Blood Reviews 31 (2017) 93–99

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

REVIEW

Recent discoveries in the molecular pathogenesis of the inherited bone marrow failure syndrome Fanconi anemia

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ARTICLE INFO

Keywords:
Fanconi anemia
Genome instability
DNA repair
Homologous recombination
Ubiquitin

ABSTRACT

Fanconi anemia (FA) is a rare autosomal and X-linked genetic disease characterized by congenital abnormalities, progressive bone marrow failure (BMF), and increased cancer risk during early adulthood. The median lifespan for FA patients is approximately 33 years. The proteins encoded by the FA genes function together in the FA-BRCA pathway to repair DNA damage and to maintain genome stability. Within the past two years, five new FA genes have been identified—*RAD51*/*FANCR*, *BRCA1*/*FANCS*, *UBE2T*/*FANCT*, *XRCC2*/*FANCU*, and *REV7*/*FANCV*—bringing the total number of disease-causing genes to 21. This review summarizes the discovery of these new FA genes and describes how these proteins integrate into the FA-BRCA pathway to maintain genome stability and critically prevent early-onset BMF and cancer.

1. Introduction

In the 1920s, Swiss pediatrician Guido Fanconi first described the disease later to become formally recognized as Fanconi anemia (FA) [1]. In a family with five children, three brothers died of a severe condition that resembled pernicious anemia. The three patients' disease manifested between the ages of five and seven, and was associated with congenital microcephaly, café au lait spots, cutaneous hemorrhage, and hypoplasia of the testes, concurrent with a current-day diagnosis of FA [2].

With an estimated incidence of 1 in 360,000 live births and a carrier frequency of approximately 1 in 181, FA is relatively uncommon and can be difficult to diagnose due to patients presenting with a wide variety of symptoms [3,4]. Symptoms that could raise suspicion of FA among physicians include various congenital anomalies including microcephaly, microphthalmia, abnormal thumbs or radii, and slow growth rate. Hematological signs can include early-onset aplastic anemia, myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) at an atypically young age, and one or more unexplained cytopenias of any cell lineage, including thrombocytopenia, neutropenia, and anemia. Additionally, clinicians should be mindful of the presentation of solid tumors at a particularly young age, specifically, head, neck, esophageal, and gynecological squamous cell carcinomas. Familial cancer predisposition or a history of chemotherapeutic hypersensitivity can also raise the suspicion of FA (see Practice points) [3,5,6].

After many decades of work, the FA genes are rapidly being identified. The *FANCC* gene was identified in 1992 [7,8]. Subsequent discoveries of the *FANCA* [9,10], *FANCG* [11], *FANCE* [12], *FANCF* [13], and *FANCD2* [14] genes followed. Correlating with rapid advances in genetic and biochemical technologies, the rate of FA gene identification has promptly increased. With the most recent additions, there are now 21 confirmed FA genes; *FANCA*, *FANCB*, *FANCC*, *BRCA2*/*FANCD1*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *BRIP1*/*FANCJ*, *FANCL*, *FANCM*, *PALB2*/*FANCN*, *RAD51C*/*FANCO*, *SLX4*/*FANCP*, *ERCC4*/*FANCQ*, and most recently, and as highlighted in this review, *RAD51*/*FANCR*, *BRCA1*/*FANCS*, *UBE2T*/*FANCT*, *XRCC2*/*FANCU*, and *MAD2L2*/*REV7*/*FANCV*. The previously designated FA-H complementation group was found to be analogous to FA-A, and as such *FANCH* was removed [15,16]. The addition of five new FA genes over the past two years is testament to the continued resolve of the international FA research community and the Fanconi Anemia Research Fund.

2. The FA-BRCA pathway

One major function of the FA-BRCA pathway is to orchestrate the repair of DNA interstrand crosslinks (ICLs) [17,18]. Examples of ICL-inducing agents include diepoxybutane (DEB) and mitomycin C (MMC). ICLs pose a direct physical block to DNA replication and RNA transcription and result in cellular cytotoxicity and chromosome structural aberrations if not properly repaired. FA patient cells from all complementation groups are characteristically hypersensitive to ICLs, and this phenotype forms the basis of the clinical FA diagnostic test [3,19].

