Mutation Research 668 (2009) 27–41

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Review

The Fanconi anemia protein interaction network: Casting a wide net

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ARTICLE INFO

Article history:
Received 14 August 2008
Received in revised form
16 November 2008
Accepted 25 November 2008
Available online 3 December 2008

Keywords:
Fanconi anemia
DNA repair
Chromosome instability
Homologous recombination
Translesion DNA synthesis

ABSTRACT

It has long been hypothesized that a defect in the repair of damaged DNA is central to the etiology of Fanconi anemia (FA). Indeed, an increased sensitivity of FA patient-derived cells to the lethal effects of various forms of DNA damaging agents was described over three decades ago [A.J. Fornace, Jr., J.B. Little, R.R. Weichselbaum, DNA repair in a Fanconi's anemia fibroblast cell strain, Biochim. Biophys. Acta 561 (1979) 99–109; Y. Fujiwara, M. Tatsumi, Repair of mitomycin C damage to DNA in mammalian cells and its impairment in Fanconi's anemia cells, Biochem. Biophys. Res. Commun. 66 (1975) 592–598; A.J. Rainbow, M. Howes, Defective repair of ultraviolet- and gamma-ray-damaged DNA in Fanconi's anaemia, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 31 (1977) 191–195]. Furthermore, the cytological hallmark of FA, the DNA crosslink-induced radial chromosome formation, exemplifies an innate impairment in the repair of these particularly cytotoxic DNA lesions [A.D. Auerbach, Fanconi anemia diagnosis and the diepoxybutane (DEB) test, Exp. Hematol. 21 (1993) 731–733]. Precisely defining the collective role of the FA proteins in DNA repair, however, continues to be one of the most enigmatic and challenging questions in the FA field. The first six identified FA proteins (A, C, E, F, G, and D2) harbored no recognizable enzymatic features, precluding association with a specific metabolic process. Consequently, our knowledge of the role of the FA proteins in the DNA damage response has been gleaned primarily through biochemical association studies with non-FA proteins. Here, we provide a chronological discourse of the major FA protein interaction network discoveries, with particular emphasis on the DNA damage response, that have defined our current understanding of the molecular basis of FA.

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doi:10.1016/j.mrfmmm.2008.11.018

1. RAD51 (December 1997)

Perhaps the earliest defined, and arguably most important, interaction between a FA protein and a non-FA protein is that of FANCD1/BRCA2 and RAD51. RAD51 is the mammalian homologue of the *Escherichia coli* RecA protein, and catalyzes the critical strand invasion step of homologous recombination (HR) DNA repair. HR is an essential cellular DNA double-strand break (DSB) repair mechanism that depends on the existence of an intact 'homologous' template sequence on the sister chromatid or homologous chromosome, to copy and synthesize lost or damaged genetic information (for reviews see refs. [5,6]). In 1997, a yeast two-hybrid screen using a T-cell cDNA library revealed that a carboxy-terminus fragment of murine Brca2 could interact with Rad51 [7]. Murine *Brca2* and *Rad51* were also found to be temporally and spatially co-expressed during early embryogenesis. Furthermore, *Rad51*−/− and *Brca2*−/− mice were characterized by early embryonic lethality, while *Rad51*−/− and *Brca2*−/− embryos were hypersensitive to the cytotoxic effects of ionizing radiation (IR) [7–9]. In the same year, Mizuta et al. described a direct interaction between the human RAD51 protein and murine Brca2 using yeast two-hybrid as well as GST pull-down approaches [10]. The critical importance of these discoveries for both clinical and molecular aspects of FA would not be revealed until five years later when biallelic mutations in the *BRCA2* gene were uncovered in two FA-D1 patients [11].

Numerous studies over the past decade have contributed to our current understanding of the mechanism by which the FANCD1/BRCA2 protein regulates the function of RAD51 in HR: FANCD1/BRCA2 encodes a 3418-amino-acid protein that contains eight evolutionarily conserved internal repeats known as BRC motifs. These BRC motifs mediate the direct binding of FANCD1/BRCA2 to RAD51 [12–15]. FANCD1/BRCA2 promotes RAD51 nucleoprotein filament formation and stimulates RAD51-mediated strand exchange (for reviews see refs. [16,17]). The recently identified FANCN/PALB2 protein (*for Partner and Localizer of BRCA2*) binds to the amino-terminus of FANCD1/BRCA2 and promotes its stabilization in chromatin [18]. Accordingly, both FA-D1 and FA-N patient cells display severely attenuated RAD51 nuclear foci formation [19–21]. The role of the upstream FA proteins (A, B, C, E, F, G, L, and M), as well as FANCD2, in the regulation of RAD51 remains unclear [19,22–24].

