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Hereditary breast cancer and the BRCA1-associated FANCD1/BRIP1

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Abstract

It is clear that *FANCD1*, also known as *BACH1* or *BRIP1*, is an essential tumor suppressor gene based on the identification of clinically relevant mutations not only in breast cancer, but also the childhood cancer syndrome, Fanconi anemia. This conclusion is further supported by the direct and functional interaction between FANCD1 and the hereditary breast cancer-associated gene product BRCA1. In the absence of the FANCD1 DNA helicase or its interaction with BRCA1, cells have defects in several aspects of the DNA damage response. In particular, the BRCA1–FANCD1 interaction is essential for promoting error-free repair, checkpoint control and for limiting DNA damage tolerance. As the number of *FANCD1* clinical mutations and affected patients accumulate, it will be critical to understand whether the associated tumors resemble BRCA-associated tumors. If so, *FANCD1* patients could also benefit from new therapies that selectively sensitize DNA repair-defective tumors and spare healthy cells. In this article, we summarize the breast cancer-associated FANCD1 mutations and discuss functional outcomes for DNA repair and tumor suppression.

Keywords

BACH1; breast cancer; BRIP1; DNA repair; FANCD1; Fanconi anemia

Hereditary breast cancer research reaches the clinic

The story of hereditary breast cancer is at a turning point. Two decades of research brings high hopes for affected patients with the advent of targeted drugs. Heterozygous carriers of mutations in the hereditary breast cancer genes *BRCA1* or *BRCA2* have a 60–80% lifetime risk of breast cancer [1]. Uncovering the functional defect associated with *BRCA* mutations was challenging given that the *BRCA* genes encode large protein products with little homology to other proteins of known function. With thousands of published manuscripts, the consensus is that these proteins have numerous and diverse functions. The context of when BRCA function is lost can affect whether cells fail to proliferate and undergo apoptosis or incur mutations and undergo tumorigenesis. The key to targeted therapy, however, is that BRCA1 and BRCA2 have common functions in DNA double-strand break repair in the subpathway of homologous recombination [2,3]. Evidence implicates BRCA1 in the initiation of recombination by facilitating the resection of broken DNA ends.

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Subsequently, these DNA ends become competent for recombination when BRCA2 binds and loads RAD51, which mediates strand invasion of duplex DNA with homologous sequences [4]. Thus, BRCA-mutated cancer cells are selectively sensitive to agents that require repair of double-strand breaks by homologous recombination, such as PARP1 inhibitors [5–7]. At present, BRCA mutation carriers, unlike other breast cancer patients, have greater insight into what type of treatment will be effective on their tumors. The question remaining is how to enhance the efficacy of these agents and whether treated tumors will develop resistance.

This bench-to-bedside story prompts one to ask whether other hereditary breast cancer genes exist and whether associated tumors will have defects in DNA repair. In support of this idea, purification of BRCA protein complexes has led to the identification of a DNA damage response pathway composed of BRCA1, BRCA2 and several other functional partners, including the BRCA1-associated C-terminal DNA helicase BACH1, also known as BRIP1 or FANCF (the subject of this article), BRCA1-interacting receptor associated protein 80, RAP80 and partner and localizer of BRCA2, PALB2 [8–11]. Correspondingly, these and other genes functioning in the DNA damage response, such as *CHEK2*, *ATM*, *NBS1*, *RAD50*, *TOPBP1* and *RAD51C*, are mutated in breast cancer patients [12–21]. These low-penetrance genes and others that remain to be identified likely account for the remaining non-BRCA familial cases of breast cancer (Figure 1). The emerging picture is that hereditary breast cancer derives from defective DNA repair, with the implication that derived tumors will have sensitivity to current therapies that are effective on BRCA tumors.

Highlighting the importance of these hereditary breast cancer genes in DNA damage repair, mutations in some of these genes also contribute to Fanconi anemia (FA). FA is a rare cancer-prone disease in which patients' cells are characterized by extreme sensitivity to agents that induce DNA interstrand crosslinks (for review, see [22,23]). Unlike breast cancer, FA patients often present as children with congenital abnormalities and develop bone marrow failure and/or leukemia. The estimated frequency of FA is approximately one in 350,000 births spanning a variety of ethnic groups [24]. To date, at least 13 FA complementation groups have been described and designated FANCA–FANCL. To develop FA, patients must inherit two mutated alleles with the exception of one X-linked gene, *FANCB*. For example, bi-allelic mutations in *BRCA2*, also known as the *FANCD1*, gives rise to FA within the FA-D1 complementation group [25]. Similarly, inheriting bi-allelic mutations in *BACH1/FANCF/BRIP1* or *PALB2/FANCL* genes results in the FA subtypes FA-J or FA-N, respectively (Figure 1) [26–30]. Conceivably, *RAD51C* will also join the BRCA-FA family given that mutations were found not only in breast cancer [21], but also in a consanguineous family with severe congenital abnormalities characteristic of FA [31].

