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Lachaud, Christophe; Rouse, John

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A route to new cancer therapies: cells and tumors with mutations in BRCA1 or BRCA2 cannot tolerate loss of the Fanconi anemia pathway

Christophe Lachaud[1] & John Rouse[1*]

[1]MRC Protein Phosphorylation and Ubiquitylation Unit, College of Life Sciences, Sir James Black Centre, University of Dundee, Dundee DD1 5EH, Scotland, UK.
Correspondence to: j.rouse@dundee.ac.uk

Mutations in the *BRCA1* and *BRCA2* genes strongly predispose carriers to breast and ovarian cancers. Two new studies reveal that FANCD2, a key component of the Fanconi anemia pathway, is essential for the survival of cells with *BRCA1/2* mutations. These findings pave the way for new "synthetic lethal" strategies to kill *BRCA*-mutated cancers.

Individuals with heterozygous mutations in the *BRCA1* or *BRCA2* tumor suppressor genes have a greatly increased risk of developing breast and ovarian cancers. Together, *BRCA1* and *BRCA2* mutations are responsible for up to 25% of hereditary breast cancers[1], and there is an urgent need for developing strategies to improve treatment of these cancers. In this light, two recent studies[2,3] have demonstrated that cells with mutations in BRCA1/2 cannot tolerate loss of components of the Fanconi anemia (FA) pathway of genome maintenance. Both studies reveal that the high levels of DNA replication stress known to occur in cells with *BRCA1/2* mutations[4] is counteracted by the FA pathway. Loss of FANCD2, a key component of the FA pathway, from *BRCA1/2*-mutated cells causes levels of replication stress to soar out of control thereby triggering replication fork catastrophe and cell death (Fig. 1). FANCD2 becomes ubiquitinated in response to replication stress, in a manner catalyzed by an E3 ubiquitin ligase comprising a group of eight FA "core complex" proteins[5]. Ubiquitination of FANCD2 is known to be required for cell tolerance of perturbations during DNA replication[6,7] and, accordingly, it is also essential for the survival of *BRCA1/2*-mutated cells[3].

Both BRCA1 and BRCA2 are important for a mode of DNA repair known as homologous recombination (HR), which repairs and resets stressed and broken DNA replication forks[4]. BRCA1 has multiple roles in HR, while BRCA2 acts primarily as a reservoir of the RAD51 recombinase, which it helps to deposit onto DNA breaks to facilitate HR[8]. Intriguingly, the deposition of RAD51 by BRCA2 has a fork-protective role that prevents replication stress in a way that is separate from HR[9]. The significance of these various fork-protective functions of *BRCA1* and *BRCA2* is underscored by the high degree of replication stress seen in cells with mutations in these genes; the resulting genetic instability probably explains why defective *BRCA1/2* function causes cancer.

Like mutations in FANCD2, some mutations in *BRCA1* and *BRCA2* can cause FA, which is characterized by bone marrow failure, developmental defects and predisposition to a range of cancers [10,11]. The FA pathway is responsible for facilitating repair of DNA inter-strand crosslinks (ICLs), which block replisome progression[5]. It is also essential for protecting replication forks from the replication stress caused by ICLs or in other circumstances[12]. The observations that mutations in *FANCD2*, *BRCA1* or *BRCA2* can cause FA suggested these genes are in some respects equivalent in function. However, the recent studies from the Tarsounas, D'Andrea and Ceccaldi labs show that this is not always the case, as FANCD2 acquires a vital role in cells with *BRCA1/2* mutations[2]. Precisely how FANCD2, and its ubiquitination by the FA core complex, prevents the replication stress seen in *BRCA*-mutated cells from rising to deadly levels is not entirely clear. However, Tarsounas and colleagues provide important clues. They show that FANCD2 limits damage to replication forks possibly by helping to arrest and then re-start forks that stall and/or collapse in *BRCA*-mutated cells (Fig. 1). Further work is needed to understand how FANCD2 enables these protective responses. D'Andrea, Ceccaldi and colleagues provide further clues by presenting evidence that the "synthetic lethality" resulting from loss of FANCD2 ubiquitination in cells with mutations in *BRCA1/2* may reflect an unanticipated role for FANCD2 in promoting a mode of DNA repair termed "alternative end-joining" (alt-EJ)[3]. Consistent with this view, it was previously shown that loss of a specialized