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http://dx.doi.org/10.1016/j.blre.2016.10.002
0268-960X/

Specifically, FA patient cells exhibit increased radial chromosome formations following ICL induction, a consequence of a molecular roadblock in ICL repair. ICL repair mediated by the FA proteins can be described as a tri-phasic process.

In the first phase, the upstream FA proteins FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, and FANCL, as well as the Fanconi anemia-associated proteins FAAP20 and FAAP100, assemble to form the FA core complex (Fig. 1). Upon DNA damage, the FA core complex is recruited to chromatin where it interacts with UBE2T/FANCT, which is constitutively chromatin localized [20,21]. Recent studies have identified three distinct modules within the FA core complex: the FANCB–FANCL–FAAP100 module, which provides the essential monoubiquitination catalytic activity, and the FANCA–FANCG–FAAP20 and FANCC–FANCE–FANCF modules, the exact functions of which have yet to be determined [22,23]. The FANCM anchor complex, comprising FANCM, FAAP24, FAAP16/MHF1, and FAAP10/MHF2, promotes the chromatin recruitment of the FA core complex [24–27]. Together, the FA core complex and UBE2T/FANCT constitute an active multi-subunit E2/E3 ubiquitination enzyme complex. The RING domain-containing FANCL subunit functions as the E3 ubiquitin ligase while UBE2T/FANCT functions as the E2 ubiquitin-conjugating enzyme [28–30]. This complex catalyzes the second phase of the pathway, the conjugation of a single ubiquitin moiety (monoubiquitin) to K561 of FANCD2 and K523 of FANCI [31–33]. FANCD2 and FANCI form a heterodimer known as ID2 [34]. Ubiquitin is a 76-amino acid protein that is covalently posttranslationally attached to target proteins. Monoubiquitin functions as a molecular signal that regulates diverse cellular processes including the targeting of proteins to distinct subcellular locations and the promotion of protein–protein interactions [35]. As evidence of the critical role the FA core complex plays in this process, FA patient cells harboring deleterious mutations in *FANCA*, *FANCB*, *FANCC*, *FANCE*, *FANCF*, *FANCG*, *FANCL*, or *FANCT* lack the capacity to monoubiquitinate the ID2 heterodimer [31–33]. Monoubiquitinated ID2 localizes to chromatin where it associates with several established DNA repair proteins, including BRCA1/FANCS, BRCA2/FANCD1, and RAD51/FANCR [31,36,37] Monoubiquitinated ID2 is thought to function in the recruitment of several DNA repair proteins, including CtIP, Fanconi anemia associated nuclease 1 (FAN1), SLX4/FANCP, and ERCC4/FANCQ [38–44]. SLX4/FANCP and the ERCC4/FANCQ endonuclease catalyze the unhooking of the ICL [39], enabling translesion DNA synthesis (TLS) beyond the ICL by the multi-subunit TLS polymerase Polζ, one subunit of which is REV7/FANCV (Fig. 2) [45,46].

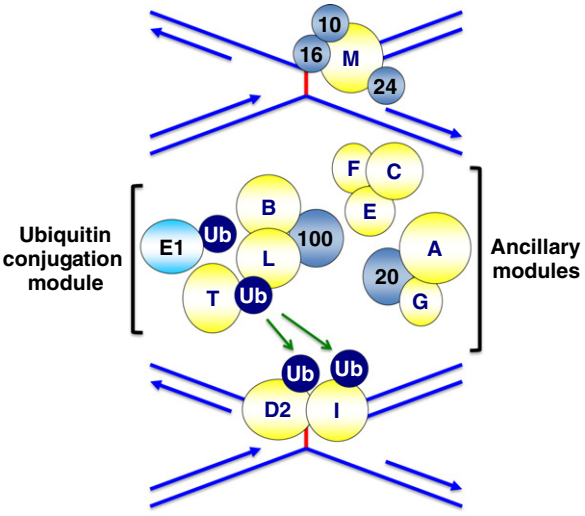

Fig. 1. Schematic of FANCD2 and FANCI monoubiquitination. Following exposure to DNA-damaging agents and during S-phase of the cell cycle, the FANCM anchor complex, comprising FANCM, FAAP24, FAAP16/MHF1 and FAAP10/MHF2, recognizes the damage, remodels the fork, and promotes the recruitment of the FA core complex. The FA core complex, which is comprised of three sub-complexes—FANCB/FANCL/FAAP100, FANCC/FANCE/FANCF, and FANCA/FANCG/FAAP100—together with the E2 ubiquitin-conjugating enzyme UBE2T/FANCT, constitutes an active multisubunit E2/E3 ubiquitination enzyme complex. This E2/E3 enzyme complex catalyzes the site-specific monoubiquitination of FANCD2 K561 and FANCI K523.

