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BRCA-FA pathway as a target for anti-tumor drugs

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Abstract

Promising research on DNA repair signaling pathways predicts a new age of anti-tumor drugs. This research was initiated through the discovery and characterization of proteins that functioned together in signaling pathways to sense, respond, and repair DNA damage. It was realized that tumor cells often lacked distinct DNA repair pathways, but simultaneously relied heavily on compensating pathways. More recently, researchers have begun to manipulate these compensating pathways to reign in and kill tumor cells. In a striking example it was shown that tumors derived from mutations in the DNA repair genes, of BRCA-FA pathway, were selectively sensitive to inhibition of the base excision repair pathway. These findings suggest that tumors derived from defects in DNA repair genes will be easier to treat clinically, providing a streamlined and targeted therapy that spares healthy cells. In the future, identifying patients with susceptible tumors and discovering additional DNA repair targets amenable to anti-tumor drugs will have a major impact on the course of cancer treatment.

Keywords

Fanconi anemia; DNA repair; anti-tumor drug; BRCA

The DNA damage response

The integrity of the genome is under constant attack from DNA damage. Even internal processes such as normal cellular metabolism create byproducts that wreak havoc on DNA. In some instances, DNA damage is severe enough to create a double strand break in the DNA helix. If breaks are not repaired, genes can be extensively rearranged and chromosomes can become unstable. To avoid this type of genomic instability, cells monitor DNA for signs of damage and respond by mounting a DNA damage response. The response is initiated by distortions in the DNA structure and eventually leads to activation of both cell cycle checkpoints and DNA damage repair pathways.

Checkpoints can be triggered by DNA damage at several stages in the cell cycle, e.g., the G1 to S-phase and the G2 to M-phase boundaries. DNA damage can also arrest the replication of DNA within S phase, when new DNA is synthesized. Arresting DNA synthesis provides cells sufficient time to repair damaged DNA before progressing into G2 and M phases. Thus, cell cycle checkpoints cooperate with DNA repair processes to ensure that the genome replicates accurately. If, however, the damage is irreparable, the cell will activate an apoptotic response

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to induce cell death. Overall, activating the signal cascade induced by DNA damage is critical to allow time for effective DNA damage repair and ultimately, maintain overall genomic stability.

If these DNA damage responses are impaired, DNA damage can fail to be recognized, repaired, and/or transduced to activate cell cycle checkpoints. These failures can sometimes allow damaged DNA to be replicated and/or tolerated creating further genetic changes that can enhance cellular transformation. However, even transformed cells ultimately rely on some form of a DNA damage response pathway for growth, viability, and ultimately to survive toxic forms of DNA damage. In some cases, toxic forms of DNA damage can be tolerated by healthy cells, but not by cancer cells. For example, cancer cells that evolved from mutations in genes regulating a DNA damage response pathway inevitably are defective in a particular response pathway compared to healthy cells. As such, cancer cells should have a greater dependence on the remaining intact DNA damage response pathways for survival. If so, inactivation of one of these remaining pathways should selectively kill cancer cells and spare healthy cells that have all pathways intact. Not surprisingly, researchers have already begun to ask whether disrupting these compensating DNA repair pathways could enhance the sensitivity of cancer cells to chemotherapy. In particular, the importance of understanding and targeting compensating DNA response pathways for therapeutic gain has been brought to light by research on the BRCA-FA DNA damage response pathway.

BRCA-FA DNA damage response pathway

Together, the BRCA-FA pathway consists of the hereditary breast cancer genes BRCA1 and BRCA2 (BRCA) and a network of at least 12 Fanconi anemia (FA) genes (designated *FANCA* to *FANCN*) [1]. Functional loss of the BRCA-FA pathway leads to increased cellular sensitivity to DNA damaging agents, defects in cell cycle checkpoints, and cancer predisposition. While heterozygous carriers of mutations in the BRCA genes are predisposed to breast and ovarian cancer [2], homozygous carriers of mutations in FA genes develop FA-related cancers, including leukemia [1].