DNA polymerase - POLθ - which promotes alt-EJ is synthetic lethal in cells with *BRCA1/2* mutations[13,14]. The implication from these data is that collapsed forks, which cannot be repaired because HR is not functional in cells with defects in *BRCA1/2*, can be rescued by alt-EJ. D'Andrea, Ceccaldi and colleagues now show that POLθ is recruited to stalled forks in a manner dependent on FANCD2 ubiquitination, and that FANCD2-depleted cells show a reduced capacity for alt-EJ[3]. These data are consistent with an essential role for alt-EJ, facilitated by ubiquitin-FANCD2, in cells with *BRCA1/2* mutations. It is interesting to note that besides POLθ two other proteins are known to be recruited to stalled forks by ubiquitin-FANCD2, and may therefore contribute to protecting BRCA-defective cells. These are the HR-promoting factor CTIP[15], and the FAN1[16,17] nuclease, which is known to work closely with ubiquitin-FANCD2 in protecting stalled replication forks. CTIP loss does not kill *BRCA*-mutated cells[3], but the effect of FAN1 loss has yet to be examined, and this will be an important area of investigation.

Synthetic lethality provides a conceptual framework for the development of drugs that are selectively toxic in specific genetic backgrounds associated with human disease. A classic example is the toxicity of PARP (poly-ADP ribose polymerase) inhibitors in cells with *BRCA1/2* mutations. PARP is involved in repairing single-strand breaks (SSB); inhibiting PARP causes the accumulation of SSBs which, if they persist into S-phase, can cause fork collapse. As the repair of collapsed forks requires HR, PARP inhibitors cannot be tolerated in BRCA-mutated cells[18]. PARP inhibitors have shown promise in clinical trials but a major limitation in their use in treating cancers is intrinsic and acquired resistance, and so there is a great need for alternatives[19]. The two new studies suggest that inhibiting FANCD2 function might represent a novel strategy for the treatment of *BRCA*-deficient cancers (Fig. 2). This notion is strongly supported by the exciting observation that FANCD2 depletion impaired the growth of tumors derived from breast cancer cells xenotransplanted into mice[3]. From a clinical perspective, how might FANCD2 function be inhibited in *BRCA1/2*-mutated cancers? FANCD2 is not an enzyme and so drugging it may not be possible but preventing its

ubiquitination by inhibiting the E3 ligase activity of the FA core complex might be a way to selectively kill BRCA-mutated tumors. Alternatively, interfering with enzymes that act downstream of ubiquitin-FANCD2 may be an option. An obvious candidate is POLθ, which is recruited to stressed forks by ubiquitin-FANCD2[3]: the synthetic lethality resulting from loss of POLθ in *BRCA*-mutated cells has already catapulted this enzyme to the forefront of anti-cancer drug discovery[13,14] (Fig. 2). FAN1 is also recruited to stalled forks by ubiquitin-FANCD2[17] and if, like FANCD2, FAN1 turns out to be required to counteract the high levels of fork stress in *BRCA*-mutated cells then small molecule FAN1 inhibitors may also be selectively toxic in *BRCA*-mutated tumors (Fig. 2). These considerations provide new hope for the development of novel treatments for breast and ovarian cancers caused by *BRCA1* and *BRCA2* mutations. It is worth considering that heightened replication stress is not unique to cancers caused by BRCA mutations. Indeed, switching on a range of oncogenes causes replication stress[20,21] and thus pathways that limit replication stress in BRCA-mutated cancers may also be vital for the survival of other cancers.

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Figure Legends:

Figure 1: FancD2 protects replication forks and prevents cell death in BRCA1/2 deficient cancers. Mutations in BRCA1/2 genes lead to high levels of DNA replication stress and genetic instability. By protecting stressed replication forks from catastrophe, FancD2 prevents cell death in BRCA1/2 deficient cancers.

Figure 2: Strategy to selectively target BRCA1/2-deficient cancers by inhibiting FancD2 function. In response to replication stress, FancD2 is ubiquitinated by the FA core complex (E3 ligase), and this in turn promotes

replication fork protection. in order to regulate downstream factors such as Poθ (polymerase) and Fan1 (nuclease). Therefore inhibiting the activity of the FA core complex, which would prevent FANCD2 ubiquitination, should be toxic in BRCA1/2-mutated cancers. Alternatively, inhibiting enzymes downstream of ubiquitin-FANCD2 such as POLθ or FAN1 may be toxic in these cancers. In this light, it has already been shown that BRCA-mutated cells cannot tolerate loss of POLθ[13,14] but FAN1 has not yet been investigated.

