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1. Introduction

In 1927, the Swiss pediatrician Guido Fanconi first reported a family with aplastic anemia and physical anomalies known as FA now, as reviewed by Lobitz et al (Lobitz and Velleuer 2006). FA is a chromosomal fragility disorder characterized by cytopenia, progressive bone marrow failure (BMF) under production, variable developmental anomalies and a strong propensity for cancer. The prevalence of FA is 1 to 5 per million, and heterozygous carrier frequency is about 1 in 300. Clinically, FA patients develop heterogeneous manifestations. 80% of FA patients develop progressive BMF with a mean age of death occurring at 19 years (D’Andrea, Dahl et al. 2002; Bagby and Alter 2006; Giri, Batista et al. 2007). The other 20% of patients usually die of malignancies resulting from the acquisition of myeloid cell leukemia particularly acute myelogenous leukemia and myelodysplastic syndrome. In addition, FA Patients are susceptible to solid tumors, including gynecologic squamous cell carcinoma, head and neck squamous cell carcinoma, esophageal carcinoma, liver tumors, brain tumors, skin tumors, and renal tumors (Kutler, Singh et al. 2003).

To date, at least 15 distinct FANC genes, including FANCA, FANCB, FANCC, FANCD1/BRCA2, FANCD2/BRCA1, FANCE, FANCF, FANCG/XRCC9, FANCI, FANCI/BRIP1, FANCL, FANCM/HEF, FANCN/PALB2, FANCO/ RAD51c and FANCP/SLX4, are found in FA patients, and FANCA, FANCC, FANCG and FANCD2 are the most frequent in clinic (Moldovan and D’Andrea 2009; Vaz, Hanenberg et al. 2010; Kim, Lach et al. 2011; Stoepker, Hain et al. 2011). Except for FANCB which is on the X chromosome (Meetei, Levitus et al. 2004), all these FANC genes are located on autosomes.

FA pathway is inactive in normal cells but turned on during the S phase of cell cycle or in the presence of DNA damage proteins, and it also plays a pivotal role in DNA repair pathway in cellular defense against DNA interstrandcrosslinkers (Moldovan and D’Andrea 2009;
Kee and D’Andrea 2010). Following the activation, eight of 15 FANC gene productions, including FANCA, B, C, E, F, G, L and M proteins assemble into a nuclear E3 ubiquitin ligase complex. This complex is known as FA core complex and one of the main functions of this core complex is to monoubiquitinate and to activate the proteins of FANCD2 and FANCI, which co-localizes with BRCA1 and BRCA2, at the sites of DNA repair (D’Andrea 2010). The monoubiquitinated FANCD2/FANCI is then translocated to chromatin and interacts with other downstream FA proteins FANCD1, J, N and O to form a nuclear DNA-repair complex (D’Andrea 2010). FA patients display spontaneous chromosomal breakage and chromosomal abnormalities (Schroeder, Anschutz et al. 1964; Sasaki 1975). In addition, these DNA instability can be significantly enhanced by DNA cross-linking agents, such as mitomycin C (MMC) (Schroeder, Anschutz et al. 1964; Sasaki 1975).

2. FANC genes regulate HSC/HPC functions

Because of the earlier bone marrow failure and the predisposition to malignancy, especially the high risk of developing acute myeloid leukemia (AML), FA has been clinically categorized as a hematopoietic disease due to hematopoietic dysfunction. The defective hematopoietic functions are known related to an excess of genetic instability. FA bone marrow cells have clonal evolution, which predispose patients to the development of malignancies. Vinciguerra et al. reported an increased number of ultrafine DNA bridges and binucleated cells in both bone marrow stromal cells from FA patients and in primary murine FA pathway-deficient hematopoietic stem/progenitor cells (HSCs/HPCs) (Vinciguerra, Godinho et al. 2010). Using primary and immortalized cell cultures as well as ex vivo materials from patients, multiple studies showed oxidant hypersensitivity of these FA cells as reviewed by Du et al. (Du, Adam et al. 2008).

3. Murine models of FA

To our best knowledge, 9 of 15 Fanca genes have been inactivated in mice, resulting in Fanca, Fance, Fancg, Fancd1, Fancd2, Fancl, Fancm, Fancn and Fancp knockout mice.