The genetic link between BRCA and FA was identified by the discovery that inheritance of two mutant copies of the *BRCA2* gene caused FA in the FA-D1 complementation group [3]. The finding that BRCA2 was FANCD1 clarified the previously proposed functional link between BRCA and FA proteins. In particular, similar to FA-deficiency, BRCA-deficiency resulted in hypersensitivity to MMC [3–6]. While *BRCA1*-mutations have not been linked to an FA complementation group, the direct BRCA1 binding partner, formally called the BRCA1-associated C-terminal helicase, BACH1, was identified as the FA gene, FANCF [7–9]. A functional link between FA and BRCA proteins was also established with the finding that FA and BRCA proteins are mutually dependent on each other for localization within nuclear structures. For example, BRCA1 is required for FANCD2 foci formation [10], and FANCD2 monoubiquitination is required for the DNA-damage induced translocation of BRCA2/FANCD1 to chromatin [11]. Furthermore, after DNA damage, BRCA and FA proteins co-localize and co-precipitate suggesting they function in a complex [12].

While the molecular function of the BRCA-FA proteins is not entirely clear, several gene products, including FANCA, -B, -C, -D, -E, -F, -G, -L, and -M, form a nuclear core complex (the FA core complex), that is required for monoubiquitination and activation of the FANCD2 protein. BRCA-FA proteins are also required to mediate the interstrand cross-link (ICL)-induced cellular response [1]. Consequently, FA cells lacking any of the BRCA-FA proteins fail to respond to ICLs, which leads to cellular sensitivity and a prolonged accumulation of cells at the late S or G2/M checkpoint. This accumulation is thought to result from a failure of FA cells to elicit a proper ICL-induced intra-S-phase checkpoint or to delayed repair in late S-

phase [13–15]. BRCA1 mutant cells also fail to respond to ICLs by arresting DNA synthesis and promoting HR [2,16,17] and are hypersensitive to ICLs, which causes profound genetic instability [18,19].

In addition to classic DNA interstrand cross-linking agents, the FA pathway may serve to process other types of DNA damage. For example, it was recently reported that ultraviolet (UV) light, which does not directly introduce DSBs or DNA interstrand cross-links, can activate the FA/BRCA pathway as evidenced by FANCD2 monoubiquitination [20]. In that study, it was suggested that the BRCA-FA pathway may be responsible for recombinational repair of stalled replication forks when nucleotide excision repair or translesion bypass fail. In further support of the notion that the FA pathway may respond to DNA damage other than ICLs, a recent report provided evidence that the BRCA-FA pathway is required for cell survival following treatment with the anti-cancer agent irifolven [21]. Irifolven, an analogue of mushroom-derived illudin toxins, has been shown in preclinical studies and clinical trials to be cytotoxic to several tumor cell types. The precise type of DNA damage induced by irifolven is not well understood; however, a recent study demonstrated that irifolven induces DSBs [22]. In that work, the authors reported that BRCA1 plays a role in the DNA damage response to irifolven by controlling cell cycle arrest in S and G2/M, and enabling repair of DSBs by HR. Furthermore, BRCA1 deficiency results in elevated chromosome damage and chemosensitivity after irifolven treatment [22].

Furthermore, BRCA-FA cells respond and are sensitive to DNA alkylating agents temozolomide (TMZ) and 1,3-bis[2-chloroethyl]-1-nitroso-urea (BCNU), two small molecule compounds frequently used in chemotherapeutic treatment of malignant glioma [23]. TMZ is a monofunctional alkylating agent that directly methylates DNA nucleotides [24]. BCNU can act as a mon- or bi-functional alkylating agent, which introduces a chloroethyl moiety to the nucleotide, which can subsequently become covalently attached to nearby proteins or DNA nucleotides [24,25]. It has been observed that TMZ and BCNU activate the FA pathway by FANCD2 monoubiquitination. Furthermore both FA-deficient cells and a glioma cell line deficient in the FA repair pathway were sensitive to TMZ and BCNU suggesting a role of the FA pathway in resistance to the cytotoxicity of the alkylating agents [23].

