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Review

Mouse models of Fanconi anemia

Kalindi Parmar[a], Alan D'Andrea[a], Laura J. Niedernhofer[b,*]

[a] Department of Radiation Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA
[b] Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine and Cancer Institute, 5117 Centre Avenue, Hillman Cancer Center, Research Pavilion 2.6, Pittsburgh, PA 15213-1863, USA

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ABSTRACT

Fanconi anemia is a rare inherited disease characterized by congenital anomalies, growth retardation, aplastic anemia and an increased risk of acute myeloid leukemia and squamous cell carcinomas. The disease is caused by mutation in genes encoding proteins required for the Fanconi anemia pathway, a response mechanism to replicative stress, including that caused by genotoxins that cause DNA interstrand crosslinks. Defects in the Fanconi anemia pathway lead to genomic instability and apoptosis of proliferating cells. To date, 13 complementation groups of Fanconi anemia were identified. Five of these genes have been deleted or mutated in the mouse, as well as a sixth key regulatory gene, to create mouse models of Fanconi anemia. This review summarizes the phenotype of each of the Fanconi anemia mouse models and highlights how genetic and interventional studies using the strains have yielded novel insight into therapeutic strategies for Fanconi anemia and into how the Fanconi anemia pathway protects against genomic instability.

Contents

1. Fanconi anemia

Fanconi anemia is a rare autosomal recessive disease with a complex spectrum of symptoms including congenital skeletal and renal anomalies, growth retardation, pigmentation abnormalities, fertility defects, aplastic anemia, and increased risk of acute myeloid leukemia and epithelial tumors (see "Fanconi Anemia and its Diagnosis" Auerbach, this issue). Progressive bone marrow failure and late-developing myeloid malignancies account for 90% of mortality in FA patients. Bone marrow failure in FA children is attributed to the excessive apoptosis and subsequent failure of the hematopoietic stem cell compartment. The disease is caused by mutation in genes encoding proteins required for the Fanconi anemia (FA) pathway, a response mechanism to replicative stress [33]. To date, 13 complementation groups of FA have been identified (FANC A, B, C, D1, D2, E, F, G, I, J, L, M, N) [63]. FA is diagnosed by clinical suspicion coupled with detecting an increased number of chromosomal aberrations in patient cells exposed to drugs that induce DNA

* Corresponding author. Tel.: +1 412 623 7763; fax: +1 412 623 7761.
E-mail address: niedernhoferl@upmc.edu (L.J. Niedernhofer).

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interstrand crosslinks (ICLs). ICLs covalently tether both strands of the DNA helix together and therefore are an absolute block to the progression of a replication fork and a potent inducer of replication stress. Crosslinking agents induce replication-dependent double-strand breaks (DSBs) [54]. These DSBs are subsequently repaired by homologous recombination (HR), which is detected cytogenetically as sister chromatid exchanges (the swapping of sister chromatids distal to a DSB) (see "Cellular and Molecular Consequences of Defective Fanconi Anemia Proteins in Replication-Coupled Repair: Mechanistic Insights" Thompson and Hinz, this issue). The diagnosis of FA is made when crosslinking agents cause chromatid breaks and radial structures rather than sister chromatid exchanges. These hallmark cytogenetic changes demonstrate that HR-mediated repair of ICLs is compromised in FA.

2. Fanconi anemia pathway

The 13 FA proteins work as a complex signaling network that facilitates HR-mediated repair of DSBs caused by DNA ICLs and other types of replication stress (see "The Genetic and Molecular Basis of Fanconi Anemia" de Winter and Joenje, this issue, for more detail and a model). FANC A, B, C, E, F, G, L and M interact to form the FA core complex [19]. The FANCL subunit is an E3 ubiquitin ligase that monoubiquitylates FANCD2 and FANCI during S phase, particularly in response to genotoxic stress [48]. After ubiquitylation, FANCD2 is stabilized on chromatin with numerous proteins required for HR including FANCD1/BRCA2 and the FA core complex [80].

Three genes encoding components of the FA core complex have been deleted in the mouse (*FancA*, *FancC* and *FancG*), as well as *FancD1* and *FancD2* [18]. Most recently, the gene that encodes the enzyme that deubiquitylates FANCD2, *Usp1*, was deleted in the mouse [36], yielding the most accurate recapitulation of FA. This review summarizes the phenotype of the various FA mouse models (see Table 1) and illustrates how genetic and interventional studies using the mice have revealed important information about how the FA pathway protects against genomic instability and how FA might be treated.

