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INSIGHTS

Predicting AID off-targets: A step forward

Claude-Agnès Reynaud and Jean-Claude Weill

In this issue of JEM, Álvarez-Prado et al. (https://doi.org/10.1084/jem.20171738) designed a DNA capture library allowing them to identify 275 genes targeted by AID in mouse germinal center B cells. Using the molecular features of these genes to feed a machine-learning algorithm, they determined that high-density RNA PolII and Spt5 binding—found in 2.3% of the genes—are the best predictors of AID specificity.

Activation-induced cytidine (AID) deaminase is the key enzyme involved in affinity maturation of the immune response, triggering somatic hypermutation and class switch recombination of Ig genes through locus-specific cytidine deamination. It is also a key factor in lymphomagenesis originating from germinal center B cells, through bystander mutagenic activity.

That AID targeting to Ig loci is not stringently controlled has long been recognized (Pasqualucci et al., 1998; Shen et al., 1998), yet a first systematic study of genes highly expressed in germinal center B cells proposed that most AID off-targets identified, 36 in total, are in fact mutated and faithfully repaired (Liu et al., 2008).

The mutation load of AID off-targets is low, much lower than mutations at the Ig loci, so that studying them by next-generation sequencing techniques, which have themselves very high mutation background, is challenging. In this issue of JEM, Álvarez-Prado et al. used a gene capture approach that allows sufficient sequencing depth for mutation detection, together with analysis of repair-deficient mice, which further restricts the pattern of mutations to transitions at G/C bases (see figure). They defined a set of 275 genes, whose expression characteristics and chromatin structure were used to feed a machine-learning algorithm, and they thus determined high-density RNA PolII and Spt5 binding as key predictive factors for AID targeting. They estimate that 2.3% of genes in the genome (i.e., ∼500 genes in total) are likely targets of AID mutations in mouse germinal center B cells.

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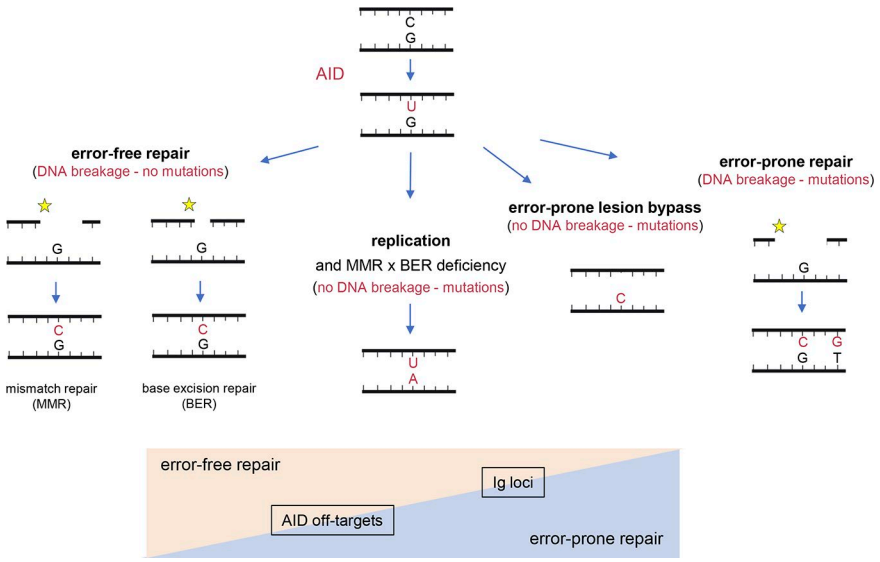

The various outcomes of AID-mediated cytidine deamination are represented, from left to right, with the relative involvement of mutagenesis and DNA strand break for each of them. (1) Error-free repair, mobilizing either BER or MMR to faithfully restore genomic information after excision of the modified base (DNA incision is represented by a yellow star). (2) Ignorance of DNA damage, copied by replication (a configuration that is the sole outcome of cytidine deamination in *Msh2xUng* KO mice, doubly deficient for the MMR and BER pathways). (3) Error-prone lesion bypass of the abasic site generated by Ung-mediated uracil excision. (4) Error-prone repair generated by MMR-mediated recruitment of DNA polymerase eta (specific to Ig gene hypermutation process). Whereas error-prone repair predominates at the Ig loci, various degrees of error-free repair take place at AID off-targets, whose precise extent remains to be determined, notably in human B cells.

Surprisingly, the number of AID mutations with a causal role in lymphomagenesis

claude-agnes.reynaud@inserm.fr or jean-claude.weill@inserm.fr.

appears relatively modest. How can it be? In fact, a large number of genes have promoter proximal regions devoid of coding capacity, with 5′ noncoding exons and large intronic sequences, a configuration that likely contributes to moderate the impact of mutations. *TP53* is also silenced during the germinal center reaction, a regulation allowing these cells to cope with the genome insults that cytidine deaminations trigger, but this regulation may also prevent its mutational inactivation by AID. There are nevertheless several oncogenic mutations documented, notably dominant-negative or up mutants that can have a strong phenotype at the heterozygous state, the best described affecting MyD88 or CD79a and CD79b (Rosenquist et al., 2016).

Translocations are indeed more drastic events for oncogene deregulation, and many recurrent translocations in B cell lymphomas of germinal center origin take place between genes targeted by AID and one of the Ig loci (Lieber, 2016). In this context, the faithful repair described at AID off-targets does not really appear as a safeguard event for the genome because, like hypermutation-associated error-prone repair, it involves breaking DNA and is thus at risk for oncogenic translocation (see figure).

Although Ig diversification with reasonable collateral damage likely results from evolutionary selection processes that made the immune response altogether beneficial, such balance between benefits and risks may differ in the case of ectopic or abnormal AID activation. Such activation events bring into AID's scope of action new set of genes harboring the transcriptional hallmarks defined by Álvarez-Prado et al. (2018). AID activation occurs in B cell lymphomas themselves, triggering mutation events from single base modifications up to "mutation storms" or kataegis, as well as in various cancers and inflammatory processes, induced notably during viral or bacterial infections (Orthwein and Di Noia, 2012; Casellas et al., 2016). The prediction tools proposed in this paper will thus be of great help to further decipher AID off-targets outside the physiological germinal center immune response.

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