The function of the BRCA-FA pathway

Understanding the function of the BRCA-FA pathway is essential to uncover how loss of this pathway leads to the development of cancer. In the absence of a functional BRCA-FA DNA damage response pathway, breast and ovarian tumors as well as leukemia can develop. Furthermore, it will be essential to understand how BRCA-FA tumors compensate for loss of this pathway when confronted with toxic DNA damage. BRCA-FA derived tumor cells must rely on alternate pathways for survival. Understanding specifically how these alternative pathways compensate for defects in the BRCA-FA pathway to promote survival, and/or resistance to DNA damage, will be essential for selectively killing these tumors. To uncover these compensating pathways, it is first essential to appreciate the function of the BRCA-FA pathway.

Several lines of evidence indicate that the BRCA-FA pathway participates in double strand break repair by promoting homologous recombination (HR). The NHEJ pathway can more readily result in the loss of genetic information, whereas HR repair more often restores the original sequence. Typically, HR repair uses the homologous sequence in a sister chromatid to repair the DSB by an error-free mechanism. The initial processing of the DSB for HR requires the formation of a single stranded DNA region at the site of the break. Here, the 5' strand of the duplex is resected and leaves a 3' single-stranded DNA region. This 3' DNA overhang is initially stabilized by the single-stranded binding protein RPA, but is then replaced by the

RAD51 recombination protein forming a nucleoprotein filament. The RAD51 nucleoprotein filament searches the genome for a region of sequence homology HR repair provides a mechanism for growing and dividing cells to repair DSBs that can arise from stalled, arrested, or broken replication forks. In particular, the BRCA-FA proteins functioning downstream of FANCD2 monoubiquitination, including BRCA1, BRCA2/FANCD1, FANCD1, and FANCD2 have been speculated to directly regulate HR [26]. However, BRCA2/FANCD1 has the most defined role in HR. Specifically, BRCA2/FANCD1 binds directly to Rad51 and promotes Rad51 loading onto ssDNA, suggesting that BRCA2 functions directly in promoting Rad51-mediated HR [27].

The reports vary as to whether the BRCA-FA proteins operating upstream of FANCD2 monoubiquitination regulate HR. While loss of the core subunits FANCA and FANCC reduced HR loss of FANCD1 did not [26]. The reduction in HR has been attributed to a more indirect role of these FA subunits in replication fork stabilization [28]. Nevertheless, FANCD1 was shown to directly interact with the Rad51 paralog XRCC3, a key component in the recombination repair pathway. This interaction facilitates the formation of the Rad51 nucleoprotein filament essential for recombination. Furthermore, FANCD1 serves to stabilize a ternary complex between itself, XRCC3 and BRCA2 [29]. Although there are discrepancies within the literature that may pertain to diversity of cell type or assay, it appears that overall the BRCA-FA proteins are critical for mediating some aspect of Rad51-mediated recombination repair. Nevertheless, loss of the BRCA-FA pathway universally leads to ICL-induced sensitivity, accumulation of cells with a 4N DNA content, and genetic instability (Figure 1).

Exploiting defects in BRCA-FA cancers

The emerging role of BRCA-FA proteins in HR allows one to speculate that tumor cells derived from mutations in these genes should have impaired HR. In support of this possibility, BRCA1, BRCA2, BRIP1/FANCD1, and PALB2/FANCD2-deficient cells all demonstrate defects in homology-mediated double strand break repair (DSBR) (reviewed in [2,7,17,30]). Consistent with a role for the BRCA-FA pathway in HR, BRCA-FA mutant cells are hypersensitive to cross-linking agents that generate DSBs that are repaired by HR [31]. A number of compounds introduce crosslinks, including nitrogen mustards, chloroethylnitrosoureas, cyclophosphamides, mitomycin C, platinum molecules. Some of these agents have been used affectively in the clinic to combat BRCA cancers [32]. Furthermore, toxic DNA DSBs can be generated directly or indirectly by anti-cancer agents such as topoisomerase inhibitors (e.g., ectoposide), bleomycin, or ionizing radiation (IR). Demonstration that FANCD2 expression and monoubiquitination are important for cancer cell resistance to irifolven [21] suggests this agent also generates DSBs that are processed by proteins of the FA/BRCA pathway. Clearly, traditional DNA damaging agents used in chemotherapy can be more toxic to BRCA-FA cancer cells compared to healthy cells. This most likely relates to the fact that BRCA-FA cells lack the necessary DNA repair response and repair pathways promoting survival in healthy cells.