Activation of the BRCA-FA pathway is typically measured by the DNA damage-induced monoubiquitination of FANCD2 and, more recently, FANCI [32]. In addition, DNA damage induces the accumulation of BRCA-FA proteins in nuclear foci [33]. These DNA damage-induced responses are likely to be essential for DNA damage recognition and repair. Because these monoubiquitination events are independent of BRCA1, BRCA2/FANCD1, PALB2/FANCL, BACH1/FANCF/BRIP1 or *RAD51C*, these proteins are considered to function downstream in the BRCA-FA pathway. Interestingly, to date, only mutations in the downstream BRCA-FA genes have been found to elevate the risk of developing breast cancer. Here, we focus on one of these players, BACH1/FANCF/BRIP1 (herein designated FANCF), and the progress made in understanding the functional consequences of breast cancer-associated mutations.

Identification of FANCI

The primary amino acid sequence of BRCA1 provided little evidence as to how BRCA1 functioned as a tumor suppressor. BRCA1 patient mutations were throughout the gene, targeting both the N-terminal RING and the C-terminal BRCT domains [34]. The idea that the BRCT domain was important for DNA damage repair first came from the recognition of this domain in other proteins that are important for genomic integrity [35]. Confirming the essential role of the BRCTs for BRCA1's DNA repair function, a breast cancer cell line that expressed a truncated BRCA1 lacking functional BRCTs was defective in DNA repair and sensitive to DNA damage; defects that were reversed upon complementation with a full-length BRCA1 protein [36]. These findings, together with the high conservation of the BRCA1-BRCT domain throughout evolution [35], suggested that the BRCT domain was essential for the tumor-suppressing function of BRCA1.

To identify how the BRCT domain mediated tumor suppression, a GST-BRCT fusion protein was used in a far western approach to screen for BRCT-interacting proteins. Among the proteins identified was a protein of approximately 130 kDa that failed to interact with a BRCA1, harboring patient mutations within the BRCT motifs (P1749R and M1775R) [8]. The purified 130-kDa protein was subject to tandem mass spectrometry and the *FANCI* gene was cloned and found to consist of 20 exons spanning greater than 180 kb of genomic sequence in chromosome 17q22. Most notably, the protein product had strong homology to the catalytic and nucleotide-binding domains of other members within the DEAH helicase family, including the xeroderma pigmentosum complementing group D (XPD) [8]. The protein was originally termed BACH1; however, a transcription factor was also cloned with an identical name. Thus, BACH1 is also referred to as BRIP1 (for BRCA1-interacting protein) or FANCI (for Fanconi anemia complementation group J). Since the cloning of FANCI, it was confirmed that the BRCA1-BRCTs mediate a direct interaction with FANCI that is dependent on FANCI phosphorylation at S990 [37]. In addition to FANCI, several proteins, including CTIP and ABRA1/Abraxas, have phospho-SXXF motifs (S is serine, F is phenylalanine and X varies) that mediate the direct interaction with the BRCA1-BRCTs, which are phospho-binding motifs [37–40].

FANCI breast cancer mutations

The idea that FANCI could be a breast cancer tumor suppressor was considered given the direct interaction with BRCA1 and the homology with XPD, which predicted a function in DNA repair. In keeping with this suggestion, two patients with a family history and early-onset breast cancer were identified that had discrete germline sequence changes, P47A and M299I, targeting the helicase domain of FANCI [8]. The familial significance of these mutations was unclear because cosegregation analysis was not possible. Furthermore, unlike the classic paradigm for tumor suppressor genes, in which the tumor exclusively lacks the wild-type allele, tumors of individuals who were heterozygous for these two germline *FANCI* mutations did not show loss of the corresponding wild-type allele [8]. Thus, cancer in these patients may have been related to the dominant negative effect of these mutant FANCI proteins. Consistent with this idea, the P47A mutant is enzyme inactive *in vitro*, similar to the helicase-inactive FANCI mutant species, K52R, or FA-associated A349P, which dominantly disrupted DNA repair when expressed in cells [41,42]. More recently, the idea that FANCI could act as a classic tumor suppressor was bolstered with the identification of a germline *FANCI* mutation, c.2992–2995delAAGA, in which the tumor had lost the corresponding wild-type allele [43]. Other groups have also screened for *FANCI* mutations and additional sequence changes have been identified [44–53]. However, in most cases, the variants have not been characterized biochemically *in vitro* or functionally *in vivo*, such that the pathological significance is still unknown. All together, *FANCI* is considered a

