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In APL, noncoding mutations and SNP converge on WT1

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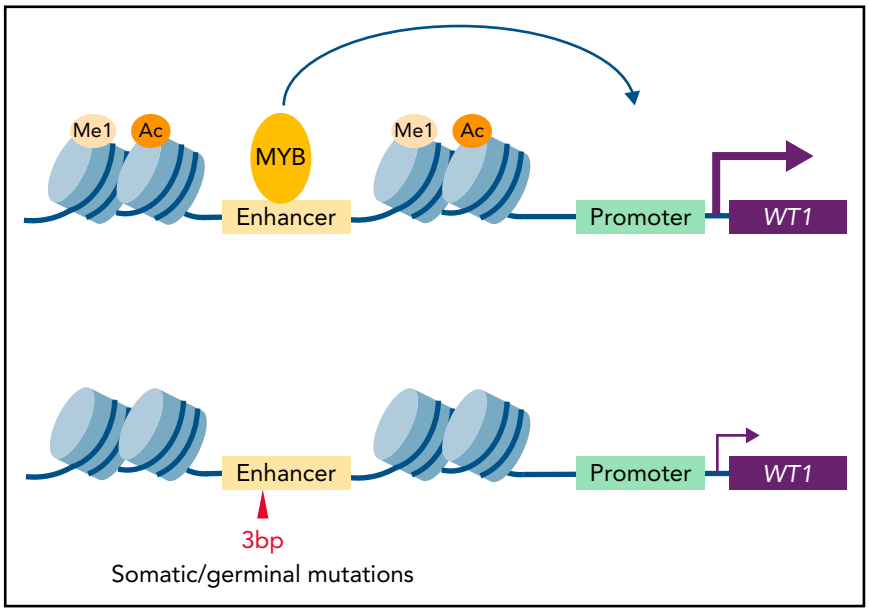
In this issue of *Blood*, Song et al[1] show that in acute promyelocytic leukemia (APL), focal noncoding enhancer mutations disrupt MYB binding and *WT1* expression, mimicking a germline single nucleotide polymorphism (SNP) that favors APL emergence.

APL is a model disease not only because of its exquisite sensitivity to the curative effect of retinoic acid and arsenic[2] but also because of its uncommon epidemiology. APL incidence is almost constant across all ages, pointing to a single key rate-limiting step. The initiating events are clearly translocation-driven RARA fusions, but extensive genomic studies, primarily exome sequencing, have revealed frequent activation of cooperating events during APL progression, primarily FLT3 mutations, but also that of many classic acute myeloid leukemia (AML) oncogenes, including WT1.[3,4] Whole-genome sequencing (WGS) of tumors has revealed a very large number of alterations, allowing detailed classification of the mutagenic processes. However, only a small fraction of these alterations in tumors are believed to be actual drivers.[5] The most common alterations include mutations in splice sites that yield truncation/destabilization of tumor suppressors.[5,6] Gain of function alterations in enhancer sequences also exist, as exemplified by mutations in the telomerase gene promoter that increase telomerase expression by allowing de novo binding of transcription factors, as found in familial melanoma and other cancers.[7] Yet, the impact of noncoding somatic mutations affecting enhancer function and directly contributing to oncogenesis appears to be limited in many cancers. Then, could WGS reveal a broader repertoire of genetic alterations that contribute to APL pathogenesis?



In APL patients, focal somatic mutations or germinal SNP in *cis*-regulatory regions disrupt MYB binding, reduce H3K27ac/H3K4me chromatin signatures of active enhancers, and disrupt interaction with the proximal promoter, all yielding *WT1* repression.

In this study, Song and colleagues investigated the mutational landscape of APL-associated noncoding mutations by performing WGS in 24 paired APL and germline samples. They subsequently focused on the *cis*-regulatory regions controlling gene expression and found that noncoding mutations were enriched in active enhancers bound by key myeloid pioneer factors, particularly MYB. When coupling analysis of these regulatory region mutations to RNA sequencing analyses, the authors discovered 38 mutated zones associated with deregulation of oncogene expression. Remarkably, in 3 patients among the 24 explored, tightly clustered mutations were identified in an intron of WT1, a gene with a complex relationship with myeloid leukemogenesis. By extending the analysis to 169 APL patients, the authors ultimately identified 6 somatic mutations in a hyperfocal 3 basepair region. Eight patients exhibited a germline polymorphism in the same sequence. As the frequency of this polymorphism in the Chinese population is 0.82%, this SNP exhibits a significant enrichment in APL patients, suggesting that it might be a risk factor for APL development. The mutant alleles opposed enhancer function, and patients displayed low WT1 expression transcribed from the mutant allele. Out of the 14 patients with this noncoding variant/mutant among the 169 APLs explored, 4 presented with biallelic functional inactivation, pointing to WT1 as a tumor suppressor in APL. Two patients displayed biallelic enhancer inactivation with homozygote variants or mutations; 2 others exhibited sharply reduced expression of one allele, while the other allele expressed a mutant WT1 protein. Mechanistically, these variants strongly reduced the binding of MYB to this intronic enhancer. MYB binding to this intronic enhancer is required for efficient interaction with the WT1 proximal promoter (see figure). These findings constitute a mirror image of T-cell acute lymphoblastic leukemia, in which gain of function intronic mutations create an MYB binding site in an enhancer of the TAL1 gene to promote its expression.[8] It will be interesting to investigate the prevalence

