Stressed Out: Endogenous Aldehydes
Damage Hematopoietic Stem Cells

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Despite a well-defined role for the Fanconi anemia (FA) pathway in mediating DNA repair, the mechanisms underlying the bone marrow failure in FA patients are poorly defined. Recently in *Nature*, Garaycoechea et al. (2012), identify aldehyde-mediated genotoxicity of hematopoietic stem cells as a cause for bone marrow failure.

Exposure to exogenous or endogenous genotoxic agents (e.g., radiation, mutagenic agents, endogenous reactive metabolites) has deleterious consequences on cells in the bone marrow, often leading to anemia, cancer, and cellular aging. In general, long-lived quiescent hematopoietic stem cells (HSCs) can undergo growth arrest and thereby survive genotoxic stress, whereas proliferating progenitors and mature blood cells undergo apoptosis and die. Nevertheless, DNA damage and genomic instability from genotoxic stress can accumulate in the surviving HSCs, which can be detrimental during their life span. The genotoxins that cause the deleterious DNA damage and the protective mechanisms that operate to repair this damage in the HSCs are poorly defined. Recently, a few specific DNA repair mechanisms, operative in HSCs, have been identified (Niedernhofer, 2008).

Endogenous aldehydes, produced during normal cellular metabolism, are known to be carcinogenic. Whether these normal metabolites also represent a specific genotoxic burden for HSCs remains unknown. A recent study published in *Nature* (Garaycoechea et al., 2012) from K.J. Patel's laboratory demonstrates that endogenous aldehydes are indeed genotoxic to HSCs and that HSCs have a novel mechanism of protection from this DNA damage. Specifically, endogenous aldehydes pose a severe threat to normal HSCs in the absence of two important protective elements: namely, the Fanconi anemia (FA) DNA repair pathway and the enzyme aldehyde dehydrogenase 2 (Aldh2).

The FA pathway consists of a network of at least 15 proteins that cooperate in the repair of DNA interstrand crosslinks (reviewed in Kim and D'Andrea, 2012). The pathway was established through the systematic study of families with the rare genetic disease Fanconi anemia, which is characterized by progressive bone marrow failure (due to HSC attrition), developmental abnormalities, heightened susceptibility to cancer (especially late developing myeloid malignancies), and cellular hypersensitivity to interstrand DNA crosslinkers. Increased chromosomal breakage and chromosome radials following exposure to DNA crosslinking agents, such as Cisplatin, is the cellular hallmark of FA.

Mouse models for FA have been generated through genetic disruption of several of the FA genes, including Fancc, Fanca, Fancl, Fancd2, Fancd1/Brca2, and Fancp/Slx4. While mice with a genetic deficiency in the FA pathway exhibit cellular hypersensitivity to interstrand crosslinking agents, they do not develop spontaneous bone marrow failure (Parmar et al., 2009), with the exception of the Fancp/Slx4 mice (Crossan et al., 2011), thus hampering pathogenesis studies of this disease. Due to the well-studied role of FA proteins in DNA cross-link repair, it was predicted that the cellular DNA repair defect is the main cause of the FA disease. Still, the nature of the endogenous crosslinking agent remained unknown. A breakthrough came last year when the Patel laboratory discovered that endogenous reactive aldehydes are the genotoxins that are largely responsible for the pathophysiology of FA (Langevin et al., 2011). Patel and colleagues had previously shown that FA pathway-deficient chicken DT-40 cells, as well as murine bone marrow hematopoietic cells, are hyper-sensitive to acetaldehyde, a by-product of natural metabolism (Langevin et al., 2011). Using genetic mouse models, these investigators elegantly showed that FA pathway counteracts the toxic effects of acetaldehydes in mice. Mice with combined deficiency in one of the FA genes (Fancd2) and Aldh2 (an enzyme that catabolizes acetaldehyde into acetate) displayed developmental defects and succumbed to acute leukemia within 3–6 months after birth. Moreover, these double knockout (KO) mice were highly susceptible to the toxic effects of ethanol—an exogenous source of acetaldehyde, and ethanol exposure induced bone marrow failure in these mice. Collectively, these findings indicated that acetaldehyde-mediated toxicity may contribute to the pathogenesis of FA.

In order to more precisely dissect the role of the FA pathway in acetaldehyde-mediated bone marrow failure, the Patel laboratory further analyzed the untreated *Fancd2*$^{-/-}$ *Aldh2*$^{-/-}$ mice for hematopoietic defects (Garaycoechea et al., 2012). While most of the double KO animals succumbed to leukemia as they aged, the surviving mice developed low blood cell counts. In addition, a small percentage of the older mice without leukemia (6/29) exhibited aplastic anemia, which is a hallmark feature of human FA and is characterized by a pantocytopenia, hypocellular bone marrow, and extramedullary hematopoiesis. Interestingly, the clinical phenotypes of *Fancd2*$^{-/-}$ *Aldh2*$^{-/-}$ mice correlated with increased DNA damage (gamma H2AX positive cells) and apoptosis in the bone marrow. Strikingly, the DNA damage response was more profound in the hematopoietic stem and progenitor (HSPC) populations,

including the long-term HSCs (LT-HSCs), than in the other progenitor populations of the bone marrow. These results suggested that perhaps the genotoxic lesions from the acetaldehyde catabolism preferentially occur in the HSCs and are repaired by the FA proteins. To confirm this, the authors systematically evaluated several bone marrow cell populations for acetaldehyde sensitivity by exposing the cells in vitro. Unexpectedly, they found that only HSPCs, and not the mature blood cells or colony-forming progenitors, require both Aldh2 and FA pathway for protection against aldehyde toxicity. Consistent with these observations, aldehyde dehydrogenase activity of HSPCs was mostly due to the Aldh2.

