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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Gong et al, page 1652

β-Hemoglobinopathies lead the way

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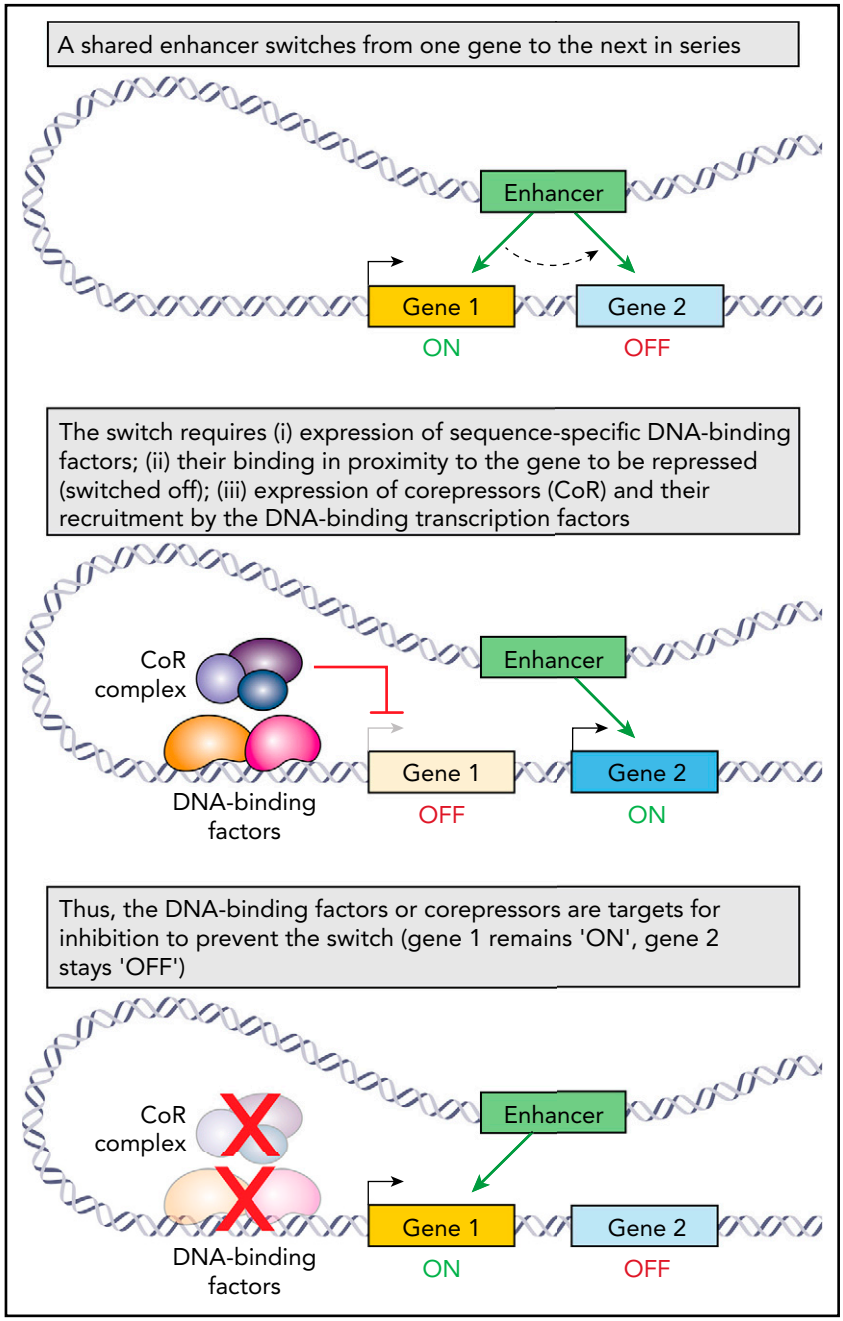
In this issue of *Blood*, guided by clinical observations and needs, Gong et al have identified a germline missense mutation in DNA methyltransferase 1 (*DNMT1*), a ubiquitously expressed key epigenetic regulator, as a cause of hereditary persistence of fetal hemoglobin (HPFH). HPFH protects against β-thalassemia and sickle cell disease (the β-hemoglobinopathies).[1] Discussed here is how these findings by Gong et al continue the pioneering role of the β-hemoglobinopathies as a model of discovery for all biomedicine. Sickle cell disease, after all, is the "first molecular disease": altered migration of sickle vs normal hemoglobin in gel electrophoresis demonstrated, for the first time, that the structure–chemical basis for disease is discoverable and knowable.[2]

