Published in final edited form as:
Cell Stem Cell. 2012 July 6; 11(1): 7–8. doi:10.1016/j.stem.2012.06.013.

Damage control and its costs: BM failure in Fanconi Anemia stems from overactive p53/p21

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Abstract

Despite having well-characterized disease-associated mutations, the mechanisms underlying progressive bone marrow failure and cancer susceptibility of Fanconi anemia have been unclear. In this issue of Cell Stem Cell, Ceccaldi et al. identify an overactive p53/p21 stress response and cell cycle arrest as an underlying cause that starts during fetal development.

Guido Fanconi originally described “pernicious anemia” in three siblings who had large red blood cells, progressively declining blood counts, and physical anomalies (Fanconi, 1927). Fanconi anemia (FA) is the paradigmatic constitutional aplastic anemia and is characterized functionally by decreased production of all circulating blood cells and pathologically by a marrow devoid of hematopoietic precursor cells. The stereotypical clinical presentation is an infant or young child with short stature, skin hyperpigmentation, hypoplastic thumbs, reduced blood cell counts, and a fatty, unregenerative bone marrow. The diagnosis is established by observing chromosomal abnormalities in cells exposed to DNA-crosslinking agents (Shimamura and Alter, 2010). FA patients die of the complications from low blood counts or from leukemia and solid cancers. This pattern of bone marrow failure (BMF) combined with late myeloid malignancies occurs in other inherited syndromes and in acquired immune-mediated aplastic anemia, making FA a useful model for studying several conditions.

The FA genes (there were 15 at most recent count) were first identified using the chromosomal phenotype apparent in cell culture along with cell line complementation, and more recently by sequencing strategies. The FA gene products have been shown to constitute a nuclear protein complex (but not yet an enzymatic activity) and to define a pathway of DNA repair that includes homologous recombination, nucleotide excision repair, and mutagenic translation synthesis (Moldovan and D’Andrea, 2009). In this issue, Soulier and colleagues reveal a link between unresolved DNA damage, an overactive p53/p21 response and the progressive BMF that occurs in FA patients.

Although the p53 pathway had been previously reported to promote leukemogenesis in FA patients (Ceccaldi et. al, 2011), in this study the authors reveal that overactive p53 also promotes a decrease in the HSPC pool of FA patients and support their claims using primitive murine bone marrow cells.

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Studies of FA are encumbered by the difficulty in obtaining patient samples of a rare disorder and because mouse models do not accurately recapitulate much of the human disease. Ceccaldi et al. use a combination of in vitro methods with FA CD34+ bone marrow cells and in vivo assays using their mouse bone marrow counterparts. First, they observed a reduction in CD34+ hematopoietic stem/progenitor cells (HSPCs) in FA patients that worsened with age. Given the role of the FA proteins in DNA repair, they hypothesized that progressive accumulation of DNA damage contributed to their bone marrow failure. Indeed, p53 activation was elevated in primary blood cells from FA patients as well as in bone marrow of *Fanc*-deficient mice. To model the endogenous DNA damage that accumulates in patients over time, the authors next treated immortalized HSPCs from FA patients with mitomycin C, a DNA cross-linking reagent known to induce the damage response. FA cells, but not control cells, arrested in G2 phase, due to unresolved DNA damage during S phase. G2 arrest was resolved in mitomycin C treated HPSCs, and the cells instead underwent G0/G1 cell cycle arrest along with strong induction of p53 and p21. Silencing of p53 in FA cells resulted in increase DNA breaks in cells arrested in G0/G1 after treatment with mitomycin C.

They next tested whether inhibiting the overactive p53 response rescued the HSPC defect. Knockdown of p53 rescued cell proliferation in both primary cells from patients and in the mouse model. Likewise, depletion of p53 or p21 increased the number of clonogenic progenitors in CD34+ cells. Although marrow failure does not occur until after birth, FA fetal liver cells obtained from medical abortions also had high levels of CDKN1A/p21. Taken together, these data show that an exaggerated physiologic stress response results in the accumulation of DNA damage, that this cellular stress underlies progressive HSC depletion in FA patients, and that genomic instability may result if critical checks like p53 induction should fail.