3. *FancA*$^{-/-}$ mice

Unlike FA patients, *FancA*$^{-/-}$ mice, created by deletion of exons 4–7, do not spontaneously display congenital anomalies or severe hematological abnormalities [12]. However, *FancA*$^{-/-}$ mice do have significantly reduced fertility due to hypogonadism [12]. Despite the mild phenotype, mouse embryonic fibroblasts (MEFs), derived from these mice are hypersensitive to mitomycin C (MMC) and accumulate large numbers of chromosomal aberrations in response to MMC [12], hallmark diagnostic criteria of FA (see "Fanconi Anemia and its Diagnosis" Auerbach, this issue). These *FancA*$^{-/-}$ mice have a mild, but significant thrombocytopenia, corresponding with impaired proliferation of bone marrow-derived megakaryocyte progenitors, but not granulocyte-macrophage progenitors, in vitro [66]. No differences are seen in the number of mature or progenitor bone marrow, spleen or thymic cells between wild type and *FancA*$^{-/-}$ mice [66]. However, cells isolated from the bone marrow of *FancA*$^{-/-}$ mice proliferate poorly under growth stimulatory conditions due to increased apoptosis [66]. To date, a (possible) hematopoietic stem cell defect for these *FancA*$^{-/-}$ mice has not yet been examined through serial transplant studies. *FancA*$^{-/-}$ and *FancG*$^{-/-}$ mice (see below) have microcephaly due to increased neuronal apoptosis [74]. Apoptosis and chromosomal instability occurs only in proliferating cells and not post-mitotic neurons, thus leads to a progressive loss of neural stem and progenitor cells [74]. This can be interpreted as accelerated aging of stem cells in FA [73].

FancA$^{-/-}$ mice, created by deletion of exons 1–6, display pre-natal growth retardation and craniofacial abnormalities (microphthalmia), common features of FA [83]. These developmental defects are strain-dependent, indicating the existence of modifier genes affecting the severity of the phenotype. The more severe phenotype of this knock-out strain corresponds with an increased sensitivity of their bone marrow progenitor cells to MMC relative to cells isolated from the other *FancA*$^{-/-}$ strain [66,83]. This supports the notion that the clinical heterogeneity in FA results at least in part from differences in sensitivity to crosslink damage.

FancA$^{-/-}$ mice are not hypersensitive to ionizing radiation [10]. In contrast, mice defective in proteins required for HR-mediated DSB repair (*e.g.* RAD54 or BRCA1) [14,71]. This suggests that FA is not caused by a generalized defect in HR (although HR defects may be cell type specific and therefore not readily detected). However, cells from *FancA*$^{-/-}$ mice do show impaired gene targeting due to a defect in single-strand annealing [87], providing evidence that the FA pathway may facilitate a subpathway of HR.

4. *FancC*$^{-/-}$ mice

Like the *FancA*$^{-/-}$ mice, genetic deletion of *FancC* in the mouse does not lead to skeletal abnormalities or spontaneous peripheral hematological abnormalities [11,82]. However, *FancC*$^{-/-}$ mice are born with sub-Mendelian frequency and have a significantly increased incidence of microphthalmia, a congenital abnormality, if they are bred into a C57BL/6J background [6]. *FancC*$^{-/-}$ mice have impaired fertility [11,82] due to impaired proliferation of germ cells during embryogenesis [51], similar to FA patients. An increased incidence of tumors in *FancC*$^{-/-}$ mice, greater than 1 year of age, has been reported [6].

As predicted from FA, hematopoietic progenitor cells isolated from *FancC*$^{-/-}$ mice have impaired function in vitro [82], as do hematopoietic stem cells [7]. Mouse embryonic fibroblasts, derived from these mice are hypersensitive to MMC and diepoxybutane (DEB) [82] and stimulated splenocytes isolated from *FancC*$^{-/-}$ mice arrest in G2/M and display a 6-fold increase in chromosomal aberrations in response to crosslinking agents [11,82], hallmark diagnostic criteria for FA (see "Fanconi Anemia and its Diagnosis" Auerbach, this issue). Bone marrow progenitor cells isolated from adult, but not juvenile *FancC*$^{-/-}$ mice have impaired proliferation in vitro [82], demonstrating a progressive, yet subclinical hematopoietic defect. This is substantiated by the observation that bone marrow cells isolated from *FancC*$^{-/-}$ mice have a significantly decreased short-term and long-term, multi-lineage repopulating ability in competitive transplantation assays [4,7,27]. This was attributed to impaired ability of *FancC*$^{-/-}$ hematopoietic stem cells to differentiate and self-renew in response to stimulatory growth factors and cytokines [3,23]. The defect in repopulating capacity of *FancC*$^{-/-}$ bone marrow cells is corrected by retroviral-mediated gene transfer of *FancC* [28], demonstrating that FANCC and the FA pathway are specifically required for the maintenance of HSC function in vivo.

