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Review

Mouse models of Fanconi anemia

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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

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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-