Figure_1

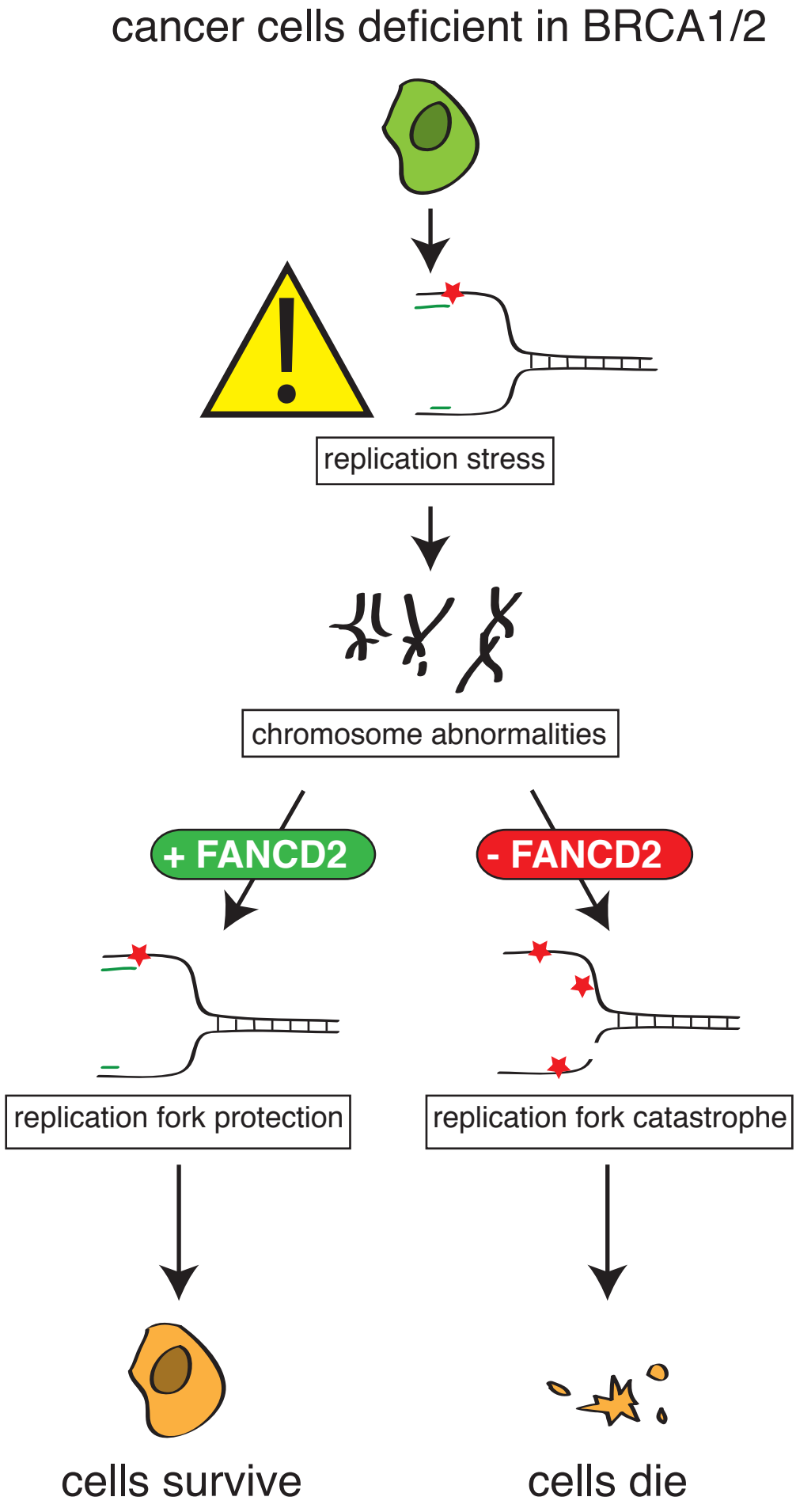
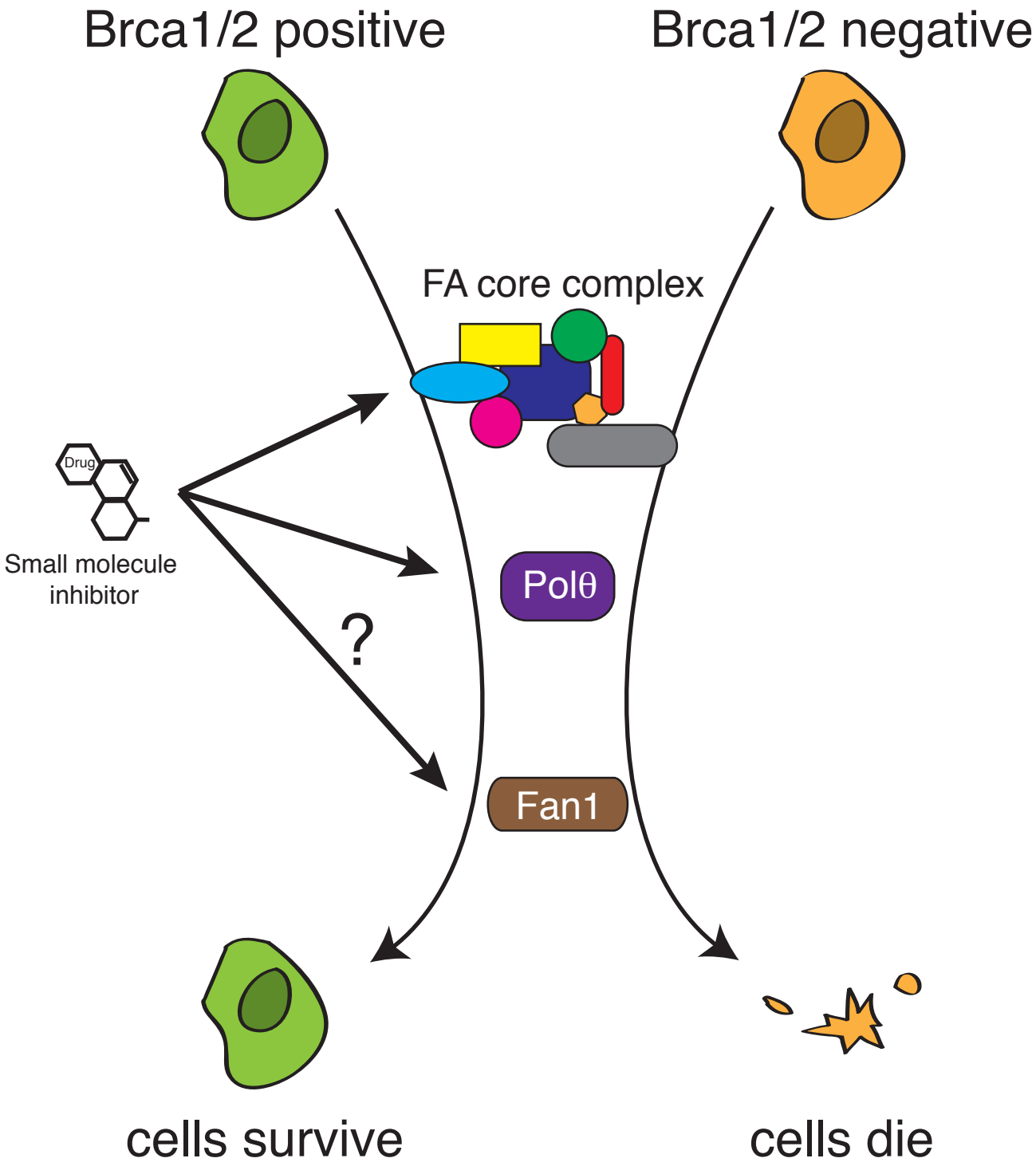

cancer cells deficient in BRCA1/2
replication stress
chromosome abnormalities
+ FANCD2
- FANCD2
replication fork protection
replication fork catastrophe
cells survive
cells die

Figure_2


Brca1/2 positive
Brca1/2 negative
FA core complex
Drug
Small molecule inhibitor
?
Polθ
Fan1
cells survive
cells die