The DNA repair defect in BRCA-FA mutant cells may be exploited as a therapeutic strategy. It was recently demonstrated that inhibiting BER increased the number of double strand breaks and also enhanced RAD51 foci formation, suggesting an increase in HR. Based on these findings, it was hypothesized that cells defective for HR would be sensitive to inhibition of BER. In fact, it was shown that BRCA-FA cancer cells were extremely sensitive to BER inhibition. In particular, treatment of BRCA-FA-deficient cells with an inhibitor of the BER enzyme, poly ADP-ribose (PARP), lead to a dramatic reduction in cell survival. In contrast, BRCA-FA proficient cells were only mildly impacted by the PARP-inhibition. Thus, inactivation of the BER pathway cooperates with inactivation of the BRCA-FA pathway to enhance cell death. Indeed, a review by Helleday and colleagues (2005) [33] summarizes the

observations that HR-deficient cells with a defect in BRCA1, BRCA2, XRCC2, or XRCC3 are hypersensitive to PARP inhibitors because of the accumulation of the toxic recombination intermediates. In summary, these landmark studies demonstrated that inhibition of a key regulatory DNA repair enzyme (PARP) provides the opportunity to selectively kill tumor cells that are defective in DNA repair (HR repair). Thus, identifying tumors with mutations in BRCA-FA genes predicts a new age of cancer treatment with targeted approaches that do not require toxic chemical therapies and spare healthy cells [34] (see Figure 2). In fact, PARP inhibitors are now in phase I/II clinical trials [32].

The ability to target the DNA repair defect in BRCA-FA tumors by increasing lesions repaired by HR, through inhibition of PARP, suggests that other tumor cells with defects in BRCA-FA associated partners or functions could be sensitized by PARP inhibition. For example, deficiency in DSS1, a protein that interacts with BRCA2 [35], leads to decreased Rad51 foci formation and genomic instability [36] and therefore, may be sensitive to PARP inhibition. Conceivably, tumors derived from defects in other HR proteins such as RAD54 or RPA1, which play critical roles in DNA strand exchange, an early event in HR [37,38], may also be amenable to PARP inhibition. Perhaps this sensitivity extends to tumors that derive from defects in activating or signaling to the BRCA-FA pathway. Components of this pathway include the phosphoinositol 3-kinase related kinases ATM and ATR and its substrates CHK1 and CHK2 that facilitate the DNA damage response and checkpoint [39,40]. NBS1 is a principal player in the MRE11/RAD50/NBS1 complex that not only acts to process DSBs, but also plays a role in DNA damage signaling by ATM or ATR [41]. Since defects in these genes are observed in a number of cancers, the PARP inhibitor strategy may be useful for treatment of malignancies other than those associated with breast or ovarian cancer. In fact, Ashworth and colleagues showed that deficiency in DNA damage response proteins including, RAD51, RAD54, DSS1, RPA1, NBS1, ATR, ATM, CHK1, CHK2, FANCD2, or FANCC resulted in sensitivity to PARP inhibition as evidenced by clonogenic survival assays [34].

