like disorder when biallelically mutated (Bogliolo & Surrallés 2015). Thus HBOC and the FA genes do overlap to some extent (Table 1 and 2). In a simplified view, it could be said that near total loss of (or hypomorphic) HR repair activities causes the FA phenotype (or sometimes FA-like, see below), while breast and ovarian cancer without constitutive symptoms (i.e., HBOC) is

caused when half of the HR repair activities are disabled. However, HR is severely disabled in the HBOC tumors, by loss of heterozygosity (LOH), promoter methylation, other mechanisms.

In this review, we provide a brief overview of the current understanding of the molecular mechanism of HR repair and ICL repair, highlighting BRCA2, and their relationship to two important human diseases, HBOC and FA.

Genome integrity is maintained by DNA repair

Each day, every cell incurs a large number of DNA lesions that threaten the integrity of the genome. These lesions originate either from an exogenous source (e.g., X-rays or ultraviolet light), or are created endogenously by metabolic pathways (i.e., free radicals or aldehydes) or by programmed cellular activities (i.e., VDJ recombination in developing lymphocytes or meiotic recombination in germ cells) (Hoeijmakers 2001). Alternatively, it is known that replication stress provoked by oncogene activation or fork collision with transcription machineries can induce stalled replication forks that may result in fork collapse in genomic regions such as common fragile sites (Debatisse *et al.* 2012).

A DNA double strand break (DSB) is among the most severe insults to the genome, and it can be repaired by two basic mechanisms termed HR or end joining (NHEJ). HR and NHEJ function in a cooperative and overlapping manner, perhaps paradoxically, can compete with each other (Takata *et al.* 1998; Prakash *et al.* 2015). While NHEJ can function throughout the cell cycle, the HR pathway only functions during S/G2 phase. In essence, NHEJ unites two DNA ends by ligation any requirement of homology, often after processing (i.e., removal or addition of short stretches of nucleotides) of the ends; therefore, the repair process can be error-prone. On

the other hand, HR functions by a "copy-and-paste" mechanism of genetic information transfer from an intact homologous template to the damaged DNA, and therefore it without sequence alteration. HR normally requires replicated DNA (sister chromatid) as template, and this is one of the reasons for HR to be restricted to the S and G2 phases of the cell cycle.

In addition to their role in DSB repair, HR proteins have an important role during S phase, where they function in restarting stalled replication forks or protecting stalled replication forks from collapsing due to nucleolytic digestion (Hashimoto *et al.* 2012; Schlacher *et al.* 2012). For example, loss of *Rad51* in mice (Tsuzuki *et al.* 1996) or chicken DT40 cells (Sonoda *et al.* 1998) causes cell lethality which is accompanied by chromosomal breaks likely due to replication fork collapse. HR is also an integral step during ICL repair, as illustrated in Figure 1A.

Basic molecular mechanisms of HR repair

The mechanisms of HR can be best explained in the context of DSB repair (Figure 1B). To initiate DSB repair through HR, the DSB end needs to be nucleolytically resected to generate 3' single-stranded DNA (ssDNA). This is quickly coated by the trimeric ssDNA binding protein complex replication protein A (RPA). Then RPA complex is displaced by RAD51, which is the central player in mammalian HR repair, resulting in formation of RAD51 nucleoprotein filaments. This reaction is facilitated by mediator proteins including BRCA2 and RAD51 paralogs, and can be monitored as the formation of subnuclear small dots (RAD51 foci) by immunohistochemical detection.

RAD51 is a homolog of *Escherichia coli* RecA, and it mediates the core enzymatic reactions in HR (West 2003). It catalyzes (1) searching for a homologous HR

template (homology search), and then (2) pairing of the ssDNA-RAD51 filament with the template DNA (strand invasion and homologous pairing) once the filament encounters the appropriate homologous double-stranded DNA. These reactions result in the formation of a D-loop that consists of heteroduplex DNA coated with RAD51 and displaced ssDNA. The next step is DNA repair synthesis initiated from the invading 3' ssDNA end. In most instances in mitotic cells, the extended ssDNA is displaced from the template strand, and is then annealed/captured by the other processed single-stranded DNA end (i.e., the other end of the DSB). This mechanism is termed "synthesis-dependent strand annealing (SDSA) pathway" and the final product does not contain crossover events (non-crossover). Less frequently than SDSA, the recombination intermediates are converted into a "double Holliday junction" and subsequently resolved by Holliday junction resolvases (e.g., Gen1 or the SLX4 complex)(Garner *et al.* 2013; Wyatt *et al.* 2013) (with or without crossover) or the BLM helicase complex (without crossover) (Wu & Hickson 2003). For more complete discussion of HR mechanisms such as their relationship with competing NHEJ (the pathway choice) or Holliday junction resolvases, readers should refer to recent excellent reviews (Chapman *et al.* 2012; Sarbajna & West 2014).

Basic molecular mechanisms of ICL repair

An ICL covalently bridges two nucleotides on opposite DNA strands and hampers critical DNA transactions such as DNA replication and transcription. Recent studies from Walter's lab using Xenopus egg extracts and plasmid DNA harboring an ICL provided a comprehensive and persuasive view on the detailed mechanisms of ICL repair (Figure 1A)(Räschle *et al.* 2008; Knipscheer *et al.* 2009; Long *et al.* 2011; Duxin & Walter 2015).

According to this model, two converging replication forks from opposite directions first stall ~20 bp away (-20) from the ICL, and then leading strand synthesis progresses to the -1 position with respect to the ICL, perhaps after removal of the MCM helicase. Next, the ICL and ssDNA regions are recognized, leading to the activation of the checkpoint kinase and the FA pathway. The exact mechanism by which these events are accomplished still remains unclear. DNA strands on both sides of the ICL are incised by recruited nucleases, resulting in DSB formation in one of the sister chromatids. The DNA replication occurs over the incised ICL by a bypass DNA polymerase specialized in translesion synthesis (TLS), such as REV1 or REV3, perhaps after PCNA monoubiquitination by the RAD18 ubiquitin ligase. The DSB is resected, and the core HR reaction is initiated with formation of RAD51 filaments. Finally, the short nucleotide fragment that contains the remnant of the ICL is recognized and removed by nucleotide excision repair (NER). How each component of the FA pathway is involved in the ICL repair is described in the following section.

FA, FA pathway, and ICL repair

So far, nineteen "FA genes" have been identified and are thought to function in the ICL repair pathway (Kitao & Takata 2011; Kottemann & Smogorzewska 2013; Bogliolo & Surrallés 2015; Ceccaldi *et al.* 2016). These FA pathway genes are classified into three subgroups by their functional roles in the ICL repair as explained below (Figure 1C).

