shown to result in increased activated Notch1 levels through alternative promoter usage in the *Notch1* locus.[6,7]

When transposon-induced mutations were initiated much later during T-cell development, however, Berquam-Vrieze and colleagues note very different mutation profiles in the CD4-Cre–dependent mouse strain: *Notch1* mutations were no longer significantly overrepresented. Instead, the tumors now predominantly harbored mutations of *Myc* and *Stat5b*, often in a mutually exclusive combination with additional CISs affecting either *Gfi1* or *Whsc1*, *Akt2*, *Jak1*, and/or *Sos1*. Inappropriate overexpression of Myc drives many cancers and has also been implicated in human T-ALL as a downstream target of Notch1.[8] Myc is also required for mouse T-ALL induced by up-regulation of the Wnt/β-catenin signaling pathway.[9]

The Lck-SB tumors, finally, segregated into 2 categories. In the one group, mutually exclusive *Gfi1* versus *Whsc1* mutations in combination with *Myc* and *Stat5b* were observed similar to CD4-SB tumors. In the other group, tumors carried *Notch1* and *Ikzf1* mutations similar to the Vav-SB model. This dichotomy might reflect a major change in cell state on completion of β-selection at the DN3 stage. Extensive chromatin reorganization associated with pre-TCR expression and signaling might result in very different genetic selection events becoming predominant. And a narrow window of time between Lck-Cre expression and β-selection might explain the emergence of these distinct subsets. It is also interesting in this regard that, to the best of our knowledge, the only 2 mouse models of T-ALL described to date that are Notch1-independent, conditional activation of β-catenin[9] and loss of Pten,[10] depend on TCR rearrangement, activate Myc, and can be induced after β-selection under the control of CD4Cre.

Interestingly, gene expression profiling of Vav-SB and CD4-SB tumors revealed an unexpected correlation between the CD4-SB model and human ETP-ALL and between the Vav-SB model and non-ETP (ie, more typical) T-ALL. In a second approach, unsupervised clustering of the 54 most differentially expressed genes between Vav-SB and CD4-SB also identified a gene set that was up-regulated in CD4-SB and ETP-ALL and a second that was up-regulated in Vav-SB and typical T-ALL. This important observation raises the possibility that ETP-ALL or at least a subset of ETP-ALL might originate from more differentiated cells than the ETP-like surface phenotype suggests. In fact, approximately 50% of the human ETP-ALL cases studied previously had rearrangements of TCR genes, likewise suggesting a more mature origin.[2]

In conclusion, this study by Berquam-Vrieze and colleagues confirms the notion that the biologic state of origin could be reflected in the genetic selection events observed in the resulting tumor. The study further demonstrates the power of the Sleeping Beauty transposon system to derive valuable new animal models that provide insights into the etiology of cancers less likely to be gained from gain or loss of function of single genes in mice and impossible to be gained in humans.

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

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● ● ● MYELOID NEOPLASIA

Comment on Li et al, page 4509

TET2: epigenetic safeguard for HSC

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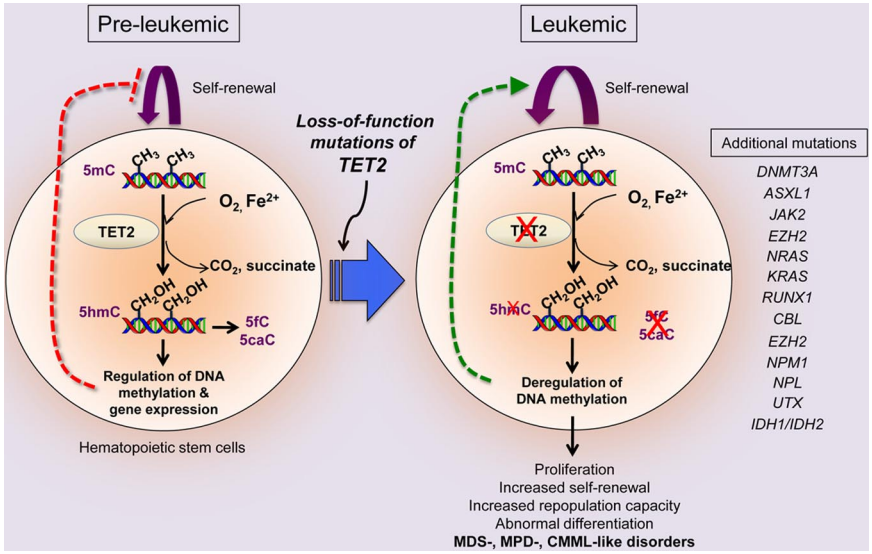
The TET-family enzymes TET1, TET2, and TET3 influence DNA methylation by modifying 5-methylcytosine.[1-3] Somatic loss-of-function mutations in *TET2* are frequently observed in myeloid neoplasms.[2,4] In this issue of *Blood*, Li et al demonstrate that ablation of *Tet2* alters the homeostasis and function of hematopoietic stem cells (HSCs) and induces leukemia in mice,[5] as also reported by other groups.[6-8]