That HSPC depletion starts during fetal development and that its circumvention later in life, as by p53 mutation or deletion, creates genomic instability and malignant hematopoietic cells is consistent with the poor outcome of FA in the clinic. Occasional FA patients do show spontaneous improvement in blood counts (Ceccaldi et al., 2011). Bone marrow function may be rescued by a somatic reverse mutation or mitotic recombination, generating a chimeric HSPC compartment, and allowing for repopulation of the depleted marrow. A second mechanism of rescue involves abrogation of the G2 DNA damage checkpoint, by lower levels or inhibition of *CHK1,* permitting cells to progress through the cell cycle despite accumulated, unrepaired DNA damage—but while blood counts may improve, escape of the checkpoint would also increase the probability of malignant transformation. In the current study, inhibition of p53/p21 signaling rescued cells from mitotic cycle arrest and, as in previous CHK1 experiments, allowed progression through cell cycle of cells with increasing numbers of chromosomal abnormalities.

These findings have several broader implications for patients with several different types of aplastic anemia. The etiologies of genetic and acquired aplastic anemia are different, but morphologically they are indistinguishable, and the pathophysiology in all types involves a drastic reduction in HSC number and a risk of malignant evolution with the emergence of leukemic clones. In FA, DNA repair pathways are implicated. In Diamond-Blackfan anemia (DBA) and in Schwachman-Bodian-Diamond syndrome (SBDS) ribosome assembly is defective due to specific genetic lesions. In DBA, as in FA, p53 activation occurs in response to stress signals and appears critical in the reduction in HSPC number (Narla and Ebert, 2010). In the telomeropathies, dyskeratosis congenita (DKC) and a variety of subtle mutations in the telomerase repair complex genes (*TERT* and *TERC*), HSC loss occurs as senescence or apoptosis is triggered by critical telomere shortening (Calado and Young, 2009).

Progression to leukemia is frequent in FA and in DKC, the most severe of the telomere diseases, in SBDS and to a lesser extent in DBA. AML that evolves in these constitutional syndromes is almost invariably associated with aneuploidy and structural rearrangements, with a high prevalence of stereotypical cytogenetic abnormalities (Rochowski et al., 2012). Why specific chromosomal patterns dominate, whether chromosomal abnormalities initiate the oncogenic process, and the relationship of chromosome aberrations to genetic mutations, especially of p53 and similarly protective genes, are not understood. Moving forward, much will be learned from sophisticated assays of telomere length in cell populations and single cells, as well as from genomic sequencing of serial samples of HSCs and their progeny in patients followed over time. It is tempting to speculate that the genetic alterations will reflect the selective pressures under which defective cells struggle, as, for example, in circumventing specific cell-cycle blocks (p53 in FA and DKC) and telomere loss (telomerase upregulation in leukemia and many cancer cells).

As highlighted in the current work in this issue, the balance between the appropriate triggering of cell death due to stress signals and its suppression by inactivation of p53, either through manipulation in the laboratory or mutations in patients, is delicate. Furthermore these new data reveal that in constitutional BMF, much or most HSC loss may occur very early in life, during fetal development or infancy. Targeting these pathways molecularly is appealing but not realistic. Gene therapy in homozygous genetic disorders has many technical obstacles, and even if successful would not target the entire pool of premalignant cells. A less elegant approach that has nonetheless been proven in practice would be HSC transplantation, which replaces defective cells. Moreover, the utility of transplant is increasing with the use of unrelated and mismatched donors and improvements in the management of complications. Taken together, the newly defined relationship between mechanisms underlying BMF and development of cancer should provide important insights into treating FA and similar diseases.

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