The FA core complex and the key downstream complex consisting of FANCD2 and FANCI

The first FA group is the "ubiquitination module", which comprises the E3 ubiquitin ligase complex (termed FA core complex) and its substrates, the FANCD2-FANCI (D2-I) complex (Figure 1C). These genes have been shown to function in HR, mainly

based on Jasin's recombination assay. In this assay, a chromosomal DSB is induced within an integrated recombination substrate by a plasmid-encoded rare restriction enzyme I, SceI, which recognizes a specific 18 bp sequence (Rouet *et al.* 1994). The HR repair pathway then uses homologous DNA segments placed either upstream or downstream of the DSB, resulting in expression of the neomycin resistance gene or GFP. In cells lacking these FA genes, the efficiency of HR repair is substantially decreased in chicken DT40 cells (Yamamoto *et al.* 2003; 2004) or mildly decreased in human cells (Nakanishi *et al.* 2005). How these proteins function in HR is still under investigation; however, milder HR defects in human cells may indicate that these "ubiquitination module" FA genes do not provide "core" HR functions. It seems more likely that they modulate the function of the core HR machineries (such as BRCA2 or CtIP, see below) or TLS polymerase (Kim *et al.* 2012), or they may regulate histone dynamics (Sato *et al.* 2012). This module is reported to be required for incision/unhooking of the crosslink during ICL repair (Klein Douwel *et al.* 2014), and thus functions in the conversion of an ICL to a DSB.

The FA core complex includes FA proteins FANCA, B, C, E, F, G, J, L, M and FA-associated proteins, such as FAAP24, FAAP20, and FAAP100 (Ciccia *et al.* 2007; Ling *et al.* 2007; Leung *et al.* 2012). In response to an ICL and/or a stalled replication fork, the FA core complex is somehow activated downstream of the checkpoint kinase ATR-ATRIP through multiple phosphorylations of FANCI (Ishiai *et al.* 2008; Aloraifi *et al.* 2015), FANCM (Singh *et al.* 2013; Caminsky *et al.* 2016) or FANCA (Miki *et al.* 1994; Wooster *et al.* 1995; Collins *et al.* 2009) and monoubiquitinates FANCD2 at lysine 561, which is a critical activating event in the FA pathway (Garcia-Higuera *et al.* 2001; Matsushita *et al.* 2005; Cancer Genome Atlas Research Network 2011). Recent

studies indicate that ubiquitin-like with PHD and RING finger domain 1 (UHRF1) protein functions as an ICL recognition factor and may participate in these steps (Ludwig *et al.* 1997; Liang *et al.* 2015; Tian *et al.* 2015). Recently, we and two different groups identified *UBE2T,* which encodes an E2 ubiquitin-conjugating enzyme, as a causative gene for FA (Howlett *et al.* 2002; Hira *et al.* 2015; Rickman *et al.* 2015; Virts *et al.* 2015). *UBE2T/FANCT* is essential for this monoubiquitination event to proceed. FANCI is a paralog of FANCD2 and its binding partner, and also undergoes monoubiquitination dependent on the core complex and monoubiquitination of FANCD2 (Sims *et al.* 2007; Smogorzewska *et al.* 2007; Ishiai *et al.* 2008; Auerbach 2009). The monoubiquitinated D2-I complex (often referred to as the "ID complex") is recruited and accumulates in foci at damaged chromatin, perhaps by binding to the stalled fork itself (with or without an ICL) (Joo *et al.* 2011; Alter 2014). Focus formation is likely to function as a platform to recruit the numerous proteins required for homologous recombination (HR) and translesion synthesis (TLS) to the damage site. However, the mechanism by which it orchestrates the repair machinery is still not entirely clear.

FANCM is a human homolog of the Archeal helicase/nuclease Hef gene (Komori *et al.* 2004; Lobitz & Velleuer 2006), which encodes a DNA translocase (Schroeder *et al.* 1964; Meetei *et al.* 2005). It is necessary for chromatin loading of the FA core complex (Sasaki & Tonomura 1973; Kim *et al.* 2008) and checkpoint activation (Huang *et al.* 2010; Duxin & Walter 2015), and it plays a distinct role in bypass replication past an ICL by promoting "traverse" of the lesion (Huang *et al.* 2013; Bogliolo & Surrallés 2015). Biallelic *FANCM* mutations were identified in a single patient, but the defects in cells from the patient could not be reversed by the expression

of wild type *FANCM* (Meetei *et al.* 2005). This was later found to be due to the presence of simultaneous mutations in *FANCA* (Singh *et al.* 2009). Thus, to date, there have been no human FA patients identified with causative mutations solely in *FANCM*. Furthermore, in the Finnish population, individuals with homozygous loss-of-function *FANCM* mutations do not show any FA phenotype (Lim *et al.* 2014), suggesting that *FANCM* is not a *bona fide* FA gene, though it clearly contributes to the function of the FA pathway (Bogliolo & Surrallés 2015) and is an HBOC gene (Kiiski *et al.* 2014).

Since FANCD2 and FANCI form a dimeric complex that seems quite stable (Sonoda *et al.* 1998; Sato *et al.* 2012; Unno *et al.* 2014), and they are mutually dependent on each other for activating monoubiquitination (West 2003; Smogorzewska *et al.* 2007; Ishiai *et al.* 2008), it has been assumed that these proteins should function together. However, it was discovered recently that FANCI, not FANCD2, has an upstream role for foci formation of the core complex components, like FANCA (Garner *et al.* 2013; Wyatt *et al.* 2013; Castellà *et al.* 2015). It would be interesting to determine which molecule (FANCM versus FANCI) is furthest upstream in localizing the core complex. Further, ATR-phosphorylated FANCI regulates the replicative helicase MCM complex, thereby suppressing dormant origin firing as a distinct function outside of the FA pathway (Wu & Hickson 2003; Chen *et al.* 2015). FANCD2 was also found to interact with MCM helicase independently of its monoubiquitination, where it restrains DNA synthesis in stressed cells, attenuating cell proliferation and carcinogenesis (Chapman *et al.* 2012; Lossaint *et al.* 2013; Sarbajna & West 2014).

The nucleases in the FA pathway

One of the essential events in ICL repair is an incision of the crosslink, which is called ‘unhooking’. In the current understanding, this event depends on the monoubiquitinated

D2-I complex, and is carried out by the nuclease complex SLX4-XPF (Räschle *et al.* 2008; Knipscheer *et al.* 2009; Long *et al.* 2011; Kim *et al.* 2013; Hodskinson *et al.* 2014; Klein Douwel *et al.* 2014; Duxin & Walter 2015). SLX4/FANCP is a large protein that itself can function as a scaffold, and is mutated in FA-P patients (Kitao & Takata 2011; Kottemann & Smogorzewska 2013; Hodskinson *et al.* 2014; Bogliolo & Surrallés 2015; Ceccaldi *et al.* 2016). SLX4 accumulates at chromatin containing DNA damage via its tandem UBZ4 domains. It has been reported that SLX4 is recruited by monoubiquitinated FANCD2 (Rouet *et al.* 1994; Yamamoto *et al.* 2011). However, there is a conflicting report (Yamamoto *et al.* 2003; 2004; Lachaud *et al.* 2014), and the UBZ domain is generally considered to bind to K63-linked polyubiquitin (Nakanishi *et al.* 2005; Kim *et al.* 2011; Lachaud *et al.* 2014). How SLX4 is tethered to the sites of damage, and how the D2-I complex affects unhooking are important issues that need to be resolved.