Like the *FancA*$^{-/-}$ mice, the relatively mild phenotype of *FancC*$^{-/-}$ mice is remarkable in view of the fact that: (1) the cellular defect in *FancA*$^{-/-}$ and *FancC*$^{-/-}$ MEFs is similar to that of FA cells [11]; (2) the mouse genes correct human FA cells of the same complementation group, demonstrating conservation of function between mice and humans [79,81,84]; (3) *FancA* and *FancC* are highly expressed in mouse embryos in tissues prone to developmental defects in FA [1,38]; (4) *FancC*$^{-/-}$ mice are hypersensitive to crosslinking agents [8] and chronic in vivo exposure to a low dose of MMC induces chromosomal aberrations and progressive bone marrow failure with pancytopenia [26,58,62,82]. Clearly mice defective in the FA pathway, like humans, are hypersensitive to DNA inter-

Table 1
Phenotypes of FA mouse models in comparison with Fanconi anemia patients.

Phenotype	Mouse strains						Human
	FancC$^{-/-}$	*FancG*$^{-/-}$	*FancA*$^{-/-}$	*FancD2*$^{-/-}$	*FancD1*/*Brca2*$^{\Delta 27/\Delta 27}$	*Usp1*$^{-/-}$	
Peripheral blood	Normal	Normal	Thrombocytopenia	Normal	Normal	Normal	Pancytopenia
Bone marrow	Impaired proliferation of progenitors in vitro, decreased short- and long-term repopulating ability of HSCs	Impaired function of mesenchymal stem/progenitor cells	Impaired proliferation of progenitors in vitro	NA	Impaired proliferation of progenitors in vitro, decreased short- and long-term repopulating ability of HSCs	Hypoplasia	Hypoplasia and aplastic anemia
Gonadal/germ cell defects	Hypogonadism, impaired fertility, impaired gametogenesis	Hypogonadism, impaired fertility, impaired gametogenesis	Hypogonadism, impaired fertility, impaired gametogenesis	Hypogonadism, impaired fertility, impaired gametogenesis	None	Male infertility, impaired gametogenesis, reduced female fertility	Hypogonadism; male infertility; impaired spermatogenesis; reduced female fertility
Developmental defects	Sub-Mendelian birth rate and microphthalmia in a C57BL/6J background	Growth retardation, microphthalmia, microcephaly	Microphthalmia microcephaly	Sub-Mendelian birth rate, growth retardation, microphthalmia, perinatal lethality in C57BL/6J background	NA	Growth retardation and severe perinatal lethality	Short stature, congenital malformations of GI, CNS, renal and skeletal systems; microcephaly and microphthalmia
Tumor development	Sarcoma and adenocarcinoma at 15 months	None in the first year of life	Lymphoma, sarcoma and ovarian tumors at 15 months	Adenomas, epithelial cancers and other carcinomas by 14–19 months	Carcinomas including mammary, gastric and squamous cell, sarcomas	NA	AML; squamous cell carcinomas (HNSCC and anogenital)
MMC sensitivity	Hypersensitivity of MEFs, BM progenitors and splenocytes; pancytopenia after systemic MMC	Hypersensitivity of MEFs, BM progenitors and splenocytes	Hypersensitivity of MEFs and BM progenitors; pancytopenia after systemic MMC	Hypersensitivity of MEFs	Hypersensitivity of BM cells	Hypersensitivity of MEFs, BM progenitors and splenocytes	Hypersensitivity of PBMNCs and BM cells
IR sensitivity	NA	Increased in vitro IR sensitivity of splenocytes	Normal in vitro IR sensitivity of MEFs, normal TBI sensitivity	Normal in vitro IR sensitivity, increased TBI sensitivity	Normal in vitro IR sensitivity, increased TBI sensitivity, impaired hematopoietic recovery after TBI	Normal in vitro IR sensitivity, increased TBI sensitivity	FA-D1 and FA-D2 patients hypersensitive
Cytokine/interferon (IFN) sensitivity	Hypersensitivity of BM progenitors to IFN-γ and TNF-α in vitro or in vivo, hypersensitivity of mice to LPS	Hypersensitivity of BM progenitors to IFN-γ in vitro or in vivo	Hypersensitivity of BM progenitors to IFN-γ in vitro or in vivo	NA	NA	NA	Hypersensitivity of BM progenitors to IFN-γ and TNF-α
References	[6,8,11,26,27,41,42,62,69,82,91]	[37,43,72,74,86]	[10,12,66,72,74,85]	[32]	[2,46,47,52]	[36]	"Fanconi Anemia and its Diagnosis" Auerbach (this issue)

