more accurate mimics of the native trimer that can be designed, as immunogens, to target the appropriate germ lines.

McGuire *et al.* compared the responses of B cells carrying germline versions of nNAbs and bNAbs to different Env immunogens. Using an assay that they developed, the authors found that a diverse array of nonengineered Envs can activate B cells that express germline nNAbs but not germline bNAbs, consistent with poor elicitation of bNAbs observed in previous vaccine studies. Envs designed to engage germline-bNAb BCRs (namely, 426c.NLGS.TM) nonetheless also activated B cells expressing germline-nNAb BCRs, and to a higher degree. Only when gp120 variable loops V1, V2, and V3 were deleted from the engineered Env did the ratio of activation reverse. Remarkably, germline-bNAb–expressing B cells were activated to a greater extent than those expressing germline-nNAb BCRs. These findings should guide the design of immunogens to identify new vaccine candidates that preferentially activate germline-bNAb–expressing B cells.

The in vitro approach developed by McGuire *et al.* may simplify certain aspects of B cell maturation that occur in lymphoid organs (germinal centers) after inoculation, including the suggested role of helper T cells. The extensive hypermutation of BCRs may not necessarily occur simply as a result of the disparate germline engagement of engineered Envs, and additional subsequent immunizations may be needed to drive the preferred maturation pathway. Further, when considering bNAbs that target other vulnerable sites on the Env trimer, which develop from a wider array of germline antibody genes, this approach may also have some limitations. However, from the perspective of vaccine design against the CD4-binding site, in which germline gene usage is critical, the study of McGuire *et al.* is an important advance in assessing vaccine immunogens for clinical development. ■

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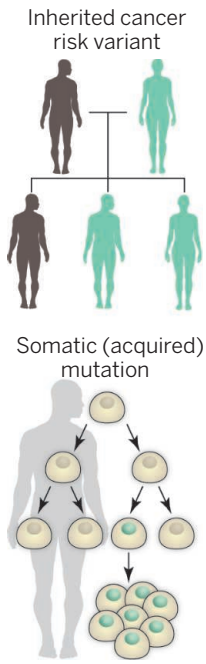
CANCER

Cancer by super-enhancer

Tiny changes in our genomes can enhance oncogene expression and contribute to tumorigenesis

By **Anna Vähärautio**[1] *and* **Jussi Taipale**[1,2]

Most recurring somatic mutations in cancer affect protein-coding regions, either through activating oncogenes or inactivating tumor suppressors (*1*). However, several classes of mutations have been identified that affect the much larger noncoding regions of the genome, leading to changes in gene expression. These include large-scale genomic rearrangements that bring a strongly active promoter next to an oncogene, or place oncogene promoters under the influence of strong transcriptional enhancers (see the figure, panel A). On page 1373 of this issue, Mansour *et al.* (*2*) identify a very small mutation in ~5% of T cell acute lymphoblastic leukemias (T-ALL; see the figure, panel B) that generates a large, powerful enhancer capable of driving tumorigenesis.



Genetics of cancer. Both inherited variants (**top**) and acquired mutations (**bottom**) can contribute to tumorigenesis.

The recent discovery that most inherited genomic variants that predispose us to disease reside in the noncoding portion of our genomes has generated considerable interest in mapping somatic (acquired) mutations in such gene regulatory regions of cancer cells. One such effort has led to the identification of causative, "driver" mutations affecting the proximal promoter of telomerase catalytic subunit (TERT) in melanoma (*3*, *4*). Despite the rapidly expanding list of causative inherited variants affecting distal enhancers (*5*), somatic driver mutations affecting enhancers had not until now been identified.