Of note, biallelic mutations in *XPF* that specifically affect cellular sensitivity to ICLs but not UV were identified among unclassified FA patients. Now this group of patients is termed FA-Q. *XPF* was originally identified as one of the causative genes for a UV-sensitive disorder, Xeroderma Pigmentosum (XP). This is an interesting example of distinct phenotypes due to specific mutations affecting different features of a single protein.

Another nuclease FAN1, which associates with monoubiquitinated FANCD2, was also thought to function for ICL repair in an FA pathway-dependent manner. However, quite recently, Lachaud et al. demonstrated that FANCD2 monoubiquitination-dependent FAN1 recruitment is dispensable for ICL repair function of FAN1 but is required for DNA replication fork progression and the prevention of

chromosome abnormalities (Kim *et al.* 2012; Lachaud *et al.* 2016).

CtIP is an important nuclease required for end resection of DSBs, and has been shown to interact with BRCA1 as well as the MRN complex. Our lab and others have identified CtIP as a novel interactor of FANCD2 (Sato *et al.* 2012; Murina *et al.* 2014; Unno *et al.* 2014). MMC-induced CtIP recruitment to damage foci is dependent on the interaction with FANCD2, and this recruitment appears to be required for end resection of the DSB generated following unhooking of the ICL. CtIP depletion mildly sensitizes cells to MMC treatment, consistent with the role of CtIP downstream of FANCD2.

The core HR genes in the FA pathway

The group of HR/FA genes that operate in the repair ICLs includes *BRCA2/FANCD1*, *Brip1/FANCJ*, *PALB2/FANCN*, *RAD51C/FANCO,* and *XRCC2*

(Klein Douwel *et al.* 2014; Bogliolo & Surrallés 2015; Ceccaldi *et al.* 2016). *BRCA1 /FANCS* (Ciccia *et al.* 2007; Ling *et al.* 2007; Leung *et al.* 2012; Sawyer *et al.* 2015) and *RAD51/FANCR* (Ameziane *et al.* 2015; Wang *et al.* 2015) genes are recent and surprising additions to this group. Many of these are HBOC genes (Table 1) and *RAD51* mediators. Thus they function as the core HR machinery.

BRCA2/FANCD1 was identified as the first core HR gene implicated in FA (Howlett *et al.* 2002). Since it is a well-known tumor suppressor and HBOC gene, this led to the exciting possibility that mutations in other FA genes would also cause HBOC. However, this prediction turned out to be a bit too simplistic. Genes encoding the core complex components and *FANCD2/FANCI* are unlikely to be a high-penetrance HBOC gene (Seal *et al.* 2003; Berwick *et al.* 2007), although there are some reports indicating *FANCM* (Kiiski *et al.* 2014) or *FANCC* (Berwick *et al.* 2007; Thompson *et al.* 2012)

could be considered HBOC genes.

PALB2/FANCN is the partner and localizer of BRCA2, and also binds BRCA1. It functions as a mediator for RAD51 together with BRCA2, and links BRCA2 and BRCA1. FA-D1 and FA-N patients develop leukemia and kidney or brain tumors at a very early age, with much higher frequency than other FA complementation groups (Hirsch *et al.* 2004; Wagner *et al.* 2004; Reid *et al.* 2007).

Five RAD51 paralogs (e.g, RAD51B, RAD51C, RAD51D, XRCC2, XRCC3) interact in two distinct complexes (Masson *et al.* 2001), Rad51B/C/D/XRCC2 (BCDX2) and Rad51C/XRCC3 (CX3). In addition to BRCA2, they also function as RAD51 mediators. It was confirmed that these two complexes are functionally different from each other (Yonetani *et al.* 2005). Furthermore, analysis of the *C. elegans* Rad51 homolog revealed that Rad51 paralogs remodel pre-synaptic filaments of Rad51 into a stabilized and flexible conformation, which prevents ssDNA degradation by nucleases and Rad51 dissociation. Among these paralog genes, *RAD51C* or *XRCC2* mutations was reported in FA-like patients with physical characteristics and chromosome breakage test results similar to FA, thus these patients are classified as FA-O (Meindl *et al.* 2010; Vaz *et al.* 2010) or FA-U group (Shamseldin *et al.* 2012; Park *et al.* 2016). Cells lacking RAD51 paralog genes generally exhibit a similar phenotype and deficiency in HR (Takata *et al.* 2000; 2001); therefore, it is possible that humans defective in any of the paralog genes may display a similar FA-like phenotype.

Recently biallelic mutations in the *BRCA1* gene have finally been identified in two patients with early-onset ovarian or breast cancer (Domchek *et al.* 2013; Sawyer *et al.* 2015). At least in one of them, a DEB-induced chromosome breakage test was found to be positive, leading to the designation of *FANCS*. BRCA1/FANCS is known to

target BRCA2/FANCD1 to the DSB site via interaction with PALB2/FANCN (Zhang *et al.* 2009). It has been reported that one of the BRCA1 interactors, Brip1 helicase, is responsible for the FA-J subgroup, and these patients display a characteristic (albeit not particularly severe) FA phenotype. It was also recently discovered that a monoallelic mutation in *RAD51* can give rise to FA-like symptoms by a dominant negative mechanism (termed FA-R subgroup) (Ameziane *et al.* 2015; Wang *et al.* 2015). Of note, however, these patients display neither bone marrow failure (BMF) nor childhood cancer.

Interestingly, while FA-D1/N/J patients display the usual constellation of FA symptoms, the patients belonging to FA-O/R/S subgroups do not appear to develop BMF (Bogliolo & Surrallés 2015). Although it is possible that they eventually develop BMF in long-term follow-up, it seems inappropriate to classify these patients as having FA, and at the moment they should be called FA-like. It will be highly interesting to clarify why these patients do not (or tend not to) develop hematopoietic stem cell failure, since patients belonging to FA-D1/N/J/O/R/S all seem to have a similar pathophysiology due to HR deficiency. Of note, a recent study demonstrated that *BRCA1* deficiency specifically in mouse bone marrow causes hematopoietic defects (Vasanthakumar *et al.* 2016).

Structure and function of BRCA2 in the HR pathway

General Features: The *BRCA2* primary structure is depicted in Figure 2. BRCA2 is a huge protein that encompasses 3,418 amino acids in humans. Shahid et al. recently revealed the structure of full-length BRCA2 as being a dimer, using cryo-EM 3D reconstruction (Shahid *et al.* 2014). The sequence conservation among BRCA2 orthologs among various species is mostly limited to the N-terminal, the middle part,

and the C-terminal regions, therefore, these regions may be more important for genome maintenance (Takata *et al.* 2002).

