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mutation acquisition, chemotherapy exposure selects for driver mutations.

In the first mode of tMN development, chemotherapy selects for a CH clone by creating an environment that is permissive to the clonal expansion of a mutated precursor. Chemotherapy also facilitates the subsequent acquisition of complex genomic drivers. Compared to de novo AML and tMNs without the chemotherapy mutation signature, tMNs with chemotherapy-related signatures were enriched for copy number aberrations (CNAs), structural variants, templated insertions, chromoplexy, and chromothripsis. In 5 of 8 tMNs with chromothripsis, the *SMARCA4* gene was amplified. Overexpression of *SMARCA4* in Ba/F3 cells supports the possibility that it plays a role in leukemia cell proliferation and cytokine independence.

In the second mode of tMN development, the preexisting CH clone escapes chemotherapy exposure via apheresis and is reintroduced by autologous stem cell transplantation (ASCT). Although difficult to prove directly, this "escape" model is supported by 4 lines of evidence, as follows: the latency between chemotherapy exposure and tMN development did not influence penetrance of mutation signatures; patients sequentially treated with platinum and then melphalan/ASCT harbored only the platinum mutation signature; postmelphalan/ASCT B-ALL samples did not harbor the melphalan signature; and tumors that did not have a route of escape from melphalan did harbor its signature. Furthermore, targeted sequencing of pre-melphalan blood cells or apheresis samples revealed preleukemic mutations in 8 of 11 patients, including one case with an antecedent *TP53*-mutated CH clone in the apheresis product.

One of the most innovative aspects of the study is the use of mutation signatures as temporal barcodes that permit dating of chromosomal gains relative to a discrete mutagenic exposure in a patient's lifetime. If gain of a chemotherapy-related mutation occurs along with a chromosomal gain, then the chemotherapy exposure preceded the chromosomal gain and vice versa. In all 8 tMN cases with chemotherapy signatures (first mode) that were amenable to temporal barcoding using this method, melphalan or platinum signatures were found within chromosomal gains, demonstrating that chemotherapy exposure preceded large CNAs. In addition to the prevalence of CNAs in tMNs with chemotherapy signatures, the authors found a significant enrichment of *TP53* mutations, compared to tMNs without chemotherapy signatures. This finding suggests that *TP53* loss may protect clonal precursor cells against chemotherapy exposure.

The authors extended temporal barcoding to 2 postplatinum MM cases, which are known to acquire chromosomal gains early.[9] They used the age-associated SBS5 mutation signature to time events leading up to MM. The authors inferred that multichromosomal gains emerged in the second decade of life and that a most recent common ancestor cell emerged prior to the primary malignancy (ie, solid tumor) and the associated platinum exposure. Although the number of patients analyzed here is limited, these observations support the use of mutation signatures to time genetic events and reconstruct clonal dynamics of blood cells.

In conclusion, Diamond et al decipher how chemotherapy can serve as a double-edged sword. Although the sparsity of direct chemotherapy-induced driver mutations may be a relief, chemotherapy exposure selects for *TP53*-mutated CH and facilitates subsequent clonal evolution. A worthwhile future undertaking is to clarify mechanisms by which chemotherapy facilitates later acquisition of complex genetics. Furthermore, the length of time over which evolutionary trajectories can be traced warrants deeper investigation into the clinical utility of monitoring genetic lesions to predict the risk of progression to disease. Finally, this work demonstrates the impact that can be achieved by pairing clinical insight with computational genomics.

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

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