low-to-moderate penetrance breast cancer susceptibility allele and is associated with modest two- to threefold increases in breast cancer risk [9,54].

It is worth considering that the breast cancer risk in individuals carrying a *FANCF* mutation will be higher for those with a strong family history [55]. When screening for *FANCF* mutations, the identification of mutations in the control population does not directly imply that the mutation in question was not causative in the disease. For example, the P47A variant identified in 2001 [8] and shown to disrupt *FANCF* enzyme function [56] was found in the controls of a subsequent study and considered to be unrelated to breast cancer [44]. This discrepancy could reflect the fact that low-penetrance alleles deviate from the traditional genetic patterns as found for highly penetrant alleles. Highly penetrant alleles will typically segregate with disease, whereas moderately penetrant alleles will not. Instead, low-penetrant alleles likely require additional modifier alleles and/or secondary hits [57]. In this model, the P47A allele would segregate with cancer in families with secondary hits. Contributing to the difficulty of tracking the segregation patterns of moderate-to-low penetrance genes, the sporadic rate of breast cancer is high. With a sufficient number of families, segregation analysis can be achieved, as carried out for *CHEK2* [12]. While issues remain regarding clinical relevance, our goal here is to summarize what is known about *FANCF* mutations, the effect on *FANCF* function and to speculate as to how these may contribute to breast cancer.

Clinically relevant *FANCF* mutations alter expression & activity

Of all the *FANCF* germline mutations, the most common mutant allele is *FANCF* R798X. This mutation was first found in patients within the FA complementation group *FANCF* [26–28], later identified in breast cancer patients [44] and more recently found in prostate cancer [58]. The R798X mutation predicts a truncated protein, but a detectable protein product was not found [26]. Similarly, at least four other distinct *FANCF*-truncating mutations have been identified in breast cancer patients [44]; however, it is not clear whether these alleles express a truncated product. While the disease pathology in tumors is not clear, expression of mutant proteins in tissue culture cells has been used to ascertain how clinically relevant *FANCF* mutations affect function. Using this approach, reduced protein expression was reported for two breast cancer-associated *FANCF* mutants, including c.2992–2995delAAGA, which generates a deletion and results in a premature stop codon after the helicase domain [43]. Moreover, the aforementioned P47A mutant had a reduced protein half-life when overexpressed in tissue culture cells [8]. Thus, reduced *FANCF* expression, as well as protein truncation, characterizes several *FANCF* breast cancer mutations.

Loss of *FANCF* function is also a consequence of clinical mutations targeting the highly conserved helicase domain (residues 1–888), which disrupt or alters *FANCF* enzyme activity. In particular, enzyme activity requires the seven ATPase/helicase motifs. This region also harbors a predicted nuclear localization sequence, as well as the iron–sulfur (Fe–S) cluster domain, which are essential for enzyme activity and present in other DEAH helicases, such as XPD, RTEL and CHL1 [59]. Outside the helicase domain, the C-terminus harbors the phospho-SXXF motif (residues 990–993) required for BRCA1 binding (Figure 2) [37]. Tumor suppression likely requires not only the helicase domain, but also nuclear co-localization with BRCA1, given that variants with a sequence change in the nuclear localization sequence (A173C) [60] or BRCA1-binding domain were detected in patients with a strong family history of breast cancer [43,53].