The β-hemoglobinopathies become manifest after a switch during development from fetal to adult hemoglobin production. Thus, patients who continue to express high amounts of fetal hemoglobin after infancy receive protection. HPFH describes those individuals with particularly generous fetal hemoglobin amounts (>10%, compared with <1% usually) and correspondingly benign natural histories (reviewed in Thein[3]). Motivated by the goal of using pharmacology to mimic the salutary effects of HPFH, there have long been efforts to dissect the mechanisms involved with the developmental switch from fetal to adult whemoglobin production. The insights gained are fascinating and of general import (see figure). The fetal (*HBG2*, *HBG1*), adult (*HBB*), and other β-globin genes are arrayed together on chromosome 11 ("the β-globin locus"), ordered 5′ to 3′ in the order of their sequential activation by a shared distal (as much as ~20 kB distant) enhancer, "the locus control region," that activates each gene in turn.[4] In erythroid precursors before 7 months gestational age, this enhancer march from gene to gene stalls at *HBG2/HBG1*. Above this age, the march proceeds onward to *HBB*. Germline mutations linked with HPFH have assisted in elucidating the biochemical mechanics of enhancer switching between genes. For example, HPFH-linked point mutations at the locus of *BCL11A* on chromosome 2, which decreased its expression in the erythroid lineage, implicated this sequence-specific DNA binding factor in switching. In this tradition of discovery, Gong et al identified another HPFH-linked mutation, again not at the β-globin locus, but in *DNMT1* on chromosome 19. DNMT1 is famous as the maintenance methyltransferase, that during S-phase, recapitulates the DNA methylation marks (a repression or "off" mark) of the parental strand onto the newly synthesized DNA strand. Notably, DNMT1 is also a corepressor, a protein recruited by sequence-specific DNA binding factors, including BCL11A, to repress, instead of activate, target genes by acting as a platform for other corepressor enzymes and possibly by methylating DNA de novo (reviewed in Gong et al and Molokie et al[5]).

Several facets of this discovery are worthy of attention and discussion. (1) The discovery was not from population-based genome-wide association study. Rather, the investigators used next generation sequencing, complemented by Sanger sequencing as needed, to examine exons and presumed regulatory regions of a shortlist of 49 genes in 1142 β-thalassemia patients. These 49 genes were already implicated in regulation of the β-globin locus. This approach increased their power to identify rare variants in these candidate genes that might explain some instances of HPFH (~50% of fetal hemoglobin variation in adult humanity remains unexplained[3]). They found the *DNMT1* mutation in 3 of the 1142 patients, all 3 of whom did have substantially elevated fetal hemoglobin levels of between ~32% and 50%. (2) The mutation they discovered is missense, c.2633G>A, S878F. That is, a nucleophilic serine is substituted with an aromatic phenylalanine; the substitution occurs in a bromo-adjacent homology domain of DNMT1 (not in the methyltransferase catalytic domain), which does influence DNMT1 protein folding in structural studies (other functions are unknown) (reviewed in Jeltsch and Jurkowska[6]). The authors moreover showed that the substituted serine can be phosphorylated, a posttranslational modification they correlated with increased DNMT1 protein stability; the substitution hence correlated with decreased protein stability. They also found decreased interactions of mutated DNMT1 S878F with BCL11A and other proteins. (3) Better natural histories of β-hemoglobinopathy patients with co-inherited HPFH implies that the phenotypic consequences of HPFH mutations, present in every cell of the body, are restricted to the erythroid lineage. Accordingly, HPFH-linked *BCL11A* mutations are not in *BCL11A* protein-coding regions but in regulatory elements that drive erythroid-specific gene activation.[7] HPFH-linked *KLF1* mutations do disrupt the DNA-binding domain of this key transcription factor, but KLF1 expression and function is inherently restricted to the hematopoietic lineage (reviewed in Borg et al[8]). How then to explain HPFH-linked mutation in *DNMT1*, ubiquitously expressed and with fundamental functions as the maintenance methyltransferase and as a corepressor? One possibility (the only possibility?) is that S878 phosphorylation



Bedside-to-bench investigation in the β-hemoglobinopathies has pioneered insights into fundamental mechanisms in biology (eg, coordinated, consecutive activation of a gene series). These discoveries have opened the door to rational, noncytotoxic methods of manipulating cell fates and functions (eg, by inhibiting corepressors) for the therapy mission in the β-hemoglobinopathies and beyond.

is an erythroid-lineage-specific post-translation modification, explaining the selection for *DNMT1* c.2633G>A, S878F in β-thalassemia pedigrees. Certainly, the logical next step is to investigate DNMT1 S878 phosphorylation further. (4) This discovery supports a model in which switching of a shared enhancer from 1 gene to another, likely a generalizable motif for regulated consecutive expression of gene series, requires that the presently activated gene is silenced first[3] (see figure). (5) Finally, the discovery aligns with active clinical and preclinical efforts using small molecules that inhibit/deplete DNMT1 to upregulate fetal hemoglobin.[5,9] The discovery also implicates the kinase-mediating phosphorylation of DNMT1 S878 as a candidate for inhibition as a potential new avenue to pharmacologic recapitulation of HPFH.

In sum, this discovery indicates that posttranslational modification of a key corepressor, by a kinase-based pathway that is amenable to sensitive command and control, is a method by which consecutive activation of a gene series is regulated. Highlighting the broader implications for biomedicine, clinical DNMT1-targeting is already approved for myeloid tumor therapy, is in evaluation for treatment of several other cancers, does prevent a switch from fetal to adult hemoglobin production in the erythroid lineage, and does affect master transcription factor "switching" to redirect lineage-fate trajectories (reviewed in Velcheti et al[10]). The β-hemoglobinopathies thus continue their tradition of revealing life's mechanisms for all biomedicine.