The discovery by Mansour *et al.* was based on the observation that a subset of T-ALL expressed high levels of the *TAL1* oncogene, a DNA-binding transcription factor, but did not contain the previously identified large rearrangements near *TAL1* that could explain the high expression level. The lymphoma cells expressed only one of the two copies (alleles) of the *TAL1* gene, suggesting the existence of a novel "cis-acting" mutation near the overexpressed copy. To identify the site of the mutation, Mansour *et al.* studied whether the surrounding regions had gene regulatory potential. They found that two T-ALL cell lines, Jurkat and MOLT-3, harbored a large 20-kb region with high levels of a histone modification associated with transcriptional enhancer and promoter activity (acetylated histone H3 lysine 27). Sequencing of this region from the cell lines and from primary T-ALL revealed small insertions of 2 to 18 base pairs (bp) that were located ~7.5 kb upstream of the *TAL1* transcription start site. All the insertions introduced one or two de novo recognition sites for transcription factors of the MYB (myeloblastosis) family. Further experiments indicated that these sites were indeed bound by MYB. MYB binding also recruited to the affected region several other transcription factors that regulate hematopoiesis (GATA3, TCF12, RUNX1, and TAL1), as well as the transcriptional coactivator CBP.

These results suggested that the mutations had generated a novel enhancer that was responsible for the allele-specific overexpression of TAL1. To test this hypothesis, Mansour *et al.* set out to study the requirement of the MYB sites using CRISPR–Cas9 genome editing methods. Specific deletion of the enhancer region resulted in loss of both the histone acetylation signal and TAL1 expression, indicating that the observed enhancer-forming mutation functions as a bona fide driver mutation in T-ALL. Furthermore, the hematopoietic transcription factors that bind to the TAL1 enhancer are known to cooperate with the TAL1 gene product, and regulate each other, forming a self-reinforcing autoregulatory loop. The gain of the MYB site(s) upstream of the *TAL1* gene may thus act by completing the loop, and generating a pathological "locked" tran-

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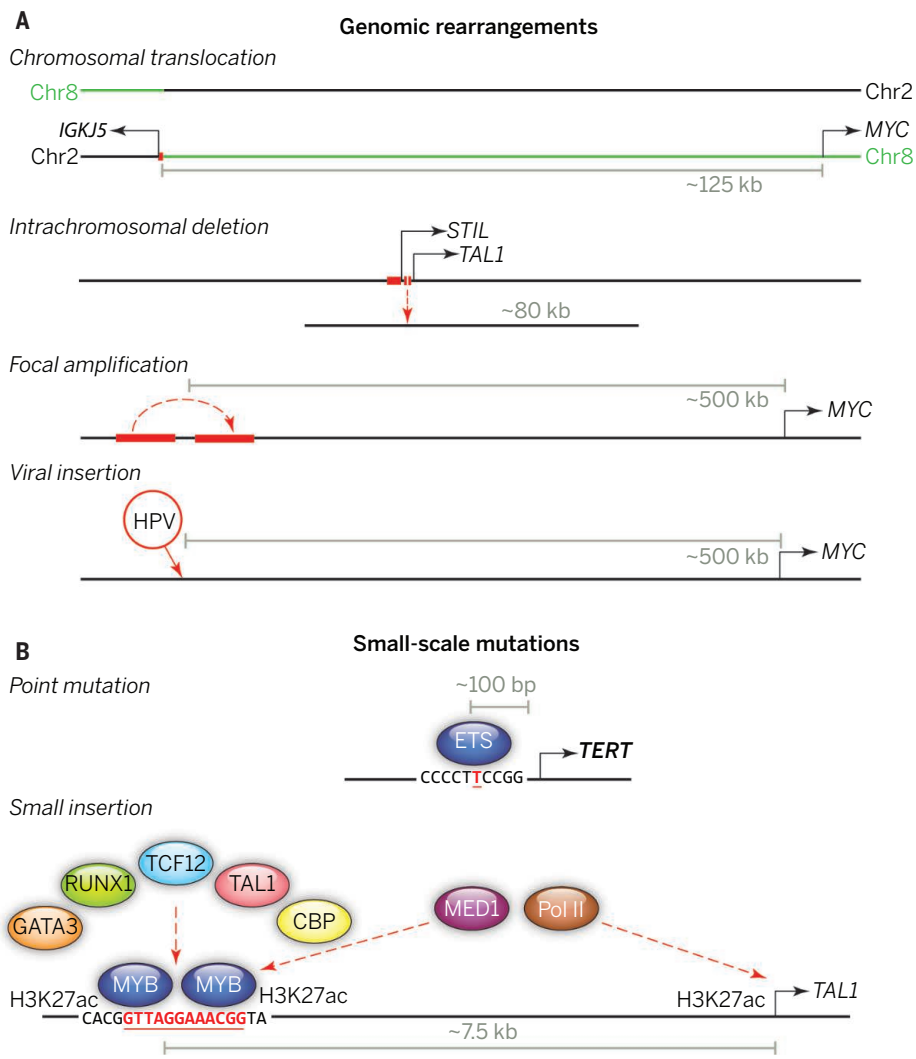
scriptional state that drives tumorigenesis. Given that similar regulatory interactions have also been found in T-ALL cases that carry different upstream lesions, it is tempting to speculate that the specific collection of driver mutations present in each individual tumor invariably results in formation of such locked transcriptional states that drive proliferation.