To address the impact of *FANCF* sequence changes on enzyme activity, recombinant proteins have been generated and tested comparatively with wild-type *FANCF*. These studies revealed that wild-type *FANCF* is a DNA-dependent ATPase, which unwinds DNA in a 5' to 3' direction [56]. Moreover, it was determined that *FANCF* preferentially binds and

unwinds forked duplex DNA substrates as well as D-loop structures that mimic an intermediate step in homologous recombination [61]. As predicted, the ATPase and helicase functions were inactivated by a single missense mutation (K52R) in the ATP-binding pocket of the FANCD1 helicase domain [56,61,62]. Consistent with enzyme activity being essential for tumor suppression, the breast cancer-associated mutation in helicase block II (P47A) or an FA-associated mutation in the Fe–S domain (A349P) reduced FANCD1 enzyme activity [42,56,63]. Interestingly, the A349P mutant protein retained some DNA unwinding activity on a set of DNA substrates, but was unable to couple ATP hydrolysis with the enzyme activity necessary to unwind forked duplex structures or translocate DNA [42]. These studies implicate that FANCD1 enzyme activity is important for tumor-suppressor function and that mutations targeting the helicase domain likely dictate or have a detrimental effect on the type of DNA substrates FANCD1 can metabolize. However, some clinical mutations are not predicted to disrupt expression or enzyme activity and therefore may not disrupt DNA repair. For example, the breast cancer-associated M299I mutant protein unwound a forked duplex DNA substrate and translocated DNA more robustly than wild-type FANCD1 (Figure 2) [62,63].

Given that changes in *BRCA1* promoter status contribute to sporadic tumors [64,65], mutations outside the reading frame or changes in promoter methylation status could alter FANCD1 expression and contribute to tumorigenesis. Along these lines, several *FANCD1* mutations targeting noncoding genomic DNA have been identified in both breast cancer and FA patients [28,43]. *FANCD1* promoter mutations that disrupt transcription factor binding affinities have also been identified [66]. It remains to be determined whether cancer is associated with changes in *FANCD1* transcription, which is cell growth-related and controlled by tumor suppressors of the E2F–retinoblastoma pathway [67]. Finally, somatic inactivation of the *FANCD1* gene could stem from its location in chromosome 17, which undergoes frequent somatic loss [68].

Predicting the physiological consequences of FANCD1 clinical mutants

At present, it is not certain that mutations altering FANCD1 enzyme function or expression are pathogenic. However, cell culture experiments revealed that FANCD1 enzyme activity is essential for DNA repair as well as checkpoint activation. In particular, overexpression of the helicase-inactive K52R mutant in cells with wild-type FANCD1 resulted in the accumulation of unrepaired DNA breaks when analyzed by pulse field electrophoresis [8], as well as reduced homologous recombination following double strand break induction [69]. Furthermore, expression of K52R attenuated replication stress-induced RPA chromatin association and checkpoint activation [70]. FANCD1 helicase activity induced in S phase is also required for FANCD1 to promote S phase progression and possibly intra-S phase checkpoints [71,72]. Experiments using FANCD1-null FA-J patient cells complemented with K52R or the A349P FA-associated mutant demonstrated that FANCD1 enzyme activity and the Fe–S domain are essential for DNA interstrand crosslink repair [42,73].

The role of FANCD1 in double-strand break repair, as well as homologous recombination, was further supported by studies in which FANCD1 expression was depleted with siRNA reagents. Reduced levels of FANCD1 correlated with delayed resolution of double-strand breaks as measured by persistent γ -H2AX foci formation following ionizing radiation [74]. Using a DNA double-strand break-induced recombination assay [75], siRNA depletion of FANCD1 was shown to substantially [26] or partially reduce homologous recombination [38], supporting the idea that FANCD1 mediates a subset of BRCA1 functions in double-strand break repair. Consistent with this idea, unlike BRCA1- or BRCA2-deficient cells, FANCD1-deficient cells are only mildly sensitive to agents that induce double-strand breaks, such as ionizing radiation [74]. Instead, FANCD1 could participate with BRCA1 in a distinct aspect of

DNA repair associated with resolving stalled replication forks or crosslinked DNA, a function that explains the extreme sensitivity and massive chromosomal rearrangements found in FANCF- and BRCA1-deficient cells in response to agents that induce interstrand crosslinks [26]. It will be important to ascertain what other functional defects are common to FANCF- and BRCA1-deficient cells in light of recent findings. In particular, BRCA1-deficient cells have defects in homologous recombination due to an overactive nonhomologous end-joining pathway. Remarkably, inactivation of one of these nonhomologous end-joining factors, 53BP1, restored homologous recombination to BRCA1-deficient cells [76,77].