During the final phase of ICL repair, the downstream FA proteins—BRCA2/FANCD1, BRIP1/FANCJ, PALB2/FANCN, RAD51C/FANCO, BRCA1/FANCS, RAD51/FANCR, and XRCC2/FANCU—function cooperatively to repair the remaining broken duplex via homologous recombination (HR) (Fig. 2). HR is predominantly a conservative and error-free process whereby DNA damage is repaired using a homologous DNA template, typically the sister chromatid [47,48]. RAD51/FANCR is the major eukaryotic HR repair protein. RAD51/FANCR forms nucleoprotein filaments on 5′–3′ resected single-stranded DNA and catalyzes homologous pairing and DNA strand invasion and exchange. Many of the downstream FA proteins, e.g. BRCA2/FANCD1, PALB2/FANCN, and RAD51C/FANCO, are known to facilitate RAD51 function [49–51]. Disruption of the FA-BRCA pathway leads to defective HR and an increased dependence on the typically error-prone nonhomologous DNA end-joining (NHEJ) repair pathway [52–54].

3. *UBE2T/FANCT* is responsible for a new FA subtype

The covalent attachment of ubiquitin to proteins regulates a variety of cellular pathways. This transfer is completed through a cascade of ubiquitin-related enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3) (Fig. 1) [55]. Monoubiquitination of FANCD2/FANCI is the central step of the FA-BRCA pathway, and it is estimated that this step is defective in >90% FA patients, and intricately tied to patients' increased BMF and cancer risk. UBE2T, newly termed FANCT, is the E2 ubiquitin-conjugating enzyme for FANCD2 and FANCI [28,29,56]. In 2000, Zhang et al. cataloged three hundred cDNAs of previously undefined genes expressed in CD34-positive hematopoietic stem cells, one of which, *HSPC150*, contained a ubiquitin-conjugating motif [57]. *HSPC150* was mapped to 1q31, a region known to be amplified in a range of cancers, including breast cancers, hepatomas, and cervical carcinomas [58]. This protein, later renamed UBE2T, was demonstrated in biochemical assays to interact with FANCL, the E3 ubiquitin ligase of the FA pathway [29]. UBE2T was subsequently shown to be required for FANCD2 monoubiquitination. Consequently, similar to other FA patient cells, loss of UBE2T leads to increased sensitivity to ICL-induced chromosome damage [29]. These data clearly implicated UBE2T as the ubiquitin-conjugating enzyme for the FA-BRCA pathway, yet stopped short of classification of a new subtype.

In 2015, Hira et al., from the DNA damage signaling laboratory of Kyoto University, reported two unrelated FA patients who, through WES and Sanger sequencing, were found to have biallelic *UBE2T* mutations [59]. Both patients presented with congenital malformations, hematological abnormalities, and early onset BMF at ages 8 and 13. A heterozygous missense mutation leading to p.Q2E was uncovered in both patients [59]. This highly conserved glutamine residue is found in an amino-terminal helix, which is part of the E2–E3 interacting interface [60]. Disruption of this interface results in less efficient FANCD2 ubiquitination [59]. Additionally, both patients harbored unique mutations in their second alleles, a 23-kilobase genomic deletion in one patient and skipped exon resulting in a frameshift and premature stop codon in the other. Complementation of patient cells with wild-type UBE2T restored efficient FANCD2 monoubiquitination and nuclear foci formation, and rescued the increased sensitivity to MMC-induced chromosome breakage [59]. Hira et al. suggested that this new FA complementation group be named FA-T.

Coinciding with this study, the Laboratory of Genome Maintenance at Rockefeller University described an individual with biallelic mutations in *UBE2T*, born with microcephaly and bilateral thumb malformations, who tested positive in the DEB chromosome breakage test [61]. However, this patient had normal bone marrow cellularity,