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NEWS AND COMMENTARY

Fanconi Anemia

Fanconi anemia, breast and embryonal cancer risk revisited

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Back-to-back publications in the February issue of Nature Genetics reveal the identity of the gene underlying the thirteenth, and arguably most severe, Fanconi anemia (FA) complementation group: biallelic mutations in *PALB2* cause FA complementation group N (FA-N). A third publication in the same issue further exemplifies the importance of this 'maintenance of genomic stability' pathway in the general (non-FA) population; like *FANCD1/BRCA2* and *FANCJ/BRIP1*, heterozygosity for *FANCN/PALB2* mutations is associated with an increased susceptibility to breast cancer.

FA is a rare autosomal and X-linked recessive disorder characterized by developmental abnormalities, progressive bone marrow failure and a pronounced susceptibility to cancer.[1] FA was first described by Swiss pediatrician Guido Fanconi in 1927, when he encountered three brothers afflicted with aplastic anemia as well as congenital anomalies, including microcephaly and skin hyperpigmentation. Decades of FA research led Fanconi to hypothesize that mutation of a single gene could not account for the clinical heterogeneity observed among FA patients. Eighty years later, Fanconi's astute observation resonates; to date, FA has been associated with mutation of any one of 12 different genes. Encoded by these genes includes a core multi-protein E3 ubiquitin ligase (comprising FANC proteins A, B, C, E, F, G, L and M), a substrate (FANCD2) and three 'downstream' proteins (FANCD1/BRCA2, FANCJ/BRIP1 and now FANCN/PALB2).

The following model for the FA pathway has been proposed: after exposure to DNA damaging agents, as well as during S phase of the cell cycle, the core FA complex catalyzes the monoubiquitination of the FANCD2 protein (Figure 1). Monoubiquitination of FANCD2 signals its translocation to discrete nuclear foci, where it colocalizes with the DNA repair proteins BRCA1 and Rad51.[2] Monoubiquitination of FANCD2 also appears to promote the loading of BRCA2 into chromatin.[3] Thus, it seems likely that the FA proteins function cooperatively in the maintenance of genomic stability by promoting the repair of damaged DNA via a homologous recombination (HR)-mediated mechanism. On the face of it, the identification of FANCN indeed supports this hypothesis.

FANCN (a.k.a. PALB2 for 'Partner and Localizer of BRCA2'), the latest addition to the ever-lengthening list of FA proteins, was recently identified by Xia *et al*[4] in a screen to uncover novel BRCA2-interacting proteins. PALB2 and BRCA2 were found to stably associate in chromatin. PALB2 binds to the N terminus of BRCA2 and is required for the intra-nuclear localization and stabilization of BRCA2. Thus, PALB2 facilitates BRCA2's previously established roles in the S phase checkpoint response and in HR-mediated DNA double-strand break repair.[4,5]

Notably, PALB2-depleted cells are also hypersensitive to the DNA crosslinking agent mitomycin C (MMC),[4] a hallmark phenotype of FA patient cells. This finding, coupled with the fact that biallelic *BRCA2* mutations underlie FA complementation group D1 (FA-D1),[6] fueled *PALB2*'s candidacy as a *bona fide* new FA gene. Thus, Xia *et al*[7] examined cells from unassigned FA patient EUFA1341 for PALB2 protein expression. Serendipitously, full-length PALB2 protein was not detected in EUFA1341 cells. Consistent with their initial observations of a role for PALB2 in the stabilization of BRCA2 in chromatin, EUFA1341 cells lacked chromatin-bound BRCA2. Genomic and cDNA sequencing subsequently uncovered biallelic *PALB2* mutations in EUFA1341. Reintroduction of wild-type PALB2 into EUFA1341 stabilized BRCA2 in the nucleus and corrected the MMC sensitivity of these cells. Intriguingly, Xia *et al* describe a MMC-resistant clone of EUFA1341 that most likely arose via a spontaneous Alu-mediated recombination event on the maternal chromosome arm harboring the mutant *PALB2* allele, 16p12. This intra-chromosomal recombination event deleted *PALB2* exon 4, which contained a nonsense stop mutation, and restored expression of a partially functional protein capable of promoting BRCA2's nuclear functions.

Supporting the findings of Xia *et al*, a second independent report by Reid *et al*[8] describe biallelic *PALB2* mutations in an additional seven unassigned FA patients. PALB2 protein expression could not be detected in cells from four of these patients, and reintroduction of wild-type PALB2 into one patient line restored PALB2 protein expression and corrected the MMC-induced G2/M accumulation of these cells. Collectively, these reports firmly establish PALB2 as FANCN.

Downstream divergence?

Classic FA is characterized by progressive bone marrow failure with a predisposition for squamous cell carcinoma (SCC) of the head and neck and anogenital regions later in life. The median age of onset for FA patient SCC is 31 years.[9] The clinical phenotype of FA-N patients is, however, strikingly different, and eerily resembles that of FA-D1 patients.[10] All eight FA-N patients developed aggressive embryonal tumors in early childhood including medulloblastoma and Wilms' tumor. Five FA-N patients developed tumors under the age of 2 years. So, while sharing some classic FA clinical manifestations, including hypoplastic thumbs and

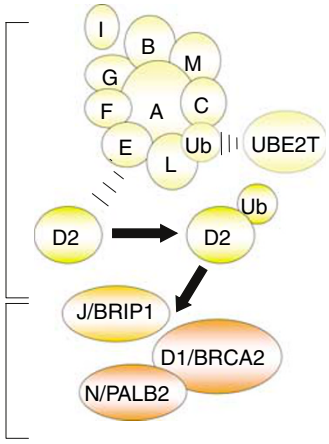

Figure 1 Schematic model of the FA pathway. After exposure to DNA damaging agents, and during S phase of the cell cycle, the core FA complex catalyzes the monoubiquitination of the FANCD2 protein. FANCD1/BRCA2, FANCJ/BRIP1 and FANCN/PALB2 act downstream, of FANCD2 monoubiquitination.