Based on the function of FANCF, it is plausible that loss of FANCF function will reduce genomic integrity not only because of reduced repair, but also because of defects in processing distinct genomic DNA structures. In particular, FANCF, similar to the *Caenorhabditis elegans* dog-1 ortholog, functions to resolve DNA structures that interfere with DNA replication, such as guanine quadriduplex (G4-DNA) structures [78]. In support of this function, FA-J patient cells accumulate deletions in regions containing G4-DNA, and the recombinant FANCF A349P mutant protein fails to unwind G4-DNA substrates [42,79]. Thus, FANCF enzyme defects could propel tumorigenesis due to loss of either G4-DNA structures and/or the expression of distinct genes harboring G4-DNA. In fact, expression of A349P in cells with wild-type FANCF had a dominant negative effect, enhancing sensitivity to the G4-DNA binding compound telomestatin [42].

Another pressing question is whether breast cancer derives from loss of BRCA1 binding to FANCF. BRCA1 clinical mutations target the BRCT region required for FANCF binding [8]. In addition, BRCA1 binding is disrupted in the FANCF breast cancer-associated mutation c. 2992–2995delAAGA [43]. The BRCA1– FANCF interaction was dependent upon phosphorylation of the FANCF residue serine 990 [37]. Thus, the BRCA1 interaction-defective mutant of FANCF (S990A) has been used to determine whether the BRCA1– FANCF interaction was important for FANCF DNA damage response functions. Initially, it was determined that BRCA1 binding to FANCF was essential for checkpoint control during the G2–M phase of the cell cycle [37]. More recently, it was shown that BRCA1 binding to FANCF was essential for regulating the mechanism of DNA damage repair. In particular, the FANCF S990A mutant reduced error-free repair by homologous recombination and enhanced error-prone DNA damage bypass through the Pol- η translesion synthesis polymerase [69]. Both mechanisms promote resistance to DNA crosslinking agents, such as mitomycin C; however, bypass is more error-prone because lesions are tolerated and not repaired. Thus, BRCA1 or FANCF patient mutations that reduce the interaction may compromise genomic stability not only because of reduced checkpoint control and DNA repair, but also owing to enhanced DNA damage tolerance. In support of this possibility, elevated FANCF expression levels were detected in grade 3 invasive breast tumors and correlated with poor prognosis [67].

Conclusion

In summary, FANCF is a disease-linked helicase. This conclusion is based on the identification of both breast cancer and FA patients with FANCF helicase-disrupting mutations, and the finding that FANCF helicase activity is important for normal cellular repair and checkpoint signaling. Conceivably, cancer will stem from not only too little, but also too much enzyme activity, as exemplified by two FANCF breast cancer-associated missense mutations (P47A and M299I). The P47A mutant had disrupted enzyme activity, whereas the M299I mutant had enhanced ATPase, helicase and translocase activity [56,61]. Clearly, the functions of FANCF in DNA repair, checkpoint and DNA damage tolerance pathways require enzyme activity. However, it remains to be determined whether these

FANCI functions are altered by mutations that hyperactivate or deregulate FANCI enzyme activity. It may be informative to consider how loss of BRCA1 binding affects FANCI function *in vivo*. Cells expressing the BRCA1 interaction- defective mutant S990A had reduced DNA repair, similar to an enzyme-inactivating FANCI mutant, K52R. However, loss of FANCI binding to BRCA1 also enhanced DNA damage tolerance [69]. Enhanced tolerance due to unregulated FANCI could contribute to cancer progression in patients lacking the interaction owing to FANCI or BRCA1-BRCT mutations.

Future perspective

The knowledge that genetic susceptibility to breast cancer is attributed to germline mutations in genes that encode DNA repair proteins has revolutionized how affected patients are treated. The therapeutic strategy is to treat patients with traditional chemotherapeutics or novel agents such as PARP1 inhibitors that generate DNA damage that cannot be efficiently repaired by DNA repair-defective tumors. The challenge will be to determine if such therapies selectively sensitize FANCI-associated tumors. In support of this possibility, similar to BRCA-deficient cells, FANCI-deficient cells are sensitive to cisplatin [69]. The role of FANCI in resolving not only DNA crosslinks, but also G4-DNA, implies that FANCI-associated tumors could also be selectively sensitive to treatment with G4-DNA ligands such as telomestatin, which stabilize G4-DNA structures.