Abbreviations: BM, bone marrow; HSCs, hematopoietic stem cells; MMC, mitomycin C; MEFs, mouse embryo fibroblasts; TBI, total body irradiation; CNS, central nervous system; IR, ionizing radiation; LPS, lipopolysaccharide; IFN, interferon; TNF, tumor necrosis factor; HNSCC, head and neck squamous cell carcinoma; PBMNCs, peripheral blood mononuclear cells; NA, not analyzed.

strand crosslinks, leading to the possibility that the mild phenotype in mice could be due to fewer spontaneous DNA lesions. This could be because of differences in metabolism between mice and humans, leading to fewer spontaneous crosslinks. For instance, humans may develop higher levels of endogenous crosslinking compounds, such as formaldehyde [64] or malondialdehyde [53]. Alternatively, and more plausibly, it is because experimental mice are bred in a bland environment consisting of a uniform, controlled diet and sterile housing material, presenting minimal exogenous genotoxic challenge. The implications of this are that much of the DNA damage that drives the FA phenotype may be environmental and therefore preventable.

5. *FancG*$^{-/-}$ mice

The phenotype of *FancG*$^{-/-}$ mice is virtually identical to that of *FancA*$^{-/-}$ and *FancC*$^{-/-}$ mice [18]. *FancG*$^{-/-}$ mice lack the characteristic congenital anomalies characteristic of FA and do not spontaneously develop hematological abnormalities or spontaneous cancer in the first year of life [37]. Like *FancA*$^{-/-}$ and *FancC*$^{-/-}$ mice, *FancG*$^{-/-}$ mice have reduced fertility due to impaired gametogenesis [37,86], a characteristic of FA (see "Fanconi Anemia and its Diagnosis" Auerbach, this issue). Primary MEFs isolated from *FancG*$^{-/-}$ mice display increased spontaneous and MMC-induced chromosomal aberrations relative to wild type MEFs [37]. Furthermore, stimulated splenic lymphocytes from *FancG*$^{-/-}$ mice display increased chromosomal aberrations in response to DEB and MMC and MMC-treated bone marrow progenitor cells have significantly impaired proliferation [86]. This growth defect is not due to accelerated telomere attrition, since *FancG*$^{-/-}$ hematopoietic stem cells, lymphocytes and MEFs, as well as FA-G human fibroblasts, have normal telomere maintenance even if cultured in the presence of MMC [16]. Interestingly, Li et al., recently demonstrated that mesenchymal stem/progenitor cells from the bone marrow of *FancG*$^{-/-}$ mice have a proliferation defect and an impaired ability to promote engraftment of hematopoietic stem cells [43]. This demonstrates that not only the hematopoietic cells but the microenvironment of the bone marrow are affected in FA.

6. *FancD1/Brca2*$^{-/-}$ mice

FANCD1 is identical to BRCA2 [34]. FANCD1/BRCA2 interacts with RAD51 [45] and is required for HR-mediated repair of DNA DSBs [49,78]. In humans, hapoloinsufficiency of BRCA2 leads to a dramatically increased risk of breast, ovarian and pancreatic cancer. Genetic deletion of *FancD1/Brca2* in the mouse results in embryonic lethality [70]. Homozygous deletion of exon 27 of *FancD1/Brca2* prevents the interaction of FANCD1/BRCA2 with FANCD2 [2]. Hematopoietic cell function is significantly compromised in *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice, including progenitor cell proliferation, HSC self-renewal and competitive repopulation capacity [52]. However the number and types of cells in the peripheral blood, spleen, thymus or bone marrow of adult *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice is normal, meaning that the mice do not recapitulate the aplastic anemia characteristic of FA. Despite this, there are several indications that the hematopoietic phenotype of *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice is more severe than that of other FA models (Table 1). Unlike *FancA*$^{-/-}$ mice, bone marrow cells from *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice display spontaneous chromosomal aberrations and are more sensitive to the crosslinking agent MMC [52]. Furthermore, spontaneous loss of hematopoietic cells (colony forming cells) occurs earlier in *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice than in *FancA*$^{-/-}$ or *FancC*$^{-/-}$ mice [7,52,66,82]. This is consistent with the fact that FANCD1 operates downstream of FANCA and FANCC in the FA pathway and that FANCD1 is more critical for the repair of replication-stalling lesions than the FA core complex.