TET1, TET2, and TET3, members of the TET family of Fe^{2+} and α-ketoglutarate–dependent dioxygenases, successively oxidise 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC; see figure).[1-3] 5caC can be excised by the base excision repair enzyme thymine-DNA glycosylase (TDG),[9] suggesting that TET proteins cooperate with TDG to effect DNA demethylation. Thus, TET proteins have the potential to be important regulators of epigenetic status in the cell types in which they are expressed.

TET2 is frequently mutated in myeloid malignancies. Microdeletions and copy number–neutral loss of heterozygosity (also called uniparental disomy) are recurrently observed in the *TET2* locus in diverse myeloid malignancies including myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), chronic myelomonocytic leukemia (CMML), and acute myeloid leukemia (AML). TET2 is expressed, and 5hmC is readily detectable, in hematopoietic stem/progenitor cells and mature blood subsets.[2,6] Moreover, leukemia-associated *TET2* mutations have been shown to impair the catalytic activity of TET2 and diminish 5hmC levels in cells from patients with MDS/MPN/CMML and AML, and shRNA-mediated depletion of TET2 in hematopoietic stem cells (HSCs) resulted in skewing toward myeloid differentiation.[2]



TET2 mutations in myeloid leukemogenesis. TET2 catalyzes the oxidation of 5mC to 5hmC, 5fC and 5caC in the genome and controls HSC self-renewal and function, presumably by regulating gene expression through effects on DNA methylation. *Tet2* deficiency in mice impairs 5mC hydroxylation and leads to skewed differentiation and enhanced self-renewal and repopulating capacity of HSCs, promoting malignant transformation to cause disorders resembling MDS, MPD, or MDS/MPD overlap syndromes including CMML. *TET2* mutations frequently coexist with other mutations in a wide spectrum of cancers including leukemias and lymphomas, suggesting that additional genetic alterations cooperate with *TET2* mutations in different phases of tumorigenesis such as tumor initiation and progression.

Together, these results suggested a causal relation between loss-of-function mutations in *TET2* and myeloid leukemias.

To address the function of TET2 in normal hematopoiesis and myeloid transformation, Li et al generated a gene-trap mouse strain in which a β-galactosidase-GFP cassette was inserted into exon 3 of the *Tet2* locus, disrupting the endogenous start codon and inducing premature termination of transcription.[5] They showed, as previously reported,[2,6] that *Tet2* was highly expressed in hematopoietic stem/progenitor cells and that ablation of *Tet2* diminished levels of genomic 5hmC. Intriguingly, $Tet2^{-/-}$ mice developed diverse myeloid malignancies: ~ 57% of deceased/moribund mice were found to have MDS with erythroid predominance and depletion of the hematopoietic stem/progenitor LSK ($Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$) population, and ~ 20% displayed MPD- or CMML-like phenotypes with enlarged LSK and granulocyte/macrophage precursor (GMP) populations as assessed by increased monocytosis, splenomegaly, hepatomegaly, elevated WBC counts, and bone marrow hypercellularity. Tumors arising in $Tet2^{-/-}$ mice with myeloid but not erythroid infiltration were highly transplantable. Consistent with the high frequency of heterozygous *TET2* mutations in myeloid malignancies in humans, $Tet2^{+/-}$ mice also developed MPD- or CMML-like leukemias. In preleukemic mice, *Tet2* deficiency increased the size of the LSK compartment in which HSCs are enriched, and conferred a significant advantage on these cells in repopulating hematopoietic lineages in a cell-autonomous manner. $Tet2^{-/-}$ LSK cells were highly proliferative, displayed enhanced cloning efficiency, and were biased to differentiate into monocyte lineage in vivo and in vitro.