Alternatively, directly targeting compensating DNA repair pathways may compromise the cellular DNA damage response and render cancerous cells more sensitive to cytotoxic drugs. Cells defective in BRCA-FA are likely to repair DSBs by compensatory pathways such as non-homologous end-joining or single-strand annealing. In fact, studies demonstrated that targeting alternative DSB repair pathways could be effective to selectively kill BRCA-FA tumors. In particular, it was demonstrated that disruption of the non-homologous end-joining (NHEJ) pathway cooperated with inactivation of the BRCA-FA pathway to enhance radiosensitivity in mouse embryonic fibroblasts (MEFs). Specifically, FANCD2 $-/-$ MEFs were sensitized to IR by suppression of a critical component of the NHEJ pathway. Thus, it appears that activation of the NHEJ pathway may compensate for loss of the BRCA-FA pathway to repair DNA damage and maintain cell viability [42]. Despite the fact that these alternative pathways are mutagenic, they may provide the opportunity for BRCA-FA cancer cells to survive. Inhibition of these alternative pathways may not only improve traditional cancer therapies (i.e. radiation), but may also be self-sufficient in killing certain cancers (i.e. inhibiting PARP in BRCA-FA cancers). The real value of these new approaches, however, is that the anti-cancer therapies targeting DNA repair pathways may spare healthy cells.

Targeting the BRCA-FA pathway in cancer

Just as BRCA-FA tumors are sensitive to persistent DSB formation (i.e inhibition of BER) and/or loss of alternative DSB repair pathways (i.e loss of NHEJ pathway), it follows that other types of tumors will be sensitive to loss of the BRCA-FA pathway. If tumors rely on the BRCA-FA pathway then targeting it should be toxic to those tumor cells. Therefore, the goal would be to identify relevant tumors, determine if the BRCA-FA pathway could be readily inhibited, and finally, identify how to target it directly.

Several lines of evidence indicate that the ability of tumors to become resistant to DNA cross-linking agents is attributed to an operational FA/BRCA DNA repair pathway. By inactivating the BRCA-FA pathway it may be possible to re-sensitize these cancer cells. In fact, BRCA-FA reversion mutations have been linked to an acquired resistance in tumor cells. For example, cisplatin sensitive ovarian cancer cells can develop cisplatin resistance by restoring expression of a previously silenced FANCF gene [43]. Similarly, it was demonstrated that restoring BRCA2 gene expression in tumor cells lead to an acquired resistance to MMC. In this case, a secondary mutation reestablished the open reading frame in one allele of the BRCA2 gene. This reversion mutation partially restored BRCA2 protein expression and also BRCA2 function. Correspondingly, enhanced activation of the BRCA-FA pathway has been shown to lead to acquired resistance in multiple myeloma cells. The ICL-resistant myeloma cells demonstrated an increase in the efficiency of ICL removal, indicative of enhanced repair. Moreover, the enhanced repair corresponded to an increase in gene expression of the BRCA-FA pathway proteins, including FANCF. The resistant phenotype observed in these cells was reversed by siRNA-mediated suppression of FANCF. Also, ICL-sensitive cell lines were made resistant by overexpression of FANCF [44]. Thus, targeting the BRCA-FA pathway may be effective in treating resistant tumors.

The question remains as to whether the BRCA-FA pathway can be readily targeted. Initial siRNA screens have identified that suppression of several BRCA-FA proteins increased cisplatin sensitivity in p53 mutant cancer cells, but did not affect the viability of p53 wild-type healthy cells. In fact, suppression of not only BRCA1 and BRCA2, but also ATR, CHK1, and RAD51 demonstrated a similar cytotoxicity suggesting that disruption of the BRCA-FA pathway is achieved by inactivating several targets. Similarly, targeted disruption of the FANCC or FANCG gene locus in adenocarcinoma cells lines resulted in enhanced sensitivity to several ICL damaging agents, further supporting the possibility that the BRCA-FA pathway can be inhibited to effectively sensitize cells to cancer therapies. The feasibility of these approaches relies on siRNA approaches reaching the clinic.