The investigators next confirmed the requirement of both the FA pathway and Aldh2 against the genotoxicity of acetaldehyde in HSCs by using the young (8- to 12-week-old) $Fancd2^{-/-}$ $Aldh2^{-/-}$ mice (Garaycoechea et al., 2012). These mice had not developed leukemia or bone marrow failure. The immunophenotyping analysis of the bone marrow showed a marked reduction in the HSPC and LT-HSC populations, with almost 250-fold reduction in the frequency of SLAM-expressing LT-HSCs compared to the wild-type littermates or single KO mice. Furthermore, the LT-HSCs from the double KO mice were less quiescent and more actively cycling. The HSCs from the double KO mice were also tested functionally in bone marrow transplantation experiments. Strikingly, the bone marrow from the double KO mice was severely compromised in the long-term repopulation ability with 638-fold reduction in competitive repopulating units, compared to wild-type bone marrow. $Aldh2^{-/-}$ bone marrow and $Fancd2^{-/-}$ bone marrow exhibited only 2.75-fold and 38-fold reduction (Parmar et al., 2010; Zhang et al., 2010), respectively. Taken together, these observations confirmed that combined deficiency of both the FA pathway and acetaldehyde catabolism can lead to profound HSC defects in the bone marrow (Figure 1).
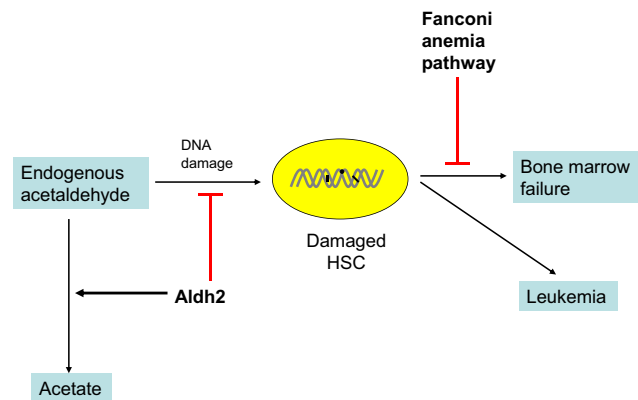

Figure 1. Acetaldehyde, an Endogenous Aldehyde, Poses a Severe Threat to HSCs in the Absence of Aldh2 Enzyme and a Functional Fanconi Anemia Pathway

Acetaldehyde is normally generated during natural cellular metabolism and is detoxified by aldehyde dehydrogenase enzymes. For instance, Aldh2 enzyme converts toxic acetaldehyde into acetate. When Aldh2 is not functional, acetaldehyde accumulates and causes DNA damage in HSCs. The Fanconi anemia (FA) DNA repair pathway protects the HSCs from this genotoxic stress. In the absence of both Aldh2 and a functional FA pathway, HSCs struggle for existence, leading to bone marrow failure and leukemia.

The HSC attrition results in bone marrow failure or leukemia. These studies therefore suggest that the progression of bone marrow failure in FA is perhaps due to the aldehyde-mediated genotoxicity in HSCs.

The findings by Patel's group highlight the importance of a functional FA pathway in resolving the cellular damage caused by endogenous aldehydes in HSCs and enhance our understanding of mechanisms for HSC loss in FA patients. Recent studies from our own laboratory, in collaboration with the Soulier group, using primary FA patient bone marrow, revealed that an exacerbated p53/p21 response caused by cellular stress and DNA damage in HSPCs may cause bone marrow failure in FA (Ceccaldi et al., 2012). HSPC depletion in FA starts during fetal development, due to the hyperactivation of the p53/p21 axis, perhaps caused by replicative stress and increased DNA damage. Taken together, the hyperactivation of p53/p21 response observed in FA fetal liver cells and bone marrow HSPCs may be due, at least in part, to an aldehyde-mediated endogenous DNA damage mechanism. Besides the DNA damage caused by aldehydes, additional injury caused by inflammatory cytokines (e.g., TNF-alpha) or oxidative stress may also lead to dysfunctional HSCs in the FA bone marrow.

These new studies by Patel's group may lead to the identification of new therapies for the bone marrow failure in FA. For instance, agents that can enhance the catabolism of endogenous aldehydes (e.g., agonists of Aldh2) may provide a new therapeutic avenue. The $Fancd2^{-/-}$ $Aldh2^{-/-}$ double KO mice may serve as a valuable model for testing new therapies for FA because they recapitulate the bone marrow failure and other pathophysiological features observed in FA patients. Several outstanding questions remain. We still do not know (1) which specific aldehyde in stem cells of FA is the most toxic, (2) the nature of the genotoxic stress (i.e., is it a DNA or protein cross-link?), or (3) how the damage ultimately weakens HSCs. Nevertheless, these recent discoveries from the Patel laboratory provide important insights into the mechanisms of genotoxin defense in HSCs and into the underlying nature of bone marrow failure in FA.

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