Cheng et al. first created Fanca<sup>-/-</sup> mice by deletion of Fanca exons 4-7 (Cheng, van de Vrught et al. 2000). The Fanca<sup>-/-</sup> mice developed a significant thrombocytopenia, while no other severe hematological abnormalities were observed. Using the same murine model, Rio and colleagues reported that megakaryocyte progenitors, but not granulocyte-macrophage progenitors from the bone marrow of the Fanca<sup>-/-</sup> mice have impaired proliferation in vitro (Rio, Segovia et al. 2002). In addition, embryonic fibroblasts (MEFs) derived from these knockout mice are hypersensitive to the crosslinker MMC and both male and female mice showed reduced fertility due to hypogonadism (Cheng, van de Vrught et al. 2000; Rio, Segovia et al. 2002). Different from FA patients, these knockout mice do not spontaneously develop congenital anomalies (Cheng, van de Vrught et al. 2000; Rio, Segovia et al. 2002). Later, Wong and
colleagues established another \textit{Fanca}^{-/-} murine model by deletion of exons 1-6 of \textit{Fanca} gene (Wong, Alon et al. 2003). The homozygous germline-line deletion of the functionally exons 1-6 of \textit{Fanca} leads to multiple developmental deficits, including growth retardation, microphthalmia, craniofacial malformations, as well as hypogonadism, resembling those observed in FA patients (Wong, Alon et al. 2003).

Two different \textit{Fancc}^{-/-} murine models were generated by deletion of \textit{Fancc} exon 9 or exon 8 (Chen, Tomkins et al. 1996; Whitney, Royle et al. 1996). Similar to \textit{Fanca}^{-/-} mice, these mice do not spontaneously develop peripheral hematological abnormalities. However the bone marrow HSCs/HPCs of these mice display impaired functions \textit{in vitro}, such as abnormal colony forming capacity, hypersensitive to interferon-\gamma. Furthermore, these \textit{Fancc}^{-/-} mice also have impaired fertility and increased incidence of a congenital microphthalmia without skeletal abnormalities, replicating some of the features of the FA patients (Chen, Tomkins et al. 1996; Whitney, Royle et al. 1996).

\textit{Fancg}^{-/-} mice were generated by deletion of \textit{Fancg} exons 2-9 (Yang, Kuang et al. 2001; Koomen, Cheng et al. 2002). Similar to \textit{Fanca}^{-/-} and \textit{Fancc}^{-/-} mice, \textit{Fancg}^{-/-} mice do not develop spontaneously hematological abnormalities and congenital anomalies either, although MEFs of \textit{Fancg}^{-/-} mice are hypersensitive to MMC (Yang, Kuang et al. 2001; Koomen, Cheng et al. 2002).

\textit{BRCA2} has a close functional relationship with the classical FA pathway. Genetic deletion of \textit{Fancd1}/\textit{Brca2} in mice results in embryonic lethality (Sharan, Morimatsu et al. 1997). Using mice with hypomorphic mutations in \textit{Brca2} (\textit{Brca2}^{Δ27/Δ27}), Navarro et al reported that bone marrow cells of \textit{Brca2}^{Δ27/Δ27} mice display spontaneous chromosomal aberrations and are more hypersensitive to MMC (Navarro, Meza et al. 2006), consistent with FANCD1/\textit{Brca2} as downstream of FA core complex in FA pathway and plays a critical role in DNA repair process. Different from \textit{Fanca}^{-/-} mice, \textit{Fancd1}^{Δ27/Δ27} mice are hypersensitive to ionizing radiation and do not have defect in fertility (Navarro, Meza et al. 2006).

\textit{Fancd2} undergoes monoubiquitylation by the complex and is targeted into nuclear foci and co-localizes with \textit{Brca1}. \textit{Fancd2}^{-/-} mice were generated by targeted deletion of \textit{Fancd2} exons 26 and 27 (Houghtaling, Timmers et al. 2003). \textit{Fancd2} mutant mice display cellular sensitivity to DNA interstrand cross-links and germ cell loss, which is similar to human FA patients and other FA mouse models. Interestingly, different from other mice carrying disruptions of proximal FA genes, these \textit{Fancd2} mutant mice exhibited phenotypes including microphthalmia, perinatal lethality, and epithelial cancers. There is similarity between \textit{Fancd2} mutant mice and \textit{Brca2}/\textit{Fancd1} hypomorphic mutation mice, implying a common function for both proteins in the same pathway.