Perhaps more feasible clinical applications will evolve from identifying dominant negative or chemical reagents that block the BRCA-FA pathway. In particular, the BRCA-FA pathway was inactivated by overexpressing-BRCA1 mutant constructs that selectively enhanced the cytotoxicity of cells to the topoisomerase inhibitor, adriamycin [45]. Likewise, the BRCA-FA pathway was disrupted by overexpression of the FANCA mutant protein, which contained the region required for FANCG binding. This exogenously expressed FANCA mutant was shown to compete with endogenous FANCA for FANCG binding and disrupted FANCD2 monoubiquitination. Thus, overexpression of this mutant protein not only disrupted the BRCA-FA pathway, but also enhanced cellular cisplatin toxicity [46]. Other reagents that disrupt FANCD2 monoubiquitination and sensitize cells to chemotherapy have been identified in high throughput screens. In particular, synthetic compounds such as curcumin, wortmannin, H-9, and alesterpaulone have been identified. While H-9 and alesterpaulone were toxic to healthy cells, wortmannin and curcumin were able to inhibit BRCA-FA pathway activation without toxicity. Wortmannin most likely disrupts the BRCA-FA pathway because it inhibits the PI3K protein kinase family members, ATR and ATM, both of which activate different proteins in the BRCA-FA pathway. On the other hand, the natural compound, curcumin is thought to disrupt the BRCA-FA pathway by blocking FANCD2 monoubiquitination [47]. Thus, curcumin is not only an effective method for BRCA-FA pathway inactivation, but also serves as a potentially potent anti-cancer treatment.

Future targets in the BRCA-FA pathway

Present attempts to target the BRCA-FA pathway have involved identifying inhibitors of FANCD2 monoubiquitination, such as curcumin [47]. If curcumin is therapeutically effective

to treat cancer, it will be important to understand what exact function of the BRCA-FA DNA damage signaling pathway is disrupted. Since the BRCA-FA proteins upstream of FANCD2 may only indirectly regulate repair, whereas BRCA-FA proteins downstream of FANCD2 may directly regulate repair, agents that impact the downstream BRCA-FA pathway may have greater therapeutic potential. In particular, targeting and interfering with FANCD2 helicase, which is essential for HR, may be a useful strategy. In an accompanying review targeting helicases is discussed (Gupta and Brosh).

It is possible that directly blocking repair may be too toxic for most cells. It is also conceivable that constitutive activation of the BRCA-FA pathway will be useful to deregulate the pathway for therapeutic gain. In particular, deubiquitination FANCD2 by the deubiquitination enzyme USP1 could be targeted. Potentially, reducing the time that FANCD2 stays monoubiquitinated will be a useful strategy to reduce but not disable the BRCA-FA pathway function. Clearly, research needs to focus on how to balance loss or gain of BRCA-FA signaling related functions to kill tumor cells but not irreversibly harm healthy cells. Conceivably, disabling the BRCA-FA pathway in the course of patient treatment with traditional cancer therapies could be used as a combined approach to weaken tumor cells and prevent the development of resistance.

Insight from understanding and targeting DNA damage response pathways, such as the BRCA-FA pathway has launched a new era in cancer therapy. Therapies have begun to attack the Achilles' heel of tumor cells. As it appears, the weakness of tumor cells is that they either lack the ability to repair a type of DNA damage and/or also rely on other compensating DNA repair pathways for survival. Thus, the new age therapy will be to attack these compensating pathways to compromise tumor cell viability. This approach promises highly targeted therapies that potentially bypass the need for traditional radiation or chemical cancer therapies.

