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SHORT REPORT

Fancd2 and p21 function independently in maintaining the size of hematopoietic stem and progenitor cell pool in mice

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Abstract Fanconi anemia patients suffer from progressive bone marrow failure. An overactive p53 response to DNA damage contributes to the progressive elimination of Fanconi anemia hematopoietic stem and progenitor cells (HSPC), and hence presents a potential target for therapeutic intervention. To investigate whether the cell cycle regulatory protein p21 is the primary mediator of the p53-dependent stem cell loss, p21/Fancd2 double-knockout mice were generated. Surprisingly double mutant mice displayed even more severe loss of HSPCs than *Fancd2*$^{-/-}$ single mutants. p21 deletion did not rescue the abnormal cell cycle profile and had no impact on the long-term repopulating potential of *Fancd2*$^{-/-}$ bone marrow cells. Collectively, our data indicate that p21 has an indispensable role in maintaining a normal HSPC pool and suggest that other p53-targeted factors, not p21, mediate the progressive elimination of HSPC in Fanconi anemia.

Introduction

Fanconi anemia (FA) is a bone marrow failure disorder caused by the disruption of FA-BRCA network, which consists of at least 15 FA genes (including *FANCD2* and *FANCC*) and FA-associated genes (Bagby and Alter, 2006; Kim and D'Andrea, 2012).

Bone marrow failure is the primary cause of early mortality for FA patients (Kutler et al., 2003). It has been reported that a subset of FA patients with lower levels of CHK1 and p53 expression and abrogation of the G2 cell cycle checkpoint display much milder bone marrow deficiency (Ceccaldi et al., 2011). Further mechanistic studies established that an overactive p53 response to cellular stress and DNA damage drives the progressive elimination of hematopoietic stem and progenitor cells (HSPC) in FA patients, suggesting p53 down-regulation as a potential target for FA drug design (Ceccaldi et al., 2012).

Abbreviations: FA, Fanconi anemia; HSPC, hematopoietic stem and progenitor cells; H&E, hematoxylin and eosin; CBC, complete blood count; KSL, cKit^{+}Sca1^{+}Lin^{-}; FITC, fluorescein isothiocyanate; qPCR, quantitative real-time PCR.

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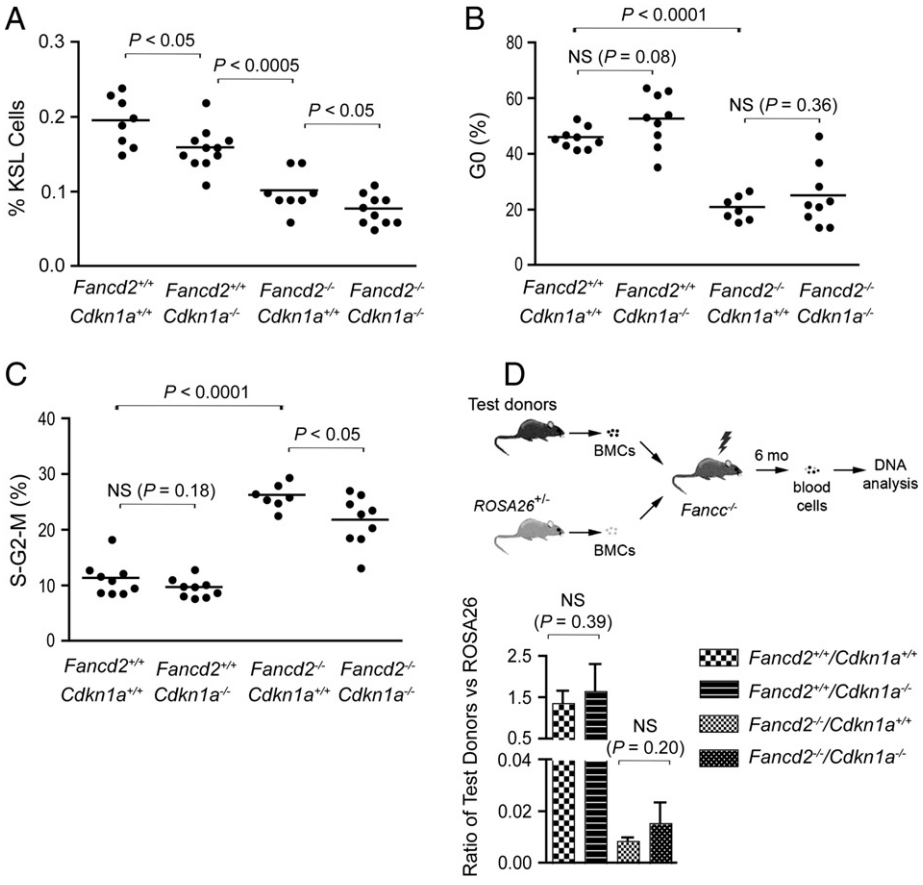

