PERSPECTIVE ON THE GENOTOXIC CONSEQUENCES OF ENDOGENOUS ALDEHYDES ON MOUSE HAEMATOPOIETIC STEM CELL FUNCTION

RESEARCH PROPOSAL

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The underlying causes of DNA damage and the corresponding protective mechanisms are yet to be fully deciphered at the moment (Garaycoechea et al. 2012). For instance, Sugrue (2012) and his colleagues report that under conditions of total body irradiation, the mechanisms used by mesenchymal stromal cells (MSCs) of the haematopoietic system to survive lethal radiation doses is poorly understood. This is reflective of the level of uncertainty in the study of the DNA damage response and the various mechanisms that aid in protecting cells and tissues from permanent damage.

Prior to the release of their paper on endogenous aldehydes and their effects on mouse haematopoietic stem cells (HSCs), Garaycoechea (2012) and his colleagues established that the aldehyde detoxifying enzyme, Aldh2 and the Fanconi anaemia DNA repair pathway are both vitally required to nullify the genotoxic effects of reactive aldehydes. Highly reactive endogenous aldehydes such as malondialdehyde, acrolein, methylgloxal (Voulgaridou et al., 2011) and acetaldehyde easily react with proteins and nucleic acids and can lead to both mutagenesis and apoptosis (Langevin et al., 2011). They observed that the majority of mice with combined deficiency in aldehyde detoxification ($Aldh2^{-/-}$) and the Fanconi anaemia DNA-repair pathway ($Fancd2^{-/-}$) are inevitably predisposed to acute T-cell leukaemia. These mice also show abnormal development and very high susceptibility to the toxic effects of exogenous acetaldehydes metabolized from ethanol (Garaycoechea et al., 2012).

Langevin (2011) and his colleagues also established that Aldh2 is essential for the development of Fancd2 gene deficient embryos. Interestingly, they also showed that double mutant ($Aldh2^{-/-}$ $Fancd2^{-/-}$) embryos can develop normally if their mothers are aldehyde-detoxification-competent ($Aldh2^{+/-}$). Surprisingly, these same embryos also succumb to ethanol exposure in the womb— probably proof positive of the essence of Aldh2 in the normal

development of Fancd2$^{-/-}$ embryos. Although the mice majority develop acute T-cell leukaemia, Garaycoechea et al. (2012) make some interesting observations about those that do not develop leukaemia and this forms the basis of their research paper under review.

First and foremost, they established that non leukaemia predisposed Aldh2$^{-/-}$ Fancd2$^{-/-}$ mice generally showed substantial deterioration in blood constituent number and quality—typically characteristic of aplastic anaemia. These changes are naturally accompanied by the accumulation of DNA damage within the haematopoietic stem and progenitor cell (HSPC) pool. This is typified by the increased number of double mutant bone marrow cells tested positive for γ-H2AX (Rogakou et al. 1998)—an indirect indicator of endogenous double-stranded DNA breaks (Wojtczak et al. 2008). Immunocytochemical analysis also revealed the presence of cleaved caspase-3—an indirect marker depicting increased cell proportion undergoing programmed cell death.

Moreover, they reported that unlike mature blood precursors, Aldh2 is necessary for protection of HSPCs from aldehyde toxification (Garaycoechea et al. 2012). The propensity of HSPCs to detoxify endogenous aldehydes is measured by the commercial assay, Aldeflour (Storms et al. 1999). Aldehyde dehydrogenase (ALDH), an intracellular enzymatic product of the Aldh2 gene, detoxifies reactive aldehydes, and the amount of fluorescence emitted by the Aldeflour stain reflects ALDH activity in the cell (NewsRx, 2005). Finally, they reported on the drastic reduction of the HSC numbers of Aldh2$^{-/-}$ Fancd2$^{-/-}$ mice. This more than 600-fold decrease in the HSC pool is typical of bone marrow hypocellularity (Garaycoechea et al. 2012).

The experimenters made a number of startling findings arising from deductions made on the experimental procedure. First, they noticed that B cells, erythroid progenitor cells and

granulocyte-macrophage progenitor cells from the three different Aldh2$^{-/-}$ Fancd2$^{-/-}$ mice strains were all more acetaldehyde sensitive compared to wild type controls. Additionally, comparisons between the acetaldehyde sensitivities of Aldh2$^{-/-}$ Fancd2$^{-/-}$ and Fancd2$^{-/-}$ mutants revealed they were slightly comparable with each other. This possibly shows that the combined Aldh2$^{-/-}$ Fancd2$^{-/-}$ mutants facilitate a synergetic HSC deficiency to acetaldehyde detoxification. This also confirms that both Aldh2 and Fancd2 are required for aldehyde detoxification. Moreover, in aged aplastic Aldh2$^{-/-}$ Fancd2$^{-/-}$ mutant mice, there is a γ-H2AX induction surge in the HSPC-enriched LKS population and in long-term HSCs compared to other haematopoietic precursors. The γ-H2AX induction surge signifies an increase in double stranded DNA damage. These results possibly suggest that HSPC DNA damage naturally accompanies aplastic anaemia in Aldh2$^{-/-}$ Fancd2$^{-/-}$ mutant mice and that both Aldh2 and Fancd2 are vital in maintaining the blood at a set functional quality. Results from the modified colony-forming unit spleen (c.f.u.-S_{10}) assay also show that ST-HSCs require both Aldh2 and Fancd2 to nullify the genotoxic effects of acetaldehyde. However, mature precursors do not require Aldh2 and this augments the role of Aldh2 and Fancd2 in specifically protecting HSPCs from aldehyde accumulation. This feature could possibly be an evolutionary mechanism to protect HSPCs since they are the most vital component of the haematopoietic system (Garaycoechea et al. 2012; Kutler et al. 2003).