N-terminus: In the BRCA2 N-terminus, there is a region that binds to PALB2/FANCN (Murzin 1993; Buisson *et al.* 2010; Menzel *et al.* 2011). PALB2 facilitates BRCA2 localization and RAD51 chromatin loading at the damage site. This region of BRCA2 was reported to also bind to EMSY(Hughes-Davies *et al.* 2003). The EMSY-binding region of BRCA2 is in exon 3, which is known to be deleted in cancer (Hughes-Davies *et al.* 2003; Cousineau & Belmaaza 2011) . The *EMSY* locus is amplified in sporadic breast cancer (13%) and higher-grade ovarian cancer (17%). At the cellular level, EMSY overexpression leads to defective HR (Cousineau & Belmaaza 2011), consistent with the notion that the EMSY binding region of BRCA2 is likely to coincide or overlap with the PALB2-interacting region.

BRC repeats: In the middle part (the residues between 990 and 2100), BRCA2 harbors eight tandem BRC repeats, each consisting of about 30 amino acids, which are well conserved across human, mouse, rat, and chicken (Takata *et al.* 2002; Yang *et al.* 2002). Not only the sequences themselves but also the spacing between them is well conserved across species. These individual repeats are the primary motifs through which BRCA2 binds to RAD51, and they are essential for BRCA2 function in HR as shown by mouse knockout studies (Connor *et al.* 1997; Ludwig *et al.* 1997; Friedman *et al.* 1998; Patel *et al.* 1998; Jonkers *et al.* 2001). BRCA2 stimulates RAD51 assembly onto ssDNA, and the BRC repeats are critical for this, perhaps by acting cooperatively. It is still unclear whether each BRC repeat can have a distinct function or they can act in a redundant manner. Supporting the former possibility, BRC missense mutations disrupting the interaction with RAD51 have been identified in breast cancer patients

(Pellegrini *et al.* 2002), and conservation between the repeats in a given species is relatively low. It was also shown that BRC1-4 have a higher affinity to RAD51 monomers than BRC5-8; therefore, BRC repeats may not be functionally equivalent (Carreira & Kowalczykowski 2011). On the other hand, it was shown that an artificial fusion gene consisting of a single BRC repeat and RPA can display HR function (Saeki *et al.* 2006).

DBD: In the C terminus of BRCA2, there is a DNA binding domain (DBD) containing three oligonucleotide binding (OB) folds, a tower domain, and a helix-turn-helix (HTH) motif. The DBD not only interacts with single-stranded DNA but also DSS1(Yang *et al.* 2002), which is a small 70 amino acid protein identified from the genomic region on chromosome 7q21.3 that was deleted in an inherited developmental syndrome, Split Hand/Foot Malformation. Recently DSS1 has been reported to target BRCA2 to RPA (Zhao *et al.* 2015), and it functions in the replacement of RPA with RAD51 on resected ssDNA (see below).

According to the breast cancer information core database, more than 25% of cancer-associated missense mutations map to the C-terminal region (Szabo *et al.* 2000) (residues 2500–2850), which includes the sequence through which BRCA2 binds to DSS1 (Jeyasekharan *et al.* 2013). Recent analysis of cancer-associated BRCA2 mutations has led to the identification of a nuclear export signal (NES) in the C terminal region of BRCA2 (Jeyasekharan *et al.* 2013). The NES is masked by the interaction with DSS1. Interestingly, a common cancer-associated BRCA2 mutation, D2723H, impairs the binding of BRCA2 to DSS1, leading to its mislocalization to the cytoplasm and disruption of Rad51 loading onto damaged chromatin. Notably, this mutation is likely to decrease Rad51 foci formation even in the presence of normal BRCA2,

suggesting that this mutation acts in a dominant negative manner.

The three OB domains in BRCA2 are structurally very similar to the canonical OB fold, like the one in RPA (Murzin 1993), consisting of a highly curved β sheet that closes on itself to form a β barrel. The OB2 and OB3 folds both have the obvious groove that is characteristic of the ssDNA binding sites of canonical OB folds (Yang *et al.* 2002). Using electron microscopy, Thorslund et al. unveiled that purified human BRCA2 selectively binds to single-stranded DNA in tailed duplexes and replication fork structures. The Tower domain is capable of binding duplex DNA; however, full-length BRCA2 is likely to interact primarily with ssDNA.

C-terminal RAD51 binding site: Esashi and her colleagues demonstrated that Rad51 directly interacts with a region near the BRCA2 C-terminal end that has no homology with the BRC repeats. Phosphorylation of this region at Ser3291 by cyclin-dependent kinase (CDK) disrupts the C-terminal BRCA2-Rad51 interaction (Esashi *et al.* 2005). The level of phosphorylation at this residue is low during S phase when Rad51 activity is high, and increases as cell enters mitosis. DNA damage elicits a block of this phosphorylation, suggesting that this modification can modulate BRCA2 activity. Unlike BRC repeats, the C terminal Rad51-binding domain selectively interacts with RAD51 oligomers and RAD51 nucleoprotein filaments. This region protects RAD51 nucleoprotein filaments formed on ssDNA from dissociation by the BRC repeats. The FA-D1 patient cell line EUFA423 expresses truncated BRCA2 lacking the C-terminal 192 amino acid residues, which means that the C-terminal RAD51–binding domain (residues 3265–3330) is lost in this patient. This cell line showed impairments in RAD51 focus formation and HR activity (Wang *et al.* 2004) . In addition, an individual with HBOC has been reported to carry a deletion of the C-terminal 224 residues of

BRCA2 (Håkansson *et al.* 1997). These observations underscore the importance to tumor suppression of the C-terminal Rad51 binding region that drives Rad51 nucleoprotein filament formation (Esashi *et al.* 2007; Ayoub *et al.* 2009).

Mediator function of BRCA2 in loading of RAD51 onto ssDNA

RAD51 protein itself can bind to both ssDNA and dsDNA. In HR, because RAD51 must initially bind to resected ssDNA tails at the DSB, and the ssDNA is quickly coated with RPA, RAD51 requires a targeting factor that mediates its interaction with ssDNA. It has been shown that full-length purified human BRCA2 is able to enhance RAD51 presynaptic assembly on RPA-coated ssDNA, promoting RPA-RAD51 exchange. BRCA2 can stimulate Rad51 ssDNA binding *in vitro*, while inhibiting the ability of RAD51 to bind dsDNA (Carreira *et al.* 2009; Shivji *et al.* 2009). Mechanistically, BRCA2 stabilizes ATP-bound RAD51-ssDNA filaments by blocking ATP hydrolysis (Carreira *et al.* 2009; Jensen *et al.* 2010). Unlike yeast Rad52, which plays a dominant mediator role for yeast Rad51, BRCA2 does not bind RPA directly. How, then, does BRCA2 regulate RPA-Rad51 exchange?