Literature Cited

1. Thompson LH. *Nat Genet* 2005;37:921. [PubMed: 16132046]
2. Scully R, Livingston DM. *Nature* 2000;408:429. [PubMed: 11100717]
3. Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD. *Science* 2002;297:606. [PubMed: 12065746]
4. Moynahan ME, Cui TY, Jasin M. *Cancer Res* 2001;61:4842. [PubMed: 11406561]
5. Yun J, Zhong Q, Kwak JY, Lee WH. *Oncogene*. 2005
6. Ohashi A, Zdzienicka MZ, Chen J, Couch FJ. *J Biol Chem* 2005;280:14877. [PubMed: 15671039]
7. Litman R, Peng M, Jin Z, Zhang F, Zhang J, Powell S, Andreassen PR, Cantor SB. *Cancer Cell* 2005;8:255. [PubMed: 16153896]
8. Levitus M, Waisfisz Q, Godthelp BC, de Vries Y, Hussain S, Wiegant WW, Elghalbzouri-Maghrani E, Steltenpool J, Rooimans MA, Pals G, Arwert F, Mathew CG, Zdzienicka MZ, Hiom K, De Winter JP, Joenje H. *Nat Genet* 2005;37:934. [PubMed: 16116423]
9. Levran O, Attwooll C, Henry RT, Milton KL, Neveling K, Rio P, Batish SD, Kalb R, Velleuer E, Barral S, Ott J, Petrini J, Schindler D, Hanenberg H, Auerbach AD. *Nat Genet* 2005;37:931. [PubMed: 16116424]
10. Vandenberg CJ, Gergely F, Ong CY, Pace P, Mallery DL, Hiom K, Patel KJ. *Mol Cell* 2003;12:247. [PubMed: 12887909]
11. Wang X, Andreassen PR, D'Andrea AD. *Mol Cell Biol* 2004;24:5850. [PubMed: 15199141]
12. Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD. *Mol Cell* 2001;7:249. [PubMed: 11239454]
13. Sala-Trepat M, Rouillard D, Escarceller M, Laquerbe A, Moustacchi E, Papadopoulos D. *Exp Cell Res* 2000;260:260–208.
14. Heinrich MC, Hoatlin ME, Zigler AJ, Silvey KV, Bakke AC, Keeble WW, Zhi Y, Reifsteck CA, Grompe M, Brown MG, Magenis RE, Olson SB, Bagby GC. *Blood* 1998;91:275. [PubMed: 9414295]