The phenotypes described by Li et al are consistent with recent reports using other $Tet2^{-/-}$ mouse models, from three independent groups.[6-8] Moran-Crusio et al generated conditional *Tet2*-deficient mice targeting exon 3 in the *Tet2* locus.[6] Quivoron et al generated two different strains of *Tet2*-deficient mice: hypomorphic gene-trap mice in which β-galactosidase-neomycin was inserted into exon 9, and mice with a conditional deletion of exon 11.[7] Ko et al generated *Tet2*-disrupted mice in which exons 8-10 were targeted.[8] Exon 3 contains the start codon and exons 7-11 in the *Tet2* locus encode the double-stranded β-helix domain, a core region of the catalytic domain[1]; thus, all mice with disruption of the *Tet2* gene showed very similar loss-of-function phenotypes. *Tet2*-deficient LSK cells showed enhanced replating capacity but diminished differentiation in vitro. Loss of *Tet2* resulted in expansion of the HSC compartment in a cell-intrinsic manner and enhanced HSC self-renewal, thereby conferring a competitive advantage relative to wild-type HSCs for reconstitution into all hematopoietic lineages in vivo. Cells obtained from serial methylcellulose cultures showed gene expression profiles similar to GMPs and had uniformly high levels of c-Kit (CD117) expression. *Tet2* gene-trap or conditional exon 3–targeted mice were susceptible to CMML-like leukemia, and *Tet2* haploinsufficiency sufficed to alter HSC properties and induce myeloid malignancies. Notably, Quivoron et al observed impaired lymphopoiesis in *Tet2*-disrupted mice.[7] Furthermore, they observed rare somatic mutations of *TET2* in B-cell (~ 2%) and T-cell (~ 11.9%) lymphoma samples and these mutations seemed to originate from $CD34^{+}$ early progenitor cells with myeloid colony-forming potential, implicating *TET2* loss-of-function in lymphoid malignancies as well.

In addition to *TET2* loss-of-function mutations, many other recurrent mutations have been noted in MDS/MPN/CMML, some of which are presumed loss-of-function or possibly dominant interfering mutations (eg, *DNMT3A*, *ASXL1*, *EZH2*), whereas others are clearly neomorphic or gain-of-function (eg, *JAK2*, *NRAS* and *KRAS*, *IDH1*, and *IDH2*).[4] Collectively, these findings suggest that oxidation of 5mC controlled by Tet2 and upstream regulators results in pleiotropic changes that protect HSCs from aberrant expansion and myeloid transformation. However, a molecular relation between *TET2* and other mutations has been suggested only in the case of recurrent gain-of-function mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 and IDH2. Mutant IDH1/2 enzymes produce a novel "oncometabolite," 2-hydroxyglutarate, which competes with α-ketoglutarate to impair the catalytic activity of Fe^{2+}/α-ketoglutarate–dependent dioxygenases including TET2.[10] As a result, *IDH1/2* mutations are rarely found together with *TET2* mutations, but have similar effects as *TET2* mutations on HSCs.[10] Additional studies will be needed to clarify the mechanisms underlying the tumor-suppressor function of Tet2 in myeloid malignancies, as well as the mechanisms by which TET2 loss-of-function synergises with other mutations to promote malignant transformation.

How *TET2* mutations affect the epigenetic landscape is still unclear. Because 5hmC can potentially be an intermediate in both passive and active demethylation,[1,3,9] it is plausible that *TET2* loss-of-function mutations impair the consumption of 5mC, leading to accumulation of 5mC at certain genomic locations. Indeed, Figueroa et al reported a correlation between *TET2* mutations and DNA hypermethylation at HpaII sites in de novo AML.[10]