A key factor turns out to be DSS1, the small and highly acidic protein that interacts with OB1 of BRCA2 (Yang *et al.* 2002). Liu et al. found that purified human DSS1 in the presence of BRCA2 stimulates RAD51 binding to RPA-covered ssDNA, compared with BRCA2 alone. In contrast, DSS1 alone does not activate RAD51 binding to RPA-ssDNA. Furthermore, Zhao and his colleagues demonstrated that DSS1 targets BRCA2 to RPA, and DSS1 functions as a DNA mimic to promote removal of RPA from ssDNA, thereby promoting exchange with RAD51 on ssDNA (Zhao *et al.* 2015).

It is known that there are other molecules involved in RAD51 regulation. These include RAD51AP1 (Wiese *et al.* 2007) and TONSL/MMS22L (Duro *et al.*

2010; O'Donnell *et al.* 2010). Further analysis will shed more light on the possible interplay between these proteins and HR mechanisms underlying disorders like FA or HBOC.

Additional regulators of HR and RAD51 function

XPG, which is affected in xeroderma pigmentsum complementation group G (XP-G), has been reported to form a complex with BRCA2 and DSS1. Trego et al. searched for novel XPG partners and unexpectedly found that XPG interacts with BRCA2, RAD51, and PALB2 (Trego *et al.* 2016). XPG forms foci in S phase, but not in G1 cells. Because XPG depletion caused a decreased presence of Rad51 and BRCA2 in the chromatin fraction, this protein is likely to contribute to HR.

Foci formation by proteins involved in HR, including BRCA1 and Rad51, is tightly regulated during the cell cycle, and they normally accumulate at the site of DNA damage in S and G2 phase, when the cell has sister chromatid DNA. The mechanism by which BRCA1 foci formation is antagonized in G1 phase by proteins that inhibit DNA end resection, such as 53BP1 and RIF1, has been revealed recently (Chapman *et al.* 2013; Escribano-Diaz *et al.* 2013; Zimmermann *et al.* 2013). In addition to this mechanism, it was recently reported that PALB2-BRCA2 cannot bind to BRCA1 specifically in G1 phase owing to the ubiquitination of PALB2. This modification is mediated by the KEAP1-CRL3 ubiquitin ligase, leading to the suppression of HR in G1 (Orthwein *et al.* 2015). Furthermore, this PALB2 ubuiquitination is antagonized by a deubiquitinase USP11. Interestingly, *KEAP1* mutations have been reported in breast cancers (Hartikainen *et al.* 2015).

Genome maintenance and tumor suppression by BRCA2

As discussed above, monoallelic *BRCA2* mutation causes HBOC, while biallelic mutations are characteristic of the FA-D1 subgroup, which displays a particularly severe form of FA, with very early onset of leukemia and solid tumors (Hirsch *et al.* 2004; Wagner *et al.* 2004). The malignancies observed in FA-D1 patients are not breast or ovarian cancer; however, this is not surprising since these patients are infants whose endocrine and reproductive systems are immature. *BRCA1* deficient breast cancers are typically "basal-like" and "triple negative" for epidermal growth factor receptor2 (HER2), progesterone receptor, and estrogen receptor, and they are more recalcitrant to conventional therapy. On the other hand, breast cancer stemming from mutated *BRCA2* is clinically categorized as the "luminal-type" which is also the case for a common sporadic form of breast cancer (Roukos & Briasoulis 2007).

In line with the two-hit hypothesis proposed by Knudson (Knudson 1971), it has been considered that the malignant cells in patients carrying monoallelic *BRCA2* mutations obligatorily harbor LOH affecting the wild type allele, leading to the loss of *BRCA2* function. However, a recent study questioned this view, and provided evidence that loss of mutant alleles can occur in *BRCA*-associated breast cancer (King *et al.* 2007). Thus, in addition to the loss of all *BRCA* function, *BRCA* haploinsufficiency may also promote carcinogenesis, and some of the cancers arising in the HBOC patients may not be HR deficient. Indeed *BRCA1*+/- mutated cells are defective in response to replication stress (Pathania *et al.* 2014). As mouse model studies showed (Ludwig *et al.* 1997; Jonkers *et al.* 2001), in carcinogenic steps, the cells tend to lose *Tp53* (or an equivalent checkpoint gene) prior to loss of BRCA/HR function in order to avoid cell death and/or senescence.

Therapeutic implications of HR defects in HBOC

Defective HR is an important target for chemotherapy in HBOC patients (Konstantinopoulos *et al.* 2015). Platinum-based chemotherapy is a well-established and widely used modality for cancer treatment. Since cisplatin and its derivative carboplatin induce intra- and interstrand crosslinks (Deans & West 2011), *BRCA*-deficient, hence ICL repair-deficient HBOC cells are naturally sensitive to these drugs (De Picciotto *et al.* 2016). Indeed, *BRCA*-mutated HBOC patients appear to have a better prognosis compared to non-BRCA patients, perhaps owing to the better response to chemotherapy (Konstantinopoulos *et al.* 2015). Furthermore, based on the discovery that PARP inhibition induces a dramatic cell killing in cells deficient in HR (Bryant *et al.* 2005; Farmer *et al.* 2005), an exciting opportunity to develop novel chemotherapeutic drugs has emerged. This is an instance where two distinct but important DNA repair activities are simultaneously inhibited, leading to cell death (synthetic lethality). An initial explanation that this lethality was due to impaired base excision repair, with an increased level of single strand breaks that are converted to toxic DSBs by replication, is now challenged, and revised models have been proposed (Helleday 2011; Konstantinopoulos *et al.* 2015).

Resistance to chemotherapy drugs invariably appears after the initial clinical response during prolonged treatment. A number of resistance mechanisms have been proposed. It has been suggested that secondary mutations in *BRCA* genes that restore the wild type reading frame, leading to recovered HR activity, are the major mechanisms for the acquired resistance (Edwards *et al.* 2008; Sakai *et al.* 2008). This is analogous to the reversion mosaicism in hematopoietic cells sometimes observed in FA patients, which may mitigate progression of bone marrow failure (Soulier *et al.* 2005). Genome instability due to HR defects and selection may contribute to these phenomena.

Another interesting mechanism for acquired resistance in *BRCA2*-deficient tumors without restoring HR is the loss of the nucleosomal remodeling factor CHD4 (Guillemette *et al.* 2015). In *BRCA1*-mutated tumors, normal levels of HR activities might be restored by the loss of *53BP1* or *REV7/MAD2L2* (Bouwman *et al.* 2010; Boersma *et al.* 2015; Xu *et al.* 2015). In the absence of *BRCA1*, these genes function to prevent end-resection of DSBs, blocking the subsequent HR reaction.