Under increasing acetaldehyde concentrations, Aldeflour activity in wild type cells is markedly reduced. There was an intermediate reduction in Aldh2$^{+/-}$ cells and the activity in Aldh2$^{-/-}$ cells reduced dramatically. This possibly suggests that Aldeflour activity is primarily due to Adh2. Since Aldeflour activity measures the aldehyde-detoxifying potential of cells, it is appropriate to note that this protection is also primarily due to Aldh2. This assumption is safe to make considering that Aldh2 is the most frequently expressed gene of the ALDH enzyme family

(Levi et al. 2009; Kiel et al. 2005). Results from the cobblestone-area-forming cell (CAFC) assay confirm the more than 600-fold reduction in the HSC pool of Aldh2$^{-/-}$ Fancd2$^{-/-}$ mice. This drastic decline in the HSC pool of Aldh2$^{-/-}$ Fancd2$^{-/-}$ mutants portrays the concomitant emergence of bone marrow failure in Fanconi anaemic patients with a link to aldehyde-facilitated genotoxicity.

Throughout their paper, the authors underscore the potentially genotoxic effects of endogenously generated aldehydes on the cells of the haematopoietic system. They outline the two co-ordinate mechanisms necessary for protection from the irreversible genetic damage caused by endogenous reactive aldehydes on HSCs. They show the relevance of these two requisite pathways—aldehyde catabolism and Fanconi anaemia DNA-repair by inactivating both genes responsible and observing the effects of reactive aldehydes on these mutant HSCs. Mutant cells encountering reactive aldehydes inevitably succumb to irreversible DNA damage leading to a reduction in the HSC pool resulting in bone marrow failure. Projecting to human cases, children with Fanconi anaemia (analogous to double mutant mice) mostly develop acute myelogenous leukaemia and bone marrow failure, owing to DNA damage by reactive aldehydes to the HSC pool. This trend of Fancd2 and Aldh2 insufficiency and DNA damage by aldehydes suggest some possible treatments for Fanconi anaemia patients—primarily by limiting the amount of endogenous reactive aldehydes generated by the body or possibly replenishing the HSC pool damaged by these aldehydes (Garaycoechea et al.2012; McCarthy 2012). Parmar and D'Andrea (2012) share a similar view regarding treatment options. They suggest that aldehyde catabolism enhancers like Aldh2 agonists could be an effective therapeutic intervention against aldehyde-mediated bone marrow failure. Lipton (2012) also suggests that a more permanent approach to eradicating the disease is by HSC transplantation. He asserts that bone marrow,

peripheral blood or cord blood stem cells may cure aplastic anaemia and aid in preventing leukaemia. Nevertheless, this treatment intervention is more effective for patients with a human leukocyte antigen (HLA)-matched sibling donor (survival rate >80%). Patients without HLA-matched related donors can have a saviour sibling conceived by preimplantation genetic diagnosis (PGD) to match their HLA type (Verlinsky et al. 2001).

F1000 Prime Faculty members, Vishva Dixit and Kim Newton (2012) support the authors' assertion of an absence of a functioning Fanconi anaemia DNA-repair pathway leading to acetaldehyde mediated genotoxicity. They believe this finding is crucial because it suggests that aldehyde-mediated DNA damage could be a trigger of bone marrow failure in Fanconi anaemia patients. This theme also resonates with faculty member Junjie Chen's recommendations on the authors' work. He affirms that the mouse model employed by the experimenters gives support to the assertion that Fanconi anaemia probably results from endogenous aldehyde-mediated genotoxicity which leads to the attrition of haematopoietic stem cells. Parmar and D'Andrea (2012) are impressed by the Fancd2$^{-/-}$ Aldh2$^{-/-}$ double mutant mice model as they believe it may serve as a valuable tool for testing new Fanconi anaemia therapies. They postulate that the mice models can help recapitulate the pathophysiological features observed in Fanconi anaemia and ultimately aid in managing or curing the disease.

Regardless of the prospects presented by the authors' work, Parmar and D'Andrea (2012) identify several outstanding shortcomings. First, they express general uncertainty about which specific aldehyde in Fanconi anaemia afflicted cells is the most toxic. They also express uncertainty over whether the genotoxic stress is of DNA or protein crosslink nature. Finally, they assert that the mechanisms through which aldehyde-induced DNA damage ultimately weakens HSCs are currently poorly understood. Nevertheless, McCarthy (2012) reiterates that the

findings from the authors' work support the hypothesis that Fanconi anaemia mutant patients develop anaemia and/or acute T-cell leukaemia because of the need of both aldehyde dehydrogenases along with the Fanconi anaemia DNA-repair pathway to protect HSCs from irreversible genotoxic damage induced by endogenous reactive aldehydes.

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