Conflict-of-interest disclosure: Y.S. has issued patents around tetrahydrouridine and decitabine, ISWI family inhibition, and cancer differentiation inducers, and has equity, consulting, and Board interest in EpiDestiny, which has licensed oral tetrahydrouridine-decitabine. ■

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THROMBOSIS AND HEMOSTASIS

Comment on Moik et al, page 1669

Checkpoint inhibitors and thrombosis: what's up?

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In this issue of *Blood*, Moik et al present data from a single-center retrospective analysis that explored both the incidence and clinical impact of thrombotic events during immune checkpoint inhibitor (ICI) therapy.[1]

A diagnosis of cancer has a major and immediate impact on the lives of patients, their families, and their friends. When the diagnosis is advanced or metastatic cancer, patients and health care providers have to face difficult treatment decisions to balance the potential (but not guaranteed) treatment benefits against the risks. The treatment regimen and intensity usually determine the side effects and the impact on quality of life, which is particularly important, because life expectancy is usually limited in this situation. With the development of modern anticancer therapies, survival of several cancer entities has dramatically improved over recent years.[2] Immune checkpoint inhibitors belong to this category of modern anticancer treatments: they impair the immune-escape mechanisms of cancer cells and allow for the immune system of the patient to help in fighting the spread of the disease.

Venous and arterial thromboembolism (VTE and ATE) are common complications of cancer[3] and represent, together with infections, the leading noncancer causes of death.[4] Thromboembolism contributes significantly to the excess mortality of patients with cancer.[5] As a consequence, extensive research has been performed to predict and prevent VTE in high-risk populations and to improve treatment in patients with cancer.[6] Modern anticancer strategies should pay attention to the risk of thromboembolism. Surprisingly, the risk of thromboembolism from ICI therapy has not been systematically studied, which is especially concerning, given that the immune system is interlinked with many other physiological processes, including the hemostatic system. Currently available evidence on thromboembolic risks of ICI treatment is limited to small cohort studies and case reports, sometimes describing extreme hemostatic responses to ICI treatments, such as disseminated intravascular coagulation or hyperfibrinolysis.[7-9]

Moik et al report a cohort of 672 patients treated with nivolumab, pembrolizumab, ipilimumab, atezolizumab, or avelumab with a median follow-up of 8 months. All patients were treated at a highly specialized cancer clinic, and nearly 15% were included in treatment studies, thus reducing the risk of unreported outcomes, despite the retrospective design of the analysis.

Baseline risks of thromboembolism and death were considerable in this cohort: median age 64 years, 13% with a history of VTE, and 10% with a history of ATE. Because of these risk factors, 16% of the patients received prolonged anticoagulant therapy, 20% were given antiplatelet therapy at baseline, 85% were in treatment for metastatic disease, and 15% were already in second- or third-line ICI therapy. Given this baseline, it may not come as a surprise that the cumulative incidence of VTE during ICI therapy was 13%, with an incidence of ATE of nearly 2%. Interestingly, rates of VTE were comparable among subgroups of tumor types and checkpoint inhibitors and across Khorana-score categories, indicating that this could be a class effect that was directly related to ICI therapy. Of 15 variables tested as potential VTE risk factors, only a history of VTE was found to be a significant independent risk factor (subdistribution hazard ratio, 3.7). In contrast, expected items such as age, cancer stage, body mass index, and expression of programmed death ligand 1 (PD-L1) on tumor cells were not associated with an increased risk of VTE. Baseline anticoagulation and antiplatelet therapy were not protective.

The authors also reported on the prognostic impact of VTE and ATE. The occurrence of VTE, but not of ATE, was associated with significantly shorter overall and progression-free survival. Furthermore, in patients developing VTE during ICI treatment, recurrent VTE and major bleeding were observed in 8.5% and 4.3%, respectively. VTE did not cause discontinuation of ICI therapy but led to a delay of the next treatment cycle of up to 3 weeks. In contrast, ATE led to ICI discontinuation in 1 patient and caused considerable treatment delays of up to 5 months in another 3 patients. The authors concluded that patients with cancer receiving ICI therapy are at high risk of thromboembolism, which would have a significant impact on their clinical course and prognosis.

First of all, these data clearly indicate that the phase 2 and 3 ICI trials should be reevaluated for safety, specifically concentrating on the risk of ATE and VTE. Moik et al and other groups correctly criticize the landmark clinical trial that did not include any information on the incidence of thromboembolism.[9] This deficiency clearly indicates an important knowledge gap. Observational studies can be regarded only as hypothesis generating, and dedicated risk assessments of modern treatments should be based on randomized controlled comparisons.

Second, issues of risk prediction and primary prevention should be evaluated. In the future, VTE risk prediction models should incorporate anticancer drugs with increased thromboembolic risks, thereby identifying more patients as candidates for thromboprophylaxis. This precaution could become especially important because better prevention of VTE reduces the need for anticoagulant therapy and could reduce the resultant bleeding risks.

Third, more research should explore the impact of adverse effects of ICI treatment