of these WT1 noncoding mutations and germline polymorphism in other AMLs.

First identified as a master gene whose loss of function drives abnormal kidney development, subsequent studies demonstrated that WT1 is mutated or overexpressed in many AMLs. Enhanced WT1 expression or mutations confers a poor prognosis, and WT1 was explored as a possible tumor antigen in AML. Mechanistically, WT1 recruits TET2 to modulate DNA methylation of WT1 target genes.[9] In animal models, WT1 haploinsufficiency contributes to transformation.[10] *WT1* modulates the all-trans retinoic acid signaling pathway, which may explain the high incidence of *WT1* alterations (coding or noncoding) in APL when compared with non-APL AML.

Collectively, these new results highlight a previously unrecognized molecular mechanism for loss of WT1 expression, possibly linked to the high prevalence of copy-neutral loss of heterozygosity in relapsing patients.[3] Once again, APL serves as a paradigm for precision oncology.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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RED CELLS, IRON, AND ERYTHROPOIESIS

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Grab and go: transferrin uptake in erythropoiesis

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In this issue of *Blood*, Chen et al[1] demonstrate that Grab, a Rab guanine exchange factor for Rab8, regulates transferrin (Tf)-bound iron uptake in mouse fetal liver erythroblasts, zebrafish embryos, and human leukemic cell lines. Prior studies found that polymorphisms in *GRAB* and its target, *RAB8*, affect the mean corpuscular hemoglobinization of red blood cells. Together these findings suggest that Grab is another potential target to regulate Tf-mediated iron uptake into erythropoietic cells.

Approximately 70% of all the iron in the body is incorporated into hemoglobin in red blood cells. Tf-mediated iron uptake into erythroblasts is regulated by extent of iron-bound Tf, the number of Tf receptors (Tfrc), and the rates of endocytosis and exocytosis of Tfrc. The mechanisms by which the Tfrc is recycled to the plasma membrane are the subject of this paper. The authors chose the *hbd* mice because these mice have a spontaneous mutation resulting in a hypochromic-microcytic anemia.[2] A previously published paper indicated that the reticulocytes of *hbd* mice showed defects in Tf uptake, and in Tfrc recycling.[3] Mapping and sequencing of the mutation identified a 24-amino-acid deletion in Sec15l1 (Sec15) as the culprit causing the anemia.[4,5] Sec15 is a subunit of the exocyst, an octameric protein complex that facilitates the tethering of recycling vesicles to the plasma membrane.[6] These studies indicate that the exocyst and proteins that interacted with the exocyst were candidate of interest.

Experiments in the present paper provide mechanistic insights as to how a guanine nucleotide exchange factor (GEF), Grab, interacts with the exocyst and affects recycling of Tfrc containing vesicles. Prior studies indicated that *Grab* was upregulated during erythroid differentiation.[7] The authors examine the consequences of erythropoietic cells deficient in Grab on the recycling of Tfrc containing vesicles to the plasma membrane. They use embryonic zebrafish to study the role of Tf-mediated uptake in the regulation of erythropoiesis. They also use erythroblasts from fetal mouse liver cells and 2 cell lines, MEL and K562, to study differentiation of erythropoietic cells. Immunoblots of Grab and Tfrc demonstrate upregulation of both proteins in erythropoietin-induced differentiation of erythroid progenitors of fetal mouse liver cells. In addition, Na butyrate–induced differentiation of K562 cells and dimethyl sulfoxide–induced differentiation of MEL cells recapitulate the upregulation of Grab. Other experiments show that *Grab* is predominantly expressed in the bone marrow. Knockdown of Grab in zebrafish embryos, MEL cells, and K562 cells decreases the hemoglobin and heme levels in these models of erythroid development and differentiation. The heme effect in Grab-deficient cells is directly related to decreased Tf-mediated iron accumulation in erythroblasts. The decrease in hemoglobin and heme levels