In contrast to the previously observed enhancer-linked driver mutations that are the result of massive genome rearrangements (see the figure), Mansour *et al.* show that even a small insertion of a few base pairs that introduces only one or two binding sites for a transcription factor can generate a large active enhancer capable of driving tumorigenesis. This exemplifies the magnitude of the challenge that lies ahead: In addition to the function of the reference genome, it is necessary to understand the regulatory activity of the unique genomes of each individual tumor. The discovery by Mansour *et al.* will also undoubtedly inspire studies aimed at identifying similar lesions in other tumor types. However, unbiased identification of small driver mutations in noncoding sequence is challenging because of our limited understanding of the gene regulatory code, and the difficulty of unequivocally identifying small insertions and deletions using short-read sequencing. Given these challenges, the targeted approach used by Mansour *et al.*—focusing on allele-specific expression and mutation-induced regulatory activity, followed by rigorous loss-of-function analysis—is especially promising.

The study by Mansour *et al.* is also notable in that it provides an example of the functional importance of genomic features that carry very strong signals for enhancer-associated chromatin marks. Such regions have been variably identified as locus control regions (*6*), DNase I super-hypersensitive sites (*7*), or more recently, super-enhancers (*8*). At present, there is no consensus on a strict definition of such regions. In addition, it is unclear whether such elements exist as a distinct biochemical entity, or whether differences between them and "common" enhancers are merely quantitative rather than qualitative. Genomics has traditionally focused on the more qualitative aspects of genomes, identifying and mapping features such as genes, transcripts, binding sites, and histone modifications. Less emphasis has been placed on the relative concentration, activity, or specific importance of the identified features. But elementary biochemistry teaches us that high-order reactions involving a large number of cooperative interactions are particularly sensitive to changes in reactant concentrations. Consistently, several studies have shown that genes that have strong enhancers are more sensitive to perturbations that decrease the activity of key transcriptional regulators, such as cohesin, mediator, chromatin modifiers (for example, BRD4), and components of the transcriptional machinery (for example, CDK7) (*8*–*10*).

Many cancer driver genes are brought under the influence of very strong enhancers during tumorigenesis (see the figure). Thus, after locating enhancer-associated genomic landmarks, researchers should pay close attention to their characteristics. Especially as super-enhancers, associated with the most extensive enhancer signatures, are particularly sensitive to perturbation, and may thus become a promising new target of antineoplastic agents. ■



Noncoding mutations in cancer. (**A**) Schematic representation of large-scale genomic rearrangements. Interchromosomal translocations (top) can result in placement of oncogene promoter (e.g., *MYC*) under the influence of a strong regulatory region (e.g., those from Igκ locus, red). Intrachromosomal deletions can similarly lead to increased expression of oncogenes, exemplified by deletion of an 80-kb sequence in chromosome 1, leading to expression of the coding sequence of the *TAL1* oncogene under the broadly active STIL1 promoter (*11*). Regions with transcriptional enhancer activity can also be affected by focal amplifications (*12*) and viral insertions (*13*). (**B**) Small-scale somatic driver mutations affecting noncoding sequence. (Top) Base substitutions at the proximal promoter of the *TERT* oncogene create recognition sequences for ETS transcription factors (*3*, *4*). (Bottom) Mansour *et al*. report noncoding insertion mutations that create binding sites for the MYB transcription factor, and lead to formation of a de novo enhancer that drives expression of the *TAL1* oncogene.

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