Although \textit{Fancg}^{-/-}, \textit{Fancc}^{-/-}, and \textit{Fancd2}^{-/-} mice do not develop spontaneous hematopoietic malignancies seen in FA patients, HSCs/HPCs from \textit{Fancg}^{-/-}, \textit{Fancc}^{-/-}, and \textit{Fancd2}^{-/-} mice have defective engraftment and reconstitution of the short and long term hematopoiesis in a competitive transplantation assay (Haneline, Gobbett et al. 1999; Parmar, Kim et al. 2010; Barroca, Mouthon et al. 2012). The defective homing and reconstitution may associated with an impaired cell migration and adhesion of \textit{Fancg}^{-/-} hematopoietic cells as reported by Barro-
ca et al (Zhang, Shang et al. 2008; Barroca, Mouthon et al. 2012). In addition, the mobilization of HSCs/HPCs in Fanca-/- mice in response to G-CSF was defective in the absence of bone marrow failure (Milsom, Lee et al. 2009).

Fancl, also known as Pog (proliferation of germ cells), belongs to the multisubunit FA complex. Fancl-/- mice were generated by deletion of Pog gene exons 4-14 (Agoulnik, Lu et al. 2002). Fancl-/- mice have defects in fertility, growth retardation, although no obvious hematological abnormalities (Agoulnik, Lu et al. 2002). Fancm-/- mice were generated by deletion of Fancm exon 2 (Bakker, van de Vrugt et al. 2009). Similar to other FA mouse models, Fancm-/- mice do not spontaneously develop hematological abnormalities and congenital anomalies, whereas, Fancm-/- mice showed increased cancer incidence.

Fancn/Palb2-/- mice were generated by insertion of a gene trap construct located between exon 1 and exon 2 of the Palb2 gene (Rantakari, Nikkila et al. 2010; Bouwman, Drost et al. 2011). Homozygous deletion of Palb2 leads to embryonic lethality which die at E9.5 at the latest (Rantakari, Nikkila et al. 2010; Bouwman, Drost et al. 2011).

As described above, differing from FA patients who often spontaneously develop bone marrow failure in their lives, most of the models have relatively normal hematological function. It is possible that FA proteins have divergent functions which are independent of FANCD2/FANCI monoubiquitination in hematopoietic cells. To test if deletion of multiple Fan genes would result in a more aggressive hematopoietic phenotype, Pulliam-Leath and colleagues generated Fancc-/-; Fancg-/- (DKO) mice by genetically intercrossing Fancc+/- mice with Fancg+/- mice (Pulliam-Leath, Ciccone et al. 2010). Combined inactivation of Fancc and Fancg leads to a defective hematopoietic stem cell function, supporting the hypothesis that besides their common role in FANCD2/FANCI monoubiquitination, FANCC and FANCG function in divergent molecular pathways of relevance to hematopoiesis. This DKO model best recapitulates the spontaneous clinical hematopoietic phenotypes of human FA, including hematopoietic malignancies and bone marrow aplasia.

4. Bone marrow microenvironmental abnormalities in hematopoietic diseases

Hematopoiesis is a dynamic and highly regulated process, which relies on the ordered self-renewal and differentiation of HSCs/HPCs within the bone marrow (BM) (Kotton, Ma et al. 2001; Krause 2002; Zhang, Niu et al. 2003; Li and Li 2006; Yin and Li 2006). This process involves intrinsic and extrinsic cues including both cellular and humoral regulatory signals generated by the HSC microenvironment, also known as “niche”. The concept of hematopoietic niche has been proposed in the 1970s (Schofield 1978). Studies have shown that the cellular composition of this “niche” contains heterogeneous populations, including endothelial cells, osteoblasts, adipocytes (Calvi, Adams et al. 2003; Zhang, Niu et al. 2003; Arai, Hirao et al. 2005; Sacchetti, Funari et al. 2007), and mesenchymal stem/progenitor cells (MSPCs) (Badillo and Flake 2006), a common progenitor for many of these cells composing the HSC niche. The regulatory signals of the BM microenvironment represent a demarcated anatomi-
cal compartment that provides stimulatory signals to HSCs via the following mechanisms: (1) cell/cell direct interactions, (2) secreting soluble factors, and (3) extracellular matrix. These cellular and humoral regulatory signals dictate HSC cell fate, such as self-renewal, proliferation, differentiation, and apoptosis.