15. Akkari YM, Bateman RL, Reifsteck CA, D'Andrea AD, Olson SB, Grompe M. *Mol Genet Metab* 2001;74:403. [PubMed: 11749045]
16. Moynahan ME, Chiu JW, Koller BH, Jasin M. *Molecular Cell* 1999;4:511. [PubMed: 10549283]
17. Venkitaraman AR. *Cell* 2002;108:171. [PubMed: 11832208]
18. Shen SX, Weaver Z, Xu X, Li C, Weinstein M, Chen L, Guan XY, Ried T, Deng CX. *Oncogene* 1998;17:3115. [PubMed: 9872327]
19. Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX. *Mol Cell* 1999;3:389. [PubMed: 10198641]
20. Dunn J, Potter M, Rees A, Runger TM. *Cancer Res* 2006;66:11140. [PubMed: 17145857]
21. Wang Y, Wiltshire T, Senft J, Wenger SL, Reed E, Wang W. *Mol Cancer Ther* 2006;5:3153. [PubMed: 17172419]
22. Wiltshire T, Senft J, Wang Y, Konat GW, Wenger SL, Reed E, Wang W. *Mol Pharmacol* 2007;71:1051. [PubMed: 17229870]
23. Chen CC, Taniguchi T, D'Andrea A. *J Mol Med*. 2007
24. Drablos F, Feyzi E, Aas PA, Vaagbo CB, Kavli B, Bratlie MS, Pena-Diaz J, Otterlei M, Slupphaug G, Krokan HE. *DNA Repair (Amst)* 2004;3:1389. [PubMed: 15380096]
25. Paik J, Duncan T, Lindahl T, Sedgwick B. *Cancer Res* 2005;65:10472. [PubMed: 16288039]
26. Taniguchi T, D'Andrea AD. *Blood*. 2006
27. Liu Y, West SC. *Breast Cancer Res* 2002;4:9. [PubMed: 11879553]
28. Thompson LH, Hinz JM, Yamada NA, Jones NJ. *Environ Mol Mutagen* 2005;45:128. [PubMed: 15668941]
29. Hussain S, Wilson JB, Blom E, Thompson LH, Sung P, Gordon SM, Kupfer GM, Joenje H, Mathew CG, Jones NJ. *DNA Repair (Amst)* 2006;5:629. [PubMed: 16621732]
30. Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM. *Mol Cell* 2006;22:719. [PubMed: 16793542]
31. Niedernhofer LJ, Lalai AS, Hoeijmakers JH. *Cell* 2005;123:1191. [PubMed: 16377561]
32. Mullan PB, Gorski JJ, Harkin DP. *Biochim Biophys Acta* 2006;1766:205. [PubMed: 16919882]
33. Helleday T, Bryant HE, Schultz N. *Cell Cycle* 2005;4:1176. [PubMed: 16123586]
34. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. *Cancer Res* 2006;66:8109. [PubMed: 16912188]
35. Li J, Zou C, Bai Y, Wazer DE, Band V, Gao Q. *Oncogene* 2006;25:1186. [PubMed: 16205630]
36. Gudmundsdottir K, Lord CJ, Witt E, Tutt AN, Ashworth A. *EMBO Rep* 2004;5:989. [PubMed: 15359272]
37. Heyer WD, Li X, Rolfsmeier M, Zhang XP. *Nucleic Acids Res* 2006;34:4115. [PubMed: 16935872]
38. Wang Y, Putnam CD, Kane MF, Zhang W, Edelmann L, Russell R, Carrion DV, Chin L, Kucherlapati R, Kolodner RD, Edelmann W. *Nat Genet* 2005;37:750. [PubMed: 15965476]
39. Harrison JC, Haber JE. *Annu Rev Genet* 2006;40:209. [PubMed: 16805667]
40. Niida H, Nakanishi M. *Mutagenesis* 2006;21:3. [PubMed: 16314342]
41. Zhang Y, Zhou J, Lim CU. *Cell Res* 2006;16:45. [PubMed: 16467875]
42. Houghtaling S, Newell A, Akkari Y, Taniguchi T, Olson S, Grompe M. *Hum Mol Genet* 2005;14:3027. [PubMed: 16135554]
43. Taniguchi T, Tischkowitz M, Ameiziane N, Hodgson SV, Mathew CG, Joenje H, Mok SC, D'Andrea AD. *Nat Med* 2003;9:568. [PubMed: 12692539]
44. Chen Q, Van der Sluis PC, Boulware D, Hazlehurst LA, Dalton WS. *Blood* 2005;106:698. [PubMed: 15802532]
45. Fan S, Yuan R, Ma YX, Meng Q, Goldberg ID, Rosen EM. *Oncogene* 2001;20:8215. [PubMed: 11781837]
46. Ferrer M, de Winter JP, Mastenbroek DC, Curiel DT, Gerritsen WR, Giaccone G, Kruyt FA. *Cancer Gene Ther* 2004;11:539. [PubMed: 15192709]
47. Chirnomas D, Taniguchi T, de la Vega M, Vaidya AP, Vasserman M, Hartman AR, Kennedy R, Foster R, Mahoney J, Seiden MV, D'Andrea AD. *Mol Cancer Ther* 2006;5:952. [PubMed: 16648566]

BRCA-FA Pathway

Figure 1. Schematic representation of the BRCA-FA pathway

Activation of the pathway is required to mediate the DNA damage response, repair, and checkpoint response to maintain genomic stability. Loss or inactivation of this pathway leads to defects in DNA repair and/or checkpoint responses that reduce cell survival following ICL-treatment.

Figure 2. Exploiting defects in BRCA-FA cancers as a therapeutic strategy

The viability of healthy cells is maintained by repair being processed by multiple DNA damage repair pathways, represented as four legs of a table. However, tumor cells that lack a distinct DNA repair pathway(s), will rely more heavily on other compensating pathway(s) for survival. Disrupting these compensating DNA repair pathways may selectively compromise tumor cell survival (table falls over), while maintaining the viability of healthy cells (table remains standing on three legs). Here, we discuss the possibility that loss or inactivation of the BRCA-FA pathway is an ideal target for this type of targeted approach.