In support of this, FA-D1 cells are the only FA complementation group in which RAD51 foci do not form in response to ionizing radiation and crosslink damage [20,21,77], demonstrating that HR-mediated DSB repair is dependent upon FANCD1/BRCA2 but not the FA core complex. Accordingly, *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice are hypersensitive to ionizing radiation [52]. However, *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice do not have a defect in gametogenesis or impaired fertility [2], indicating that the interaction of FANCD1 with FANCD2 is important for HR-mediated repair of DNA damage, but not meiosis.

In addition to hematopoietic defects, *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice are prone to epithelial tumors, including gastric cancer and squamous cell carcinomas [47]. Thus the *FancD1/Brca2*$^{\Delta 27/\Delta 27}$ mice mimic the cancer predisposition syndrome of carriers of BRCA2 mutations as well as many aspects of the hematopoietic defects of FA.

7. *FancD2*$^{-/-}$ mice

FancD2$^{-/-}$ mice are viable [32], indicating that FANCD2 is not required for mammalian development. *FancD2*$^{-/-}$ mice are born with sub-Mendelian frequency and display pre- and post-natal growth retardation. The severity of the phenotype of the mice is dependent upon their genetic background, with a more severe phenotype emerging in C57BL/6J than in 129S4, indicating the existence of modifying loci. Like *FancA*$^{-/-}$, *FancC*$^{-/-}$ and *FancG*$^{-/-}$ mice and FA patients, cells from *FancD2*$^{-/-}$ mice are hypersensitive to crosslinking agents and the mice show hypogonadism with impaired gametogenesis (Table 1). The majority of *FancD2*$^{-/-}$ mice in a C57BL/6J background have microphthalmia due to impaired development of the lens and retina [32]. *FancD2*$^{-/-}$ mice have a significantly increased incidence of tumors including ovarian, gastric and hepatic adenomas as well as hepatocellular, lung, ovarian and mammary carcinomas [32]. The tumor spectrum, including primarily epithelial tumors is similar to *FancD1/Brca2*$^{-/-}$ mice and BRCA2 patients.

The phenotype of these mice is modestly more severe than that of mice genetically deleted for the FA core complex proteins (FANC-A, -C or -G), suggesting that FANCD2 has some activity even in the absence of activation by the FA core. *FancD2*$^{-/-}$ mice are mildly hypersensitive to ionizing radiation [32], indicating a role, albeit non-essential, in protecting against oxidative stress or DNA DSBs, which appears to be independent of the FA core complex. This is consistent with the observation that RAD51 foci formation, an essential step in HR-mediated repair of DNA DSBs, requires FANCD1/BRCA2, but not FANCD2 [21]. Recently, using a retroviral insertional mutagenesis, a mouse model for *FancD2* was generated in our laboratory. Similar to the *FancD2*$^{-/-}$ mouse model reported by Houghtaling et al. [32], the *FancD2*$^{-/-}$ strain developed in our laboratory display FA phenotypes including cellular hypersensitivity to MMC, hypogonadism and reduced fertility. In addition, bone marrow from *FancD2*$^{-/-}$ mice exhibit hematopoietic stem cell defects, including reduced numbers and long-term repopulating ability (Parmar et al., unpublished data).

8. *Usp1*$^{-/-}$ mice

The deubiquitylating enzyme, USP1 (ubiquitin-specific protease 1), was recently demonstrated to regulate the level of monoubiquitylated FANCD2 and FANCI proteins [55,76]. Inhibition of *Usp1* by siRNA knockdown in human cell lines leads to an accumulation of monoubiquitylated isoforms of FANCD2 and FANCI proteins. Unexpectedly, disruption of *Usp1* in chicken DT40 cells results in hypersensitivity to DNA interstrand crosslinking agents, similar to

that observed in cells with mutations in FA genes [57]. USP1 is not an FA gene *per se*, since no human FA patients have been observed to carry germ line mutations in the *USP1* gene. Thus to further understand the biological significance of USP1, we recently generated a *Usp1* knockout mouse [36].