Hypomorphic *FANCD1/BRCA2* mutations underlie the overwhelming majority of FA-D1 patients characterized to date [11,25,26]. This is consistent with the observation that disruption of murine *Fancd1*/*Brca2* results in early embryonic lethality [7]. Furthermore, the clinical phenotypes of FA-D1 (*as well as FA-N*) patients are typically markedly more severe than classic FA, and are characterized by pronounced susceptibility to early childhood cancers [11,20,21,27]. In total, 23 biallelic *FANCD1/BRCA2*−/− patients have been described to date. Among these FA-D1 patients, five cases of Wilms tumor, nine brain tumors, including medulloblastoma, glioblastoma, and astrocytoma, seven cases of acute myeloid leukemia (AML), and three cases of acute lymphoblastic leukemia (ALL), have been recorded [11,25,26,28]. The increased clinical severity of FA-D1 and FA-N patients may be attributable to the direct roles of the FANCD1/BRCA2 and FANCN/PALB2 proteins in the regulation of essential RAD51-dependent HR processes. Conversely, while the upstream FA proteins, as well as FANCD2, may not function directly in HR repair, it seems likely that they at least co-operatively promote this conservative, error-free DNA repair pathway under certain conditions.

2. BRCA1 (August 1998)

An important association between the protein products of the two major familial breast cancer susceptibility genes, *BRCA1* and *FANCD1/BRCA2*, was suggested by several studies in the latter half of the last decade [29]. In 1996, Rajan et al. demonstrated that murine *Brca1* and *Fancd1/Brca2* mRNA expression were coordinately regulated during mammary epithelial proliferation and differentiation, suggesting that these proteins might function in overlapping pathways [30]. In addition, both proteins were demonstrated to interact with Rad51 [7,10,31]. Furthermore, *Brca1*−/− mice, like *Fancd1*/*Brca2*−/− and *Rad51*−/− mice, were also characterized by early embryonic lethality [7–9,32–34]. In 1998, Chen et al. demonstrated that BRCA1 and FANCD1/BRCA2 co-immunoprecipitate, and co-localize in nuclear foci during S phase of the cell cycle. BRCA1, FANCD1/BRCA2, as well as RAD51 were also shown to co-localize on the axial element of developing synaptonemal complexes in human spermatocytes, strongly suggestive of a cooperative role for these proteins in both mitotic and meiotic HR processes [35].

The seminal FA breakthrough, establishing a biochemical connection between BRCA1 (*and hence FANCD1/BRCA2* and *RAD51*) and the FA pathway, occurred early in 2001 with the positional cloning of the *FANCD2* gene and the functional characterization of the FANCD2 protein [36,37]. Garcia-Higuera et al. discovered that the FANCD2 protein was post-translationally modified *via* the covalent linkage of a single ubiquitin molecule to internal K561 [36]. The mono-ubiquitination of FANCD2 was demonstrated to be required for its translocation to discrete nuclear foci following exposure to both IR and UV-irradiation [36]. As the BRCA1 protein had previously been demonstrated to translocate to discrete nuclear foci, where it co-localized with known DNA repair proteins, the association between FANCD2 and BRCA1 was examined [31,38–40]. FANCD2 and BRCA1 were demonstrated to co-localize in nuclear foci, as well as co-immunoprecipitate, following exposure to IR [36]. FANCD2 was subsequently demonstrated to co-localize with BRCA1 and RAD51 during S phase of the cell cycle [41]. Thus, at the molecular level, the FA pathway was simultaneously and unequivocally linked to both ubiquitin-mediated post-translational modification and HR DNA repair.

Using several complementary approaches, the FANCA protein and BRCA1 were also shown to interact [42]. Indeed, a direct interaction between the amino-terminus of FANCA and a central portion of BRCA1 was established using yeast two-hybrid analysis. The FANCA–BRCA1 interaction was confirmed using *in vitro* transcription/translation and immunoprecipitation analyses. Furthermore, the association between FANCA and BRCA1 was demonstrated to be independent of DNA damage. Interestingly, using this yeast two-hybrid system, Folias et al. failed to detect a direct interaction between FANCD2 and BRCA1, suggesting that FANCA may provide a structural link between these proteins [42].

Further highlighting the important connection between BRCA1 and the FA pathway, the FANCJ protein, a DEAH helicase, also known as BRIP1 (*for BRCA1-interacting protein*) was originally identified through its association with BRCA1 [43]. FANCJ binds directly to the carboxy-terminus BRCT repeats of BRCA1 and facilitates BRCA1s known function in DNA DSB repair [43,44]. It has been hypothesized that the FANCJ/BRIP1 helicase may play a role in the timely displacement of RAD51 from nucleoprotein filaments following RAD51-mediated strand exchange [45]. BRCA1 may directly regulate FANCJ/BRIP1 helicase activity, thereby preventing the premature termination of RAD51-dependent HR processes [45]. FANCJ/BRIP1 is also known to interact with several additional non-FA proteins including the mismatch repair proteins MLH1 and PMS2 [46]. Readers are referred to the Ali et al. review article in this issue for a comprehensive description of FANCJ function.

2.1. BRCA1 and FANCD2 mono-ubiquitination

As the BRCA1 protein harbors a RING finger domain, a domain associated with E3 ubiquitin ligase activity [47], Garcia-Higuera