The osteoblastic niche and the vacular niche are well described by independent groups (Heissig, Hattori et al. 2002; Calvi, Adams et al. 2003; Zhang, Niu et al. 2003; Avecilla, Hattori et al. 2004). Studies have shown that BM microenvironment is critical for the physiologic as well as pathologic development of hematopoiesis through the following mechanisms: cell/cell interactions, soluble factors and extracellular matrix (Koh, Choi et al. 2005; Williams and Cancelas 2006). There is increasing evidence suggesting a role of the hematopoietic microenvironment in initiating hematopoietic disorders, such as myeloproliferative disorders (MPD).

Recently, using a murine model in which Dicer1 was specifically deleted in osteoprogenitors, Raaijmakers et al demonstrated that bone marrow microenvironment plays a causative role in the development of myelodysplasia and secondary leukaemia (Raaijmakers, Mukherjee et al. 2010). The vascular microvessel density is increased in the bone marrow of many hematopoietic disorders including AML, acute lymphoblastic leukemia (ALL), myelosyndromes (MDS) and myeloproliferative neoplasms (MPN). The adipocytes are also found to be accumulated in BMF (Li, Chen et al. 2009). Although the mechanism for the accumulation of adipocytes in bone marrow is still largely unknown, the accumulated adipocytes may act as negative regulators in the hematopoietic microenvironment (Naveiras, Nardi et al. 2009).

5. Dysregulated bone marrow microenvironment in FA patients and FA murine models

Besides the hematopoietic defects, mesenchymal tissue-derived congenital malformations are also prevalent in FA patients, such as the renal/limb abnormalities and short stature. Despite these clinical observations suggesting multiple mesenchymal defects, little attention has been directed to the association between the pathological HSC functions and the microenvironment in FA.

Using a murine model with targeted disruption of the Fancg gene (Fancg<sup>-/-</sup>), Li and colleagues first reported that Fancg<sup>-/-</sup> MSPCs have decreased clonogenic growth, diminished proliferating capability and increased apoptosis and senescence (Li, Chen et al. 2009). Fancg<sup>-/-</sup> MSPCs have impaired function in supporting the proliferation, recruitment, adhesion and homing of HSPCs in vitro and in vivo. Importantly, some cellular defects such as survival and proliferation of murine MSPCs can be restored by introduction of human Fancg cDNA (Li, Chen et al. 2009).

Consistently, study by Zhang et al showed that MSPCs derived from the bone marrow of Fancd2<sup>-/-</sup> mice showed less support for progenitor growth than that from wild type mice in a CAFC assay (Zhang, Marquez-Loza et al. 2010).
Using the MSPCs derived from patients with FANCA mutation, Lecourt et al showed that human FA MSPCs also have poor proliferation and increased senescence, while no defective hematopoietic supportive activity was observed in vitro (Lecourt, Vanneaux et al. 2010), suggesting a species-specific effect between human and murine system.