BRCA-deficient tumors may accumulate an enormous number of mutations due to HR defects and genome instability during the carcinogenic process. Thus, these cells may carry higher numbers of tumor-specific peptide antigens that are presented to tumor infiltrating lymphocytes. This hypothesis has been tested in clinical samples, leading to the conclusion that *BRCA1/2*-mutated high-grade serous ovarian cancer may be more sensitive to recently developed immune checkpoint inhibitors, such as anti-PD-1 or anti-PD-L1 antibodies (Strickland *et al.* 2016).

How defects in HR and ICL repair affect hematopoietic stem cells or promote cancer development?

Deficiencies in DNA repair limit the renewal capacity of aging hematopoietic stem cells (Rossi *et al.* 2007), and FA patients have higher levels of DNA damage in these cells, leading to upregulation of p53 and cell death/senescence (Ceccaldi *et al.* 2012). The origin of the endogenous damage in FA is an important issue that needs to be resolved (Garaycoechea & Patel 2014), because this knowledge may allow us to develop a novel strategy for preventing bone marrow failure and cancer. Likewise it is important to know by what mechanism the genome is destabilized in *BRCA* mutation carriers.

Endogenous aldehydes and lipid peroxidation products have been proposed as major sources of spontaneous DNA damage in FA (Garaycoechea *et al.* 2012). Using a

mutant cell panel derived from the chicken DT40 cell line, it was shown that cells lacking *Fancd2* or *Brca2* are particularly sensitive to formaldehyde at concentrations similar to those in normal human serum (Ridpath *et al.* 2007). KJ Patel and colleagues produced a series of papers indicating that aldehyde detoxifying enzymes ALDH2 (which mainly catalyzes acetaldehyde) and ADH5 (which mainly catalyzes formaldehyde) play critical roles in FA model mice in the suppression of bone marrow failure and leukemogenesis (Langevin *et al.* 2011; Garaycoechea *et al.* 2012; Oberbeck *et al.* 2014; Pontel *et al.* 2015). These results clearly indicate that endogenous aldehydes can damage DNA in hematopoietic stem cells. Since East Asians often carry an enzymatically defective *ALDH2* variant allele（*ALDH2*2*）, we examined *ALDH2* genotypes in our cohort of Japanese FA patients (Hira *et al.* 2013). In line with the mouse studies, our results indicated that the *ALDH2* variant allele accelerates the progression of bone marrow failure in these patients. Strikingly, we identified several FA children who had homozygous *ALDH2* mutations; these patients displayed particularly grave symptoms, including an extremely early onset of myelodysplasia (Hira *et al.* 2013; Yabe *et al*. in press). This combined FA-ALDH2 deficiency could be considered to be a distinct disease entity. It will be exciting to see whether *ALDH2* can be a drug target to prevent bone marrow failure in FA patients. A compound that stimulates ALDH2 activity has already been developed (Perez-Miller *et al.* 2010). It will also be interesting to test how *ALDH2* status can affect cancer development in HBOC among East Asian populations.

Another source of DNA damage can be DNA replication fork stalling, which likely contributes to genome instability in FA or HBOC. The nascent DNA strand at the blocked fork is protected by RAD51 filaments stabilized by the C-terminal domain of

BRCA2 in a manner independent of HR (Schlacher *et al.* 2011). In the absence of BRCA2, BRCA1, or FANCD2, the stalled fork cannot be protected and is degraded by MRE11 nuclease, leading to the loss of genetic information or genome rearrangements (Schlacher *et al.* 2012). This mechanism might be important for genome stability and tumor suppression provided by *BRCA1/2* or FA genes.

Genes involved in pre-mRNA splicing and in the biogenesis and export of messenger ribonucleoprotein (mRNP) also have an important role for genome stability (Paulsen *et al.* 2009). R-loops consisting of DNA–RNA hybrids and a displaced single-stranded DNA often arise when transcription is perturbed (e.g., upon collision of transcription bubbles and replication forks). Thus, R-loops may be a chief source of replication stress and cancer-associated genome instability. Bhatia et al. demonstrated the accumulation of R-loops in *BRCA2*-depleted cells (Bhatia *et al.* 2015). Furthermore, recent studies asked whether the FA pathway coordinates transcription-replication conflicts and is involved in R-loop resolution (García-Rubio *et al.* 2015; Schwab *et al.* 2015). Indeed, human and mouse cells deficient in FA gene function accumulate R-loops, indicating that the FA pathway does play a critical role in R-loop resolution. MMC-induced FANCD2 foci levels are reduced by the expression of RNaseH1, which digests RNA in RNA-DNA hybrids. These studies imply that the accumulation of R-loops might contribute to hematopoietic stem cell exhaustion in FA. It will also be interesting to know how R-loops trigger the activation of the FA pathway.

Of note, it has also been proposed that cytokines that are upregulated in FA, such as TNF-α or TGF-β, may directly harm hematopoietic stem cells or modulate DNA damage repair in the stem cell compartment (Du *et al.* 2014; Zhang *et al.* 2016).

This line of investigation may inform the development of novel therapeutic strategies for FA.

Conclusions

As summarized in this review, there has been a lot of progress toward the mechanistic understanding of HR repair and genome stability in this decade. Furthermore, we have seen an exciting development of PARP inhibitors as novel and promising cancer therapeutics. Detailed knowledge about the pathogenesis of HBOC and FA has been obtained.

Despite this progress, obvious questions are still lingering in the field. It is a true enigma that HR deficiency leads to carcinogenesis in a tissue-specific manner, although such specificity is also often the case for the other hereditary cancer syndromes. From a practical point of view, a large number of variants of unknown significance (VUS) generated from genetic testing of *BRCA1* or *BRCA2* pose a significant problem in the interpretation of the test results. In the long run, accumulated knowledge about segregation of the genotype and an individual's cancer susceptibility within families may eventually clarify the significance of VUS. At the moment, careful evaluation of DNA repair capacity in lymphocytes from cases with VUS might be useful (Pathania *et al.* 2014; Vaclová *et al.* 2015). It would be particularly useful to construct a collection of isogenic knock-in cells with candidate variants using the CRISPR-CAS9 system (Paquet *et al.* 2016). Endogenous aldehydes may include at least several molecular species (Xie *et al.* 2016), and they may induce various types of DNA damage, such as monoadducts, interstrand crosslinks, or DNA-protein crosslinks. Which of these actually contributes to FA pathology, and how endogenous aldehydes are generated in cells should be elucidated in the near future.

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Table 1 Hereditary breast and ovarian cancer (HBOC) risk genes.

