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Alcohol and endogenous aldehydes damage chromosomes and mutate stem cells

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Haematopoietic stem cells renew blood. Accumulation of DNA damage in these cells promotes their decline, while misrepair of this damage initiates malignancies. Here we describe the features and mutational landscape of DNA damage caused by acetaldehyde, an endogenous and alcohol-derived metabolite. This damage results in DNA double-stranded breaks that, despite stimulating recombination repair, also cause chromosome rearrangements. We combined transplantation of single haematopoietic stem cells with whole-genome sequencing to show that this damage occurs in stem cells, leading to deletions and rearrangements that are indicative of microhomology-mediated end-joining repair. Moreover, deletion of p53 completely rescues the survival of aldehyde-stressed and mutated haematopoietic stem cells, but does not change the pattern or the intensity of genome instability within individual stem cells. These findings characterize the mutation of the stem-cell genome by an alcohol-derived and endogenous source of DNA damage. Furthermore, we identify how the choice of DNA-repair pathway and a stringent p53 response limit the transmission of aldehyde-induced mutations in stem cells.

The consumption of alcohol contributes to global mortality and cancer development[1]. Most of the toxic effects of alcohol are probably caused by its oxidation product acetaldehyde, which is highly reactive towards DNA[2]. The enzyme aldehyde dehydrogenase 2 (ALDH2) prevents acetaldehyde accumulation by oxidizing it efficiently to acetate, but around 540 million people carry a polymorphism in *ALDH2* that encodes a dominant-negative variant of the enzyme[3]. Alcohol consumption in these individuals induces an aversive reaction and predisposes them to oesophageal cancer[4]. Nevertheless, ALDH2 deficiency is surprisingly well tolerated in humans. This could be because of the additional tier of protection provided by FANCD2, a DNA-crosslink-repair protein. In fact, genetic inactivation of *Aldh2* and *Fancd2* in mice leads to cancer and a profound haematopoietic phenotype[5,6]. In humans, deficiency in DNA-crosslink repair causes the inherited illness Fanconi anaemia, a devastating condition that leads to abnormal development, bone-marrow failure and cancer[7]. Acetaldehyde genotoxicity is likely to contribute to this phenotype, as Japanese children who are afflicted with Fanconi anaemia and carry the *ALDH2* polymorphism display earlier-onset bone marrow failure[8]. Together, these data suggest that endogenous aldehydes are a ubiquitous source of DNA damage that impairs blood production.

It is likely that some of this damage occurs in haematopoietic stem cells (HSCs), which are responsible for lifelong blood production. HSC attrition is a feature of ageing, and mutagenesis in the remaining HSCs promotes dysfunctional haematopoiesis and leukaemia. Moreover, both humans and mice that lack DNA repair factors are prone to HSC loss, and in some cases, bone marrow failure[9,10]. HSCs employ DNA repair and respond to damage in a distinct manner compared to later progenitors[11,12]. While these observations point to a fundamental role for DNA repair in HSCs, recent work has highlighted that the response to replication stress maintains HSC function and integrity[13]. However, there is a key gap in our knowledge regarding the identity of the endogenous factors that damage DNA and lead to replication stress. Here we show that alcohol-derived and endogenous aldehydes damage the genomes of haematopoietic cells, and we characterize the surveillance and repair mechanisms that counteract this. We also establish a method that allows us to determine the mutational landscape of individual HSCs, and in doing so, provide new insight into the p53 response in mutagenized stem cells.

Ethanol stimulates homologous recombination repair

Aldh2$^{-/-}$*Fancd2*$^{-/-}$ mice develop severe HSC attrition, causing spontaneous bone marrow failure, which can also be induced by exposing these mice to ethanol[5,6]. This genetic interaction suggests that in the absence of aldehyde catabolism (such as in *Aldh2*$^{-/-}$ mice), DNA repair is engaged to maintain blood homeostasis. To test this theory, we set out to monitor DNA repair activity *in vivo*. The Fanconi anaemia pathway repairs DNA crosslinks by using a replication-coupled excision mechanism that is completed by homologous recombination[14,15]. We therefore used a method to visualize sister-chromatid exchange (SCE) events in bone marrow cells of living mice; these represent recombination repair transactions coupled to replication (Fig. 1a). The number of SCE events is elevated 2.3-fold in *Aldh2*$^{-/-}$ mice, indicating that recombination repair is stimulated in response to endogenous aldehydes (Fig. 1b, c). Moreover, a single exposure to alcohol causes a fourfold increase in SCE events in *Aldh2*$^{-/-}$ mice (Fig. 1b, c, Extended Data Fig. 1a), suggesting that physiological acetaldehyde accumulation in blood cells is not sufficient to inactivate the homologous recombination repair factor BRCA2[16]. *Fancd2*$^{-/-}$ mice do not show a similar induction following exposure to ethanol; therefore, detoxification is the primary mechanism that prevents DNA damage by aldehydes and alcohol. Finally, the number of SCE events in *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ mice is indistinguishable from that in *Aldh2*$^{-/-}$ mice, showing that homologous recombination repair occurs despite inactivation of FANCD2 (Fig. 1c, Extended Data Fig. 1b).

The repair of aldehyde-induced DNA damage is therefore not limited to the Fanconi anaemia crosslink-repair pathway. As the recombination machinery is essential for mouse development, we used the isogenic chicken B-cell line DT40, which has been used to define the involvement of homologous recombination in crosslink repair[14].

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