6. Current treatments for FA

The long-term curative therapy for the BMF of FA patients is HSC transplantation, ideally from an HLA-matched sibling (Gluckman, Broxmeyer et al. 1989; Davies, Khan et al. 1996; Guardiola, Pasquini et al. 2000; Kutler, Singh et al. 2003; Mathew 2006) Allogeneic BM transplantation (BMT) or cord blood (CB) transplantation is available to up to 30% of FA patients. However, allogeneic BMT or CB transplantation is frequently associated with an increased risk of secondary cancers, particularly squamous cell carcinoma of the head and neck (Kutler, Auerbach et al. 2003; Rosenberg, Socie et al. 2005). Since the conditioning regimens such as irradiation clearly heightens the risk of transformation of the ongoing genetic susceptibility of non-hematopoietic tissue. This complication is even more severe in high-risk FA patients, transplanted with non-matched donors and those develop chronic graft-versus-host disease. Therefore, even with successful allogeneic transplantation for BMF, the risk of secondary malignancies results in a high mortality over 10-15 years. Gene therapy using autologous HSCs is a second theoretical modality to correct defects in the HSC compartment. Transplantation of genetically corrected autologous HSCs without genotoxic conditioning regimens could provide a therapeutic strategy that avoids the increased risks of secondary cancer (Si, Ciccone et al. 2006). However, a significant obstacle for this therapy is the limited number of HSCs that can be harvested from mobilized blood or BM. In addition, in preliminary phase 1 clinical trials in FANCC and FANCA patients using retroviral mediated gene transfer, despite an efficient gene transfer of the mobilized progenitors (40-80%), and no long-term engraftment of retroviral marked stem cells was achieved (Liu, Kim et al. 1999; Williams, Croop et al. 2005; Kelly, Radtke et al. 2007). Although inefficient gene transfer of repopulating HSCs can not be excluded, inefficient engraftment and homing of exogenous genetically modified cells could also be contributory, particularly given the low numbers of HSC targets that are available for gene transfer/transplantation (Gothot, Pyatt et al. 1998; Glimm, Oh et al. 2000; Orschell-Traycoff, Hiatt et al. 2000). Since mesenchymal stem/progenitor cells were excluded in these studies, it is possible that the lack of an appropriate microenvironment could have impaired the ability of transduced cells to home and proliferate in vivo.

7. Biology of MSPCs and their potential clinical application in transplantation therapy for FA patients

Friedenstein and colleagues first reported a rare, plastic-adherent and fibroblast-like subpopulation expanded from the culture of bone marrow in 1970s (Friedenstein, Chailakhjian et al. 1970), this type of stromal cells, now commonly known as MSCs/MSPCs, has captivated...
more and more investigators, especially in the past two decades. As a group cells with heterogeneity, three criteria have been proposed to define human MSPCs, including plastic-adherence, surface expression of CD105, CD73 and CD90, and the absence of CD45, CD34, CD14 or CD11b, CD79a, CD19, CD14 or CD11b and HLA-DR, and trilineage differentiation to osteoblasts, adipocytes and chondrocytes in vitro (Dominici, Le Blanc et al. 2006).

It is well known that MSPCs lack expression of MHC class II and most of the classical co-stimulatory molecules such as CD80, CD86, or CD40 (Pittenger, Mackay et al. 1999; Tse, Pendleton et al. 2003). This phenotypic characteristic endows MSPCs with nonimmunogenicity, and therefore transplantation of MSPCs into allogeneic host could be implemented without using immunosuppressive agents. MSPCs are known promote the reconstitution of hematopoiesis. We have recently provided evidence for the first time that Fancg−/− MSPCs exhibited profoundly diminished supportive activity for normal HSCs, and intratibial injection of WT or genetically corrected FA MSPCs enhanced donor HSC reconstitution (Li, Chen et al. 2009). This data suggests that normal MSPCs transplantation may have a potential clinical application in FA patients.

8. Future directions

FA is an inherited disease caused by germ-line mutations in FANC genes. Investigators are now paying more attentions on the emerging role of the Fanc gene inactivation-caused defective bone marrow microenvironment in the pathogenesis of FA. Co-transplantation of HSCs and MSPCs is hypothesized as a potentially more effective option than HSC transplantation alone for treating the hematopoietic abnormalities in FA. Therefore, much more effort is warranted to understand the mechanisms of the utility of MSPCs to treat FA patients. These efforts should include: 1) unveiling the cellular fates of the co-injected MSPCs in vivo, such as at what degree these MSPCs are able to reconstitute the marrow microenvironment and how long a significant degree of MSPC engraftment could persist; 2) clarifying whether an immune suppression activity mediated by the injected MSPCs contributes to the enhanced HSC engraftment by co-transplantation of MSPCs; and 3) elucidating at what extend the co-injected MSPCs could normalize the altered marrow microenvironment cytokine/growth factors profiling in FA patients.

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