Gene	Syndrome with germ line mutations	Functions	Cancer type	Penetrance
BRCA1	HBOC	Homologous recombination	Breast and ovarian cancer	High
BRC*A2*	HBOC	Homologous recombination	Breast and ovarian cancer	High
PTEN	Cowden syndrome, PTEN hamartoma	Phosphatidylinositol 3-phosphate, suppresses AKT signaling	Breast cancer	High
TP53	Li-Fraumeni syndrome	Transcription factor, regulates cell cycle, apoptosis, senescence	Breast and ovarian cancer	High
CDH1	Hereditary diffuse gastric cancer syndrome	E-cadherin gene, maintains cell adherence	Breast and ovarian cancer	High
STK11	Peutz-Jeghers syndrome	Serine/threonine kinase, regulates cell polarity	Breast and ovarian cancer	High
NBS1	Nijmegen breakage syndrome	Cell cycle checkpoint after DNA damage, member of the MRN complex	Breast cancer	High
NF1	Neurofibromatosis type I	Negative regulator of Ras signaling	Breast cancer	High
ATM	Ataxia Telangiectasia	PI3 kinase-related kinase, cell cycle checkpoint and DSB repair	Breast cancer	Moderate
CHK2	Li-Fraumeni syndrome	Activation of cell cycle checkpoint after DNA damage	Breast and ovarian cancer	Moderate
FANCJ	Fanconi anemia	Interstrand crosslink repair	Breast and ovarian cancer	Moderate

FANCM	Fanconi anemia	Interstrand crosslink repair	Breast cancer	Moderate
PALB2	Fanconi anemia	Interstrand crosslink repair, homologous recombination	Breast and ovarian cancer	Moderate
RAD51C	FA-like syndrome	Interstrand crosslink repair, homologous recombination	Breast and ovarian cancer	Moderate

Table 2 Fanconi anemia (FA) and FA-like syndrome genes.

Gene	Synonym	Functions	Symptoms	Heterozygous germline mutation
FANCA		Component of the FA core complex	FA pathologies	
FANCB		Component of the FA core complex	FA pathologies	
FANCC		Component of the FA core complex	FA pathologies	
FANCD1	*BRCA2*	HR repair, recruits RAD51 onto DNA, interacts with FANCN, Stalled replication fork protection	FA pathologies, not all patients show bone marrow failure	HBOC
FANCD2		Ubiquitinated after DNA damage, Stalled replication fork protection	FA pathologies	
FANCE		Component of the FA core complex	FA pathologies	
FANCF		Component of the FA core complex	FA pathologies	
FANCG	*XRCC9*	Component of the FA core complex	FA pathologies	
FANCI		Ubiquitinated after DNA damage, required for FA core complex activation	FA pathologies	
FANCJ	*BACH1,BRIP1*	ICL repair, HR repair, 3'to 5' helicase, interacts with BRCA1	FA pathologies	HBOC
FANCL	*PHF9*	Component of the FA core complex, E3 ubiquitin ligase	FA pathologies but no cancers	
FANCM	*Hef*	DNA translocase, required for FANCI-D2 ubiquitination	Unknown, the only known patient also has a FANCA mutation	HBOC
FANCN	*PALB2*	HR repair, interacts with BRCA1 and BRCA2, facilitates BRCA2 function	FA pathologies	HBOC
FANCO	*RAD51C*	RAD51 paralog, HR repair, RAD51 nucleoprotein filament stability	FA-like syndrome, no bone marrow failure and cancer	
FANCP	*SLX4*	Coordinates XPF-ERCC1, interacts with Mus81-EME1 and SLX1 nucleases	FA pathologies	

FANCQ	*XPF,ERCC4*	Endonucleases, associates with ERCC1, ICL unhooking	FA pathologies	HBOC
FANCR	*RAD51*	HR repair, stalled fork protection	FA-like syndrome, no bone marrow failure and cancer	
FANCS	*BRCA1*	HR repair, promotes RAD51 recruitment, interacts with FANCN	FA-like syndrome, no bone marrow failure	HBOC
FANCT	*UBE2T*	E2 ubiquitin-conjugating enzyme for FANCD2 complex, interacts with FANCL	FA pathologies	
FANCU	*XRCC2*	RAD51 paralog, HR repair, RAD51 nucleoprotein filament stability	FA-like syndrome, no bone marrow failure	

Figure legends

Figure 1. Homologous recombination (HR) repair and Fanconi anemia (FA) pathway-mediated interstrand crosslink (ICL) repair.

(A) An overview of the ICL repair pathway. When a replisome collides with an ICL (I), the leading strand initially stalls -20 nucleotides away from the lesion (II). After a second fork converges at the ICL, BRCA1 facilitates dissociation of the CMG complex (consisting of Cdc45, MCM2-7 and GINS) from chromatin at the stalled fork, allowing the leading strand to approach to the -1 nucleotide adjacent to the ICL (III). In the next step, the SLX4X-XPF-ERCC1 complex incises the DNA and unhooks the lesion in a FA pathway-dependent manner (IV). Translesion synthesis (TLS) polymerase (Polζ, or possibly Polκ,Polη, or Polι) extends the leading strand synthesis past the unhooked, ICL-associated nucleotide (V), and HR and the nucleotide excision repair (NER)

pathway repair the remaining lesion (VI).

(B) Schematic of HR pathway. When a double-strand break (DSB) is generated after DNA replication during S and G2 phase, both strands are resected in the 5' to 3' direction to generate 3' overhangs. Almost immediately, replication protein A (RPA) is loaded onto the single-stranded (ss) DNA, and then replaced by a RAD51 nucleoprotein filament in a process requiring BRCA1-PALB2- BRCA2. RAD51 carries out strand invasion of the sister chromatid by the ssDNA tail and extends the resulting D-loop formation. In synthesis-dependent strand annealing (SDSA), the D-loop structure quickly dissociates from the ssDNA after synthesis of a complementary single strand, and then another strand anneals with a processed ssDNA. An alternate pathway forms a double Holliday junction (dHJ). After second end capture and fill-in synthesis, the Holliday junction is dissociated by the TopIIIα-BLM complex or resolved by resolvase complexes that contain SLX4, Mus81, and GEN1.

(C) The mechanism of FA pathway activation. Upon replication fork stalling, FANCM-FAAP24- MHF1/2 complex binding at the ICL lesion activates ATR signaling, followed by the recruitment of the FA core complex. FANCI is phosphorylated by ATR and subsequently the FA core complex with UBE2T mediates monoubiquitination of the FANCI-FANCD2 (D2-I) complex. This modification targets the D2-I complex to chromatin and leads to multiple events that repair the ICL lesion.

Figure 2. The primary structures of human BRCA1 and BRCA2. BRCA1 consists of 1863 amino acids. The RING domain is in the N terminus and partly overlaps with the BARD1 binding region. BRCA1 interacts with PALB2 via a coiled-coil region in the C terminus. The BRCT motif is a phosphorylated protein binding sequence, and this motif

mediates the association of BRCA1 with Abraxas, CtIP, and BACH1, which are all known factors in DSB repair. BRCA2 comprises 3418 amino acids. The PALB2 binding region is located in the amino (N) terminus. In the center, BRCA2 harbors eight of BRC repeats, which constitute a binding region for RAD51 monomers. In the carboxy (C) terminus, a DNA-binding domain contains three OB folds and a region that interacts with DSS1. The C-terminal region of BRCA2 is also important for the formation of the RAD51 nucleoprotein filament and BRCA2 nuclear localization.