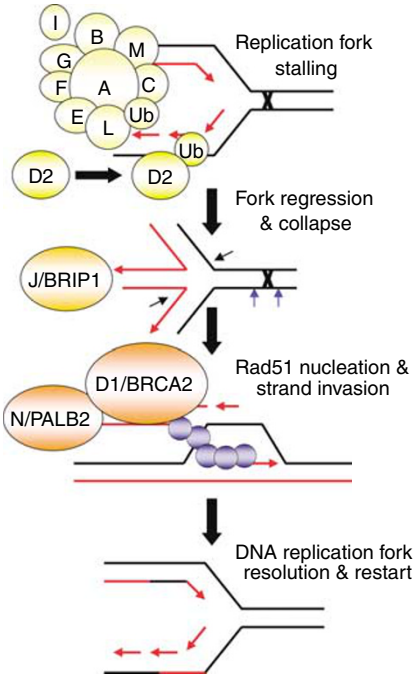

Figure 2 Speculative model of the role of the FA pathway during DNA crosslink repair. A moving DNA replication fork stalls at a DNA crosslink leading to the activation of the FA pathway via FANCD2 monoubiquitination. The stalled replication fork may reverse to form a 'chicken foot' intermediate structure, enabling nucleotide excision repair to unhook the crosslink (blue arrows). Endonucleolytic cleavage of the reversed fork (black arrows) generates a single-ended DNA double-strand break, which can act as a substrate for Rad51-dependent homologous recombination repair. FANCD1/BRCA2 and FANCN/PALB2 promote Rad51 (blue circles) nucleoprotein filament formation and stimulate Rad51-mediated strand invasion. The resulting Holliday junction(s) are resolved by endonucleolytic cleavage to re-establish the replication fork.

growth retardation, FA complementation groups D1 and N are clearly unique, possibly warranting their clinical sub-classification.

In a third report in the same issue, Nazeem Rahman and colleagues demonstrate that monoallelic truncating *PALB2* mutations are associated with an approximate two-fold increased risk of familial breast cancer, representing another point of divergence. To date, mutations in only the three 'downstream' (of FANCD2 monoubiquitination) FA genes, *FANCD1/BRCA2*, *FANCJ/BRIP1*, and *FANCN/PALB2*, have now been clearly associated with an increased risk for breast cancer.[11,12]

Several important cellular features also distinguish FA-D1 and FA-N patient cells from cells from all other FA complementation groups. These include phenotypes of considerable recent debate, including the ability of FA cells to form Rad51 nuclear foci,[3,13–15] and consequently, the role of the FA pathway in HR DNA repair. Rad51 is the major mammalian DNA-strand recombinase. The Rad51 protein forms a nucleoprotein filament on single-stranded DNA and catalyzes strand invasion of a homologous intact sister chomatid during the conservative 'gene conversion' pathway of HR repair (Figure 2). The influence of the upstream FA proteins (A, B, C, E, F, G, L and M), as well as FANCD2, on the stabilization of Rad51 in nuclear foci is ambiguous.[3,13–15] However, cells from FA-D1 and FA-N patients' display severely attenuated Rad51 nuclear foci formation.[7,8,14] Furthermore, FANCD1/BRCA2 and FANCJ/BRIP1, and now FANCN/PALB2, all appear to be required for the gene conversion-mediated repair of a DNA double-strand break.[4,5,16] The role of the other FA proteins in this process is tenuous.[15,17]

The devil may be in the details

While the upstream FA proteins, as well as FANCD2, may not function directly in gene conversion HR repair, it seems likely that they at least cooperatively promote this conservative, error-free DNA repair pathway under certain conditions (Figure 2). Conversely, FA proteins FANCD1/BRCA2 and FANCN/PALB2, and possibly FANCJ/BRIP1, function directly in this mode of DNA repair. The increased clinical severity of FA-D1 and FA-N may be a consequence of these proteins functioning 'beyond a point of no return', that is, following a cellular commitment to a doomed gene conversion HR repair mechanism. Furthermore, many DNA lesions, and not solely the DNA crosslink, will be

repaired via a DNA double-strand break intermediate, necessitating their function. Nevertheless, in the absence of an intact FA pathway, non-conservative, error-prone DNA repair pathways, such as single-strand annealing or inter-chromosomal HR, may predominate. The former pathway would be expected to give rise to intra-chromosomal deletions, possibly due to recombination between mobile repetitive sequences such as Alu elements (see EUFA1341 reversion event above). Indeed, an increased frequency of deletion mutations at the *HPRT* locus in FA lymphoblasts was described by Papadopoulo *et al*[18] many years ago. Furthermore, failed inter-chromosomal recombination events may underlie the trademark FA cytogenetic anomalies, the tri- and quadriradial chromosome formations.

While many mechanistic details remain to be resolved, what is clear is that our continued research into this rare debilitating disease continues to have far-reaching implications for our understanding of cancer susceptibility in the general (non-FA) population■

Note added in proof

The author wishes to acknowledge that since going to press two new publications have revealed the identity of the *FANCI* gene as *KIAA1794*, encoding a previously uncharacterized FANCD2 paralog.

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