In contrast, Ko et al examined bone marrow samples from diverse myeloid malignancies and found that *TET2* mutations (and more generally, low 5hmC levels) were associated with DNA hypomethylation at most differentially methylated CpG sites.[2] This discrepancy may reflect differences in the cancer subtypes examined, the genomic CpG sites sampled, or the tools used for 5mC quantification and statistical analyses. It is noteworthy, however, that *DNMT3A* mutations are also associated with myeloid malignancies, and would also be predicted to be associated with DNA hypomethylation at certain CpG sites in the genome. Furthermore, 5hmC in embryonic stem cells was recently shown to be enriched in regions associated with specific chromatin modifications.[11] Because of these associations, and because DNA methylation at promoters is often associated with transcriptional repression, *TET2* mutations may alter gene expression programs that are critical for homeostasis and self-renewal of HSCs. Further studies will be necessary to resolve the epigenetic impact of *TET2* mutations on regulated expression of genes that control the function, self-renewal, and differentiation of HSCs.

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● ● ● RED CELLS & IRON

Comment on Fridkis-Hareli et al, page 4705

Thanks for the complement (inhibitor)

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Developing an effective inhibitor of the alternative pathway of complement (APC) with potential for safe clinical use is a daunting undertaking, but Fridkis-Hareli and colleagues were up for the challenge.[1]

The straightforward strategy of generating an antibody that inhibits complement C3, the protein that serves as the nidus for formation of the APC C3 convertase (see figure) is doomed from the beginning because C3 is present in high concentration in the plasma and has a rapid turnover rate that is enhanced by inflammation. Further, continuous depletion of C3 would be associated with a high degree of morbidity and mortality as the protein plays a central role in protection of humans against bacterial infection and immune complex disease.[2] To circumvent these intimidating obstacles, Fridkis-Hareli and colleagues used a cleverly designed chimeric, recombinant protein (TT30) that targets C3 selectively at the site of activation of the APC (see figure).

TT30 is composed of 2 functional domains derived from distinct complement proteins (see figure).[3] One component of the chimeric protein functions as the recognition subunit, and the other serves as the inhibitory subunit. The recognition subunit is derived from complement receptor type 2 (CR2). CR2 (CD21) is a cellular receptor for degradation products of C3 (iC3b and C3dg) that are generated at sites of complement activation (see figure). The inhibitory component of TT30 is a truncated version of complement factor H, the primary plasma inhibitor of the APC. Factor H binds to activated C3 (C3b) and both prevents binding of complement factor B and decay-accelerates the catalytic subunit (activated factor B) from the APC C3 convertase.[4] In addition, factor H acts as a cofactor for the degradation of C3b to inactive iC3b by complement factor I (see figure). The idea behind design of the chimeric protein was that the CR2 domain would localize binding to sites of APC activation, positioning the factor H component to bind to nascent C3b, thereby preventing APC amplification. A unique feature of C3 is that when activated, an internal thioester bond is exposed that allows the molecule to bind covalently to membrane glycoproteins through ester bond formation (see figure).[5] In this way, activated C3b becomes fixed to the surface of the structure (eg, the red cell membrane) on which complement has been activated. The very short half-life (milliseconds) of the exposed thioester bond restricts the diffusion capacity of activated C3, resulting in clustering of C3b around a C3 convertase. Such a process creates a favorable microenvironment for an inhibitor like TT30, as the molecule would be positioned to reach multiple molecules of C3b within the cluster.

Using a broad, rigorous, experimental design, Fridkis-Hareli and colleagues investigated the capacity of TT30 to inhibit specifically the APC.[1] Ex vivo studies demonstrated that TT30 was a potent inhibitor of the APC with activity observed in the nanamolar concentration range. Specificity was shown in ex vivo experiments in which the inhibitory capacity of TT30 for the APC was found to be approximately 100-fold greater than for the antibody-dependent classical pathway of complement (CPC).[5] Another set of ex vivo experiments produced the remarkable finding that the APC inhibitory activity of TT30 was 150-fold greater than that of factor H, the primary plasma regulator of the APC. This observation supports Fridkis-Hareli and colleagues' hypothesis that the potency of factor H would be enhanced by targeting the protein to the site of APC activation through the CR2 binding domain.

When incubated in normal human serum, rabbit erythrocytes undergo explosive spontaneous hemolysis because the sialic acid recognition site for factor H binding to C3b is deficient (see figure). Fridkis-Hareli et al used this system to demonstrate the capacity of