Table 1. Hereditary breast and ovarian cancer (HBOC) risk genes.

Table 2. Fanconi anemia (FA) and FA-like syndrome genes. There are already 20 distinct genes identified in these syndromes, and all proteins are required for interstrand crosslink (ICL) repair. Heterozygous germline mutations in several genes are also related to HBOC.

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Figure 1

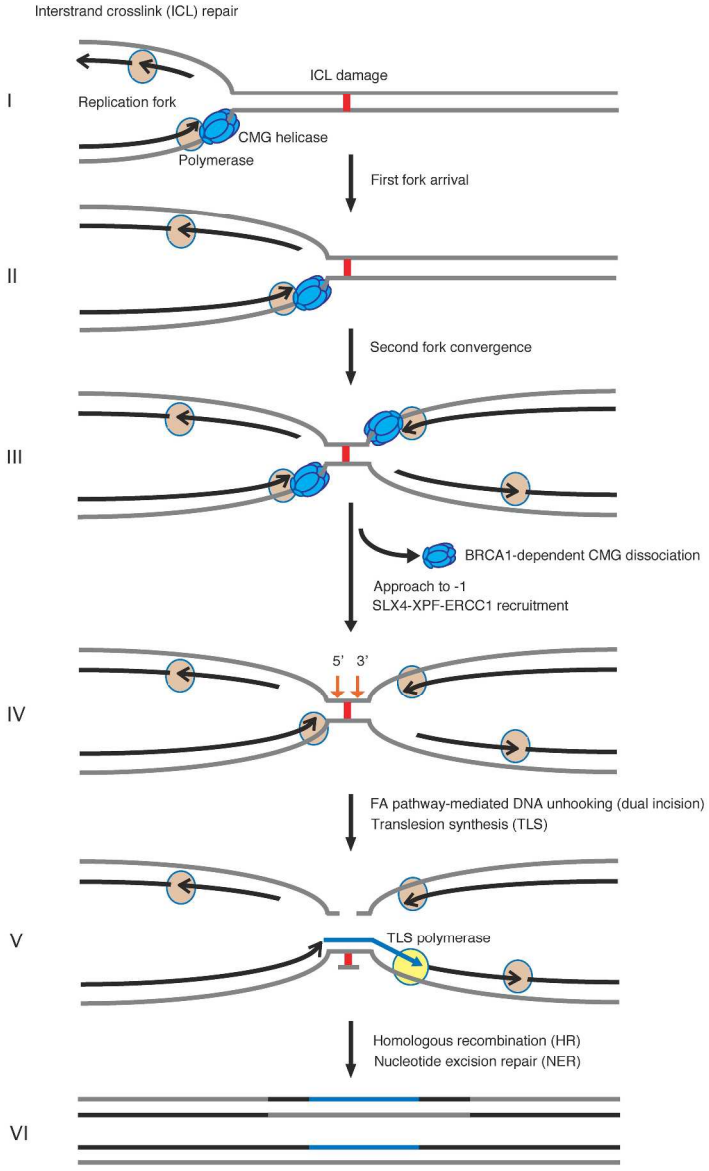

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Figure 1

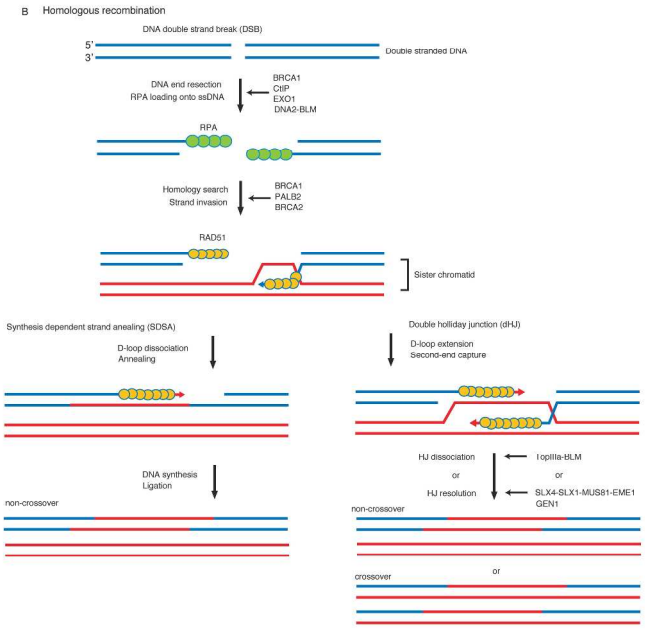

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Figure 1

C Fanconi anemia (FA) pathway

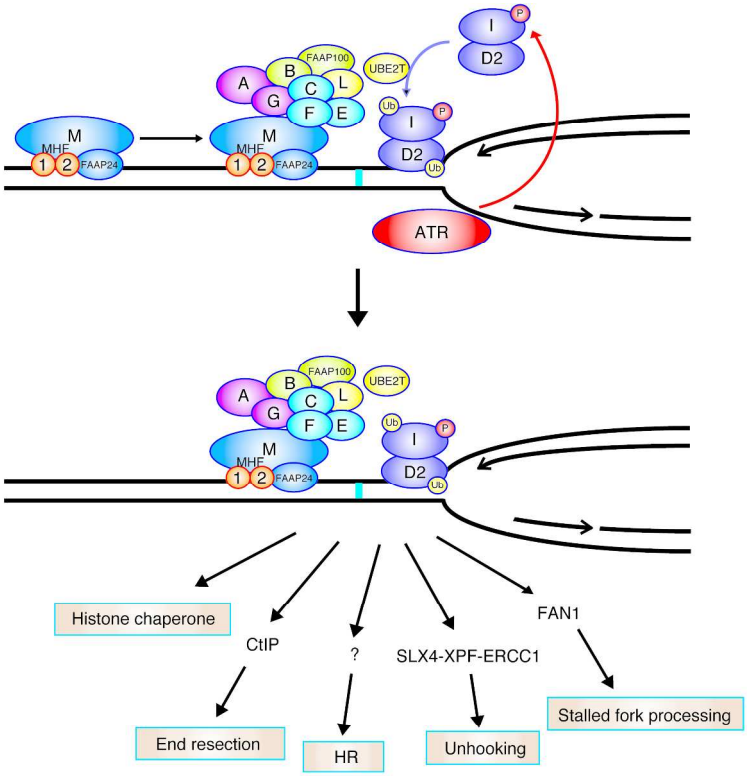

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Figure 2 Primary structures of human BRCA1 and BRCA2