Even though the location of FANCI mutations can provide information about functional consequences, it will be essential to develop fast and reliable assays to determine whether a mutation is deleterious and/or generates a tumor that is vulnerable to new or traditional therapies. Strategies that are found to be useful for deciphering pathological variants of unknown significance for other DNA repair genes will be worth considering, such as introducing human bacterial artificial chromosomes containing *BRCA1*-mutant species into mouse cells and embryos [80]. Perhaps we will learn that distinct *FANCI* mutations disrupt disparate functions and have diverse disease outcomes. Mutations in the xeroderma pigmentosa helicase XPD can lead to at least three disease states including xeroderma pigmentosa, Cockayne syndrome and trichothiodystrophy [81]. A distinction of disease states could relate to whether a mutation in FANCI is enzyme inactivating or hyperactivating (Figure 3).

A complete understanding of how FANCI functions as a tumor suppressor will be essential for designing therapies. If an overactive helicase is associated with unscheduled tolerance pathways akin to the BRCA1 interaction-defective mutant S990A, targeting the enzyme itself could be a useful strategy in cancer prevention. In this context, it is worth considering whether BRCA1-BRCT mutation carriers who have a defect in the BRCA1-FANCI interaction could benefit from therapies that reduce FANCI enzyme activity. Furthermore, if an unregulated FANCI contributes to unscheduled DNA damage tolerance, targeting FANCI could prevent chemoresistance akin to targeting the translesion synthesis polymerase REV3, which restores cisplatin sensitivity to lung tumors [82]. With numerous DNA helicases, however, the challenge will be to determine if the FANCI enzyme can be selectively targeted. Perhaps the somewhat unique and essential Fe-S domain holds the key.

Executive summary

DNA repair genes & breast cancer

- Mutations in *BRCA1* and *BRCA2* genes render derived tumors selectively sensitive to agents that generate lesions requiring DNA repair by homologous recombination.

- FANCI, also known as BACH1 or BRIP1, was identified as a direct BRCA1-BRCT interacting protein and functions in DNA repair and checkpoint control.
- Bi-allelic inheritance of mutations in FANCI, similar to PALB2 and BRCA2, contributes to Fanconi anemia, a rare cancer-prone disease characterized by extreme cellular sensitivity to agents that induce DNA interstrand crosslinks.

Towards defining the function of FANCI in breast cancer suppression

- The most common germline *FANCI* mutant allele (R798x) is found in both breast cancer and Fanconi anemia patients.
- The predicted physiological consequence of clinically relevant *FANCI* loss-of-function mutants is genomic instability due to reduced DNA repair and checkpoint activation.
- The physiological consequence of clinically relevant *FANCI* mutants that overactivate the enzyme is not known.
- Studies on a BRCA1 interaction-defective mutant FANCI S990A predict that loss of FANCI binding to BRCA1 will enhance genomic instability not only owing to reduced checkpoint and repair functions, but also owing to enhanced DNA damage tolerance.

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Website

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www.rockefeller.edu/fanconi/mutate

Figure 1. DNA repair genes associated with hereditary breast cancer and linked to Fanconi anemia

Genetic susceptibility to breast cancer is attributed to germline mutations in either *BRCA1* or *BRCA2* as well as other genes, notably some genes that encode for BRCA1- and BRCA2-interacting proteins, *FANCD1* and *PALB2*. The percentage of contribution of the non-*BRCA* genes is unclear and not indicated. Inheriting bi-allelic mutations in some breast cancer genes, which are underlined, results in Fanconi anemia associated with complementation groups *BRCA2/FANCD1*, *PALB2/FANCD1* and *BACH1/FANCD1/BRIP1*.

Figure 2. *FANCF* mutation sites and functional domains

Shown are some *FANCF* clinically relevant germline mutations: breast cancer mutations (pink), Fanconi anemia mutations (blue; see [101]) one mutation shared by both diseases is shown (pink/blue). In addition, the 7-helicase blocks are depicted, with noted DEAH residues, Fe-S domain, NLS, the K52R mutation that inactivates its enzyme activity and phosphorylation on serine 990 that is required for binding to BRCA1. Fe-S: Iron-sulfur; NLS: Nuclear localization sequence.

Figure 3. FANCD1 tumor suppression function

FANCD1 tumor suppression and DNA repair functions require the ability to interact with BRCA1 for its enzyme activity to be regulated. Too little (loss of function) or too much enzyme activity and/or loss of BRCA1 binding (gain of function) will promote tumorigenesis.