Interestingly, *Usp1*$^{-/-}$ mice share many phenotypic features with other FA gene knockout mice (Table 1). *Usp1*$^{-/-}$ mice are small, have decreased fertility and display cellular hypersensitivity to MMC and other crosslinking agents. The mice exhibit significant perinatal lethality and 80% of *Usp1*$^{-/-}$ mice die from cyanosis in the perinatal period [36]. MEFs derived from *Usp1*$^{-/-}$ embryos have been particularly useful in examining the function of the USP1 in the FA pathway. *Usp1*$^{-/-}$ MEFs have a bonafide defect in HR. Also, although the level of monoubiquitylated FANCD2 protein is normal in *Usp1*$^{-/-}$ MEFs, FANCD2 is not assembled in DNA repair foci [36]. This indicates that FANCD2 must be coordinately monoubiquitylated by the FA core complex and deubiquitylated by USP1 for efficient assembly of the protein into nuclear complexes to facilitate DNA repair.

Finally, *Usp1*$^{-/-}$;*Fancd2*$^{-/-}$ mice were generated [36]. Double knockout mice are viable, but primary cells derived from them are hypersensitive to MMC relative to either single knockout. These results provide critical evidence that the *Usp1* gene may have additional functions outside of the classical FA pathway that facilitates the repair or tolerance of genotoxic stress. Consistent with this, recent studies indicate that USP1 regulates the level of monoubiquitylated PCNA, suggesting that it is also an important modulator of translesion DNA synthesis, a mechanism that facilitates DNA damage tolerance [35].

9. Genetic studies: double mutant mice

FancA$^{-/-}$;*FancC*$^{-/-}$ mice and cells derived from them are phenotypically identical to single mutants [56]. This provides crucial genetic evidence that FANCA and FANCC are epistatic. *FancC*$^{-/-}$ mice were crossed into a *Sod1*$^{-/-}$ background to delete Cu/Zn superoxide dismutase and increase endogenous oxidative stress [25]. The double mutant mice display bone marrow hypocellularity due to a loss of committed progenitor cells, resulting in anemia and leucopenia. *FancC*$^{-/-}$;*Sod1*$^{-/-}$ bone marrow progenitor cells proliferate poorly in vitro. These data provide experimental evidence that oxidative stress contributes to bone marrow failure in FA.

In other studies, *FancC*$^{-/-}$ were crossed into a p53-deficient background to determine if ablation of p53-dependent apoptosis caused tumorigenesis [17]. *FancC*$^{-/-}$;*p53*$^{-/-}$ mice develop spontaneous tumors more rapidly than *p53*$^{-/-}$ mice (median survival 105 and 185 days, respectively). This confirms that FANCC and the FA pathway act to suppress tumorigenesis. Importantly, *FancC*$^{-/-}$;*p53*$^{-/-}$ mice and *FancC*$^{-/-}$;*p53*$^{+/-}$ mice displayed a wide variety of tumors including sarcomas, lymphomas and adenocarcinoma, whereas *p53*$^{-/-}$ mice were prone to thymic lymphomas [17]. Many of the tumor types seen in *FancC*$^{-/-}$;p53-deficient mice are also seen in FA patients in addition to the syndrome's most common cancer which is acute myeloid leukemia (see "Fanconi Anemia and its Diagnosis" Auerbach, this issue). Therefore these double mutant animals provide a good animal model for identifying strategies to prevent malignancies in FA.

FancD2$^{-/-}$*p53*$^{+/-}$ mice also have a significantly increased incidence of tumors relative to either single mutant strain [30]. Tumors were detected significantly earlier in female double mutant animals compared to controls (approximately 13 vs. 15 months of age). The tumor spectrum includes sarcomas typical of p53-deficient mice and epithelial tumors seen in *FancD2*$^{-/-}$ mice. The median survival of *FancD2*$^{-/-}$*p53*$^{+/-}$ than *FancC*$^{-/-}$*p53*$^{+/-}$ mice is similar (~1 year), but the tumor spectrum is vastly different, with approximately a third of the former having adenocarcinomas, while the latter have exclusively lymphomas and sarcomas [17,30]. Abrogation of p53 expression in *FancD2*$^{-/-}$ MEFs prevents arrest of the cells in S phase in response to genotoxic stress [30]. This demonstrates that p53-dependent cell cycle arrest occurs even if the FA pathway is not activated in response to DNA damage and that this arrest is important for cancer protection.

Prkdc encodes the catalytic subunit of DNA-PK, an essential protein for non-homologous end-joining repair (NHEJ) of DNA DSBs. Nonsense mutations in *Prkdc* affect DNA-PK activity and lead to severe-combined immunodeficiency [5]. FANCD2 is implicated in HR-mediated DSB repair. *FancD2*$^{-/-}$ mice were crossed into a *Prkdc*$^{sc/sc}$ background, and the double knockout mice were discovered to be even more sensitive to ionizing radiation than either single mutant strain [31]. This supports a role for FANCD2 in HR-mediated repair of DNA DSBs and not NHEJ. The tumor incidence in these mice was not reported.