Figure 1 p21 deletion reduced KSL pool but did not change stem cell function. (A) Quantification of KSL hematopoietic stem and progenitor cell frequencies in the bone marrow of *Fancd2*$^{-/-}$/*Cdkn1a*$^{-/-}$ mice and controls. The percentage of the KSL gate refers to the proportion of KSL cells in the whole nucleated bone marrow. n = 8 for *Fancd2*$^{+/+}$/*Cdkn1*$^{+/+}$ mice, n = 11 for *Fancd2*$^{+/+}$/*Cdkn1a*$^{-/-}$ mice, n = 8 for *Fancd2*$^{-/-}$/*Cdkn1*$^{+/+}$ mice, and n = 10 for *Fancd2*$^{-/-}$/*Cdkn1a*$^{-/-}$ mice. (B, C) Cell cycle profiles of bone marrow KSL cells. n = 9 for *Fancd2*$^{+/+}$/*Cdkn1*$^{+/+}$ mice, n = 9 for *Fancd2*$^{+/+}$/*Cdkn1a*$^{-/-}$ mice, n = 7 for *Fancd2*$^{-/-}$/*Cdkn1*$^{+/+}$ mice, and n = 9 for *Fancd2*$^{-/-}$/*Cdkn1a*$^{-/-}$ mice. NS denotes not significant. (D) Upper panel: Strategy used in the competitive repopulation experiment. BMCs denotes bone marrow cells. Lower panel: *In vivo* competitive repopulation of *Fancd2*$^{-/-}$/*Cdkn1a*$^{-/-}$ (or control) donor and *ROSA26*$^{Tg/O}$ bone marrow cells. qPCR analyses were performed to evaluate donor contribution to the peripheral blood cells from each donor. Three independent qPCR analyses were performed for each sample and results from 5 animals were pooled together for each experimental group. Data are presented as mean ± SD. NS denotes not significant.

It is also known that loss of p53 promotes carcinogenesis in both FA mouse models and human FA patients (Ceccaldi et al., 2011; Houghtaling et al., 2005; Freie et al., 2003). Given the detrimental tumorigenic effects of p53 loss, it is desirable to shut down only those p53-target genes specifically involved in HSPC elimination in FA. Further understanding the molecular targets of p53-mediated HSPC elimination could guide the future design of therapeutic regimens exploiting this approach. p21, encoded by the *Cdkn1a* gene, is a key p53-target gene and the main factor responsible for p53-mediated cell cycle arrest and apoptosis (Abbas and Dutta, 2009). p21 could even be the sole mediator of the overactive p53 response to DNA damage in FA HSPC, pointing to p21 inhibition as a way to prevent the progressive HSPC loss in FA (Murray et al., 2010; Sax et al., 2002). Interestingly, p21 deletion is already known to rescue stem cell self-renewal from mice suffering from DNA damage provoked by dysfunctional telomerase (Choudhury et al., 2007). On the other hand, it has also been reported that p21 has an important role in the regulation of FA-BRCA pathway activation (Rego et al., 2012). Therefore, the precise outcome of p21 deletion in FA patients warrants further investigation.

Fancd2$^{-/-}$ mice on the 129S4 genetic background recapitulate major FA patient phenotypes, displaying tumor susceptibility and hematopoietic defects (Houghtaling et al., 2003; Zhang et al., 2010). Here we characterized p21 and Fancd2 double-knockout mice to understand whether p21 is involved in the FA pathway and whether the rescue effects of p53 deletion on HSC maintenance are p21-related.

Methods

Mice

Fancd2 and *ROSA26* transgenic mice were maintained on the 129S4 background. *Cdkn1a* deficient mice were originally ordered from Jackson Laboratories (Bar Harbor, Maine) and backcrossed to the 129S4 background for more than 10