10. Interventional studies to elucidate the function of the FA pathway

FancC$^{-/-}$ mice chronically exposed to a sublethal dose of the crosslinking agent MMC develop progressive pancytopenia due to bone marrow failure [8], identical to the spontaneous symptoms of FA. MMC exposure depletes the bone marrow of CD34^{+} cells, but not CD34^{-} [7], suggesting that early hematopoietic progenitors are particularly vulnerable to crosslink damage. *FancC*$^{-/-}$ mice chronically treated with MMC offer a good model in which to study therapeutic interventions for FA. Transplantation of these mice with *FancC*$^{-/-}$ bone marrow cells transduced with *FANCC* cDNA corrected pancytopenia [22]. Similar results were achieved by correcting *FancA*$^{-/-}$ hematopoietic stem cells using a lentiviral vector and using a limited dilution of the transduced cells to correct the hematopoietic defect in MMC-treated *FancA*$^{-/-}$ mice [85]. This model was also used to test whether or not cytokines can be used to treat bone marrow failure in FA [9]. G-CSF by itself or in combination with erythropoietin delayed the onset of pancytopenia in *FancC*$^{-/-}$ mice chronically treated with MMC, but was unable to prevent bone marrow failure.

Haneline et al. demonstrated that correction of *FancC*$^{-/-}$ hematopoietic stem cells with *FancC* cDNA restored the repopulating capacity of these cells in a competitive bone marrow transplantation assay [28]. Interestingly, the negative control for this experiment, uncorrected *FancC*$^{-/-}$ mouse bone marrow, revealed important mechanistic information about FA. Ex vivo culture of *FancC*$^{-/-}$ mouse cells prior to bone marrow transplantation caused an increase in the incidence of hematological abnormalities in recipient mice, including bone marrow failure, myelofibrosis, splenomegaly, myelodysplastic syndrome, myeloproliferative disease and acute myeloid leukemia, all characteristic of FA [28,40]. Culture of *FancC*$^{-/-}$ mouse bone marrow also led to a time-dependent increase in cytogenetic abnormalities, apoptosis and the emergence of a cell population that is resistant to pro-apoptotic cytokines. This provides experimental evidence that the hematological abnormalities in FA arise because the bone marrow cells are proliferating under stress, leading to clonal selection of cells with growth advantage. The incidence of hematologic abnormalities is further exacerbated if the *FancC*$^{-/-}$ mouse bone marrow cells are exposed to the immunoregulatory cytokine TNF-α [39], and TNF-α levels have been reported to be elevated in the bone marrow of FA patients [13,67,68].

There is also evidence that cells isolated from FA mice are hypersensitive to oxidative stress. Bone marrow progenitor cells isolated from *FancC*$^{-/-}$ mice undergo premature replicative senescence when exposed to repetitive hypoxia-hyperoxia conditions [89]. Exposure of *FancC*$^{-/-}$ hematopoietic progenitor cells to TNF-α in vitro negatively affects clonogenic proliferation and competi-

tive hematopoietic repopulation, but this can be blocked by the ROS scavenger N-acetyl-L-cysteine (NAC) [69]. Similarly, exposure of *FancC*$^{-/-}$ mice to TNF-α induces ROS production, oxidative DNA damage, chromosomal aberrations and premature replicative senescence in hematopoietic stem cells, which is alleviated by pretreating the mice with NAC [91]. These data suggest that much of the hypersensitivity of FA cells to pro-inflammatory cytokines is driven by oxidative stress. Accordingly, primary cells and tissue from *FancA*$^{-/-}$ mice are hypersensitive to H_2O_2 as demonstrated by persistent overactivation of p53 [61].

This is further supported by a recent study demonstrating that the radical scavenger, superoxide dismutase mimetic, Tempol, significantly delays the onset of cancer and increases survival of *FancD2*$^{-/-}$*p53*$^{+/-}$ mice [88]. Tumor-free survival was increased by >25%. These data provide strong evidence that endogenous oxidative stress plays a causative role in carcinogenesis in FA. Tempol had no adverse effect on the function of hematopoietic stem cells in competitive repopulating bone marrow transplantation out to 6 months. However, it is not clear, if the radical scavenger had a beneficial effect on hematopoietic stem cells.

11. Utilizing the mouse models to discover therapeutic options for Fanconi anemia

Hematological disease and malignancies are the most common cause of death in FA (see "Fanconi Anemia and its Diagnosis" Auerbach, this issue). This can be prevented by bone marrow transplantation, indicating that FA is a prime candidate disease for gene therapy (see "Finding the Needle in the Hay Stack: Hematopoietic Stem Cells in Fanconi Anemia", Muller and Williams, this issue, for more information on gene therapy in FA). Indeed, retroviral transduction of *FancA*$^{-/-}$ hematopoietic stem cells (lineage negative; Sca1^{+}) with human FANCA corrects the hypersensitivity of the hematopoietic stem cells to crosslink damage and their ex vivo proliferation defect [66], demonstrating the feasibility of gene therapy. Two major hurdles to gene therapy with autologous bone marrow transplant in FA are that: (1) patients are hypersensitive to cytotoxic agents used to condition the host for bone marrow engraftment and (2) hematopoietic stem cells isolated from FA patients are limited in number and are hypersensitive to ex vivo culture conditions necessary for expansion. Solutions to both hurdles have been identified with FA mouse models. Transplantation of non-ablated *FancC*$^{-/-}$ mice with a 50:50 ratio of wild type and *FancC*$^{-/-}$ bone marrow cells leads to repopulation of hosts, although selectively with wild type cells [27]. Furthermore, lentivirus-mediated gene therapy of BM cells from *FancD1*/*Brca2*$^{\Delta 27/\Delta 27}$ mice with *Brca2* was used to successfully treat the hematopoietic defect in the mutant animals that were only mildly conditioned [65]. These studies reveal that host bone marrow ablation with its associated toxicity may not be necessary in FA. In addition, Muller et al., recently demonstrated that "rapid" lentiviral-mediated transduction of *FancA*$^{-/-}$ mouse hematopoietic stem cells, to minimize ex vivo manipulation of cells, resulted in engraftment levels equivalent to wild type cells [50]. This offers great promise for autologous bone marrow transplantation to treat FA.

Interferon γ (IFN-γ) and TNF-α are immunoregulatory cytokines that inhibit cell proliferation and are implicated in the pathogenesis of aplastic anemia [44]. *FancC*$^{-/-}$ mice have fewer CD4^{+} IFN-γ secreting splenic lymphocytes [15]. *FancC*$^{-/-}$ mice also display hypersensitivity to lipopolysaccharide-induced septic shock, resulting in prolonged anemia, leucopenia and bone marrow hypoplasia, as a well as enhanced secretion of pro-inflammatory cytokines [69]. *FancC*$^{-/-}$ mouse and human FA-C hematopoietic progenitor cells are hypersensitive to IFN-γ due to increased apoptosis [26,42,58,59,62,82], as are FA-A and FA-G hematopoietic progenitor cells [72,90]. This is attributed to the fact that FANCA, FANCC and FANCG interact with heat shock protein 70 (HSP70) and the pro-apoptotic kinase dsRNA-dependent protein kinase PKR [60,90]. Mutations in any of these FA genes cause an accumulation of PKR in response to IFN-γ and TNF-α, which promotes apoptosis [90]. Hypersensitivity to TNF-α is rescued by genetic deletion of p53, a downstream effecter of PKR [17], indicating that cytokine-induced apoptosis is p53-dependent. Alternatively, IFN-γ hypersensitivity has been attributed to the induction of nitric oxide synthase (iNOS) [24]. iNOS produces nitric oxide, a free radical that can stimulate lipid peroxidation [29], a potential source of endogenous DNA interstrand crosslink damage [53].

Experiments in mice demonstrated that IFN-γ hypersensitivity can be exploited as a nongenotoxic method for myelopreparation to improve engraftment of hematopoietic stem cells [42,72]. Similarly, hematopoietic stem cells isolated from *FancA*$^{-/-}$, *FancC*$^{-/-}$ and *FancD2*$^{-/-}$ mice grown under hypoxic conditions (1% O_2) home to and engraft better in the bone marrow of myeloablated hosts [75].

12. Summary

In conclusion, FA mouse models now exist, resulting from targeted disruption of the *FancA*, *FancC*, *FancG*, *FancD1*, *FancD2*, or *Usp1* gene. The mouse models exhibit some but not all of the developmental and hematologic manifestations of human FA patients (Table 1). While FA patients develop spontaneous hematologic failure, most FA mouse models have relatively normal hematologic function, though anemia can be elicited by *in vivo* exposure to crosslinking agents. Mouse models will be especially useful in the next few years for the assessment of chemoprotective agents, which limit oxidative stress, and the development of strategies to enhance the survival and expansion of FA hematopoietic stem cells with underlying DNA repair deficiency.

Conflict of interest

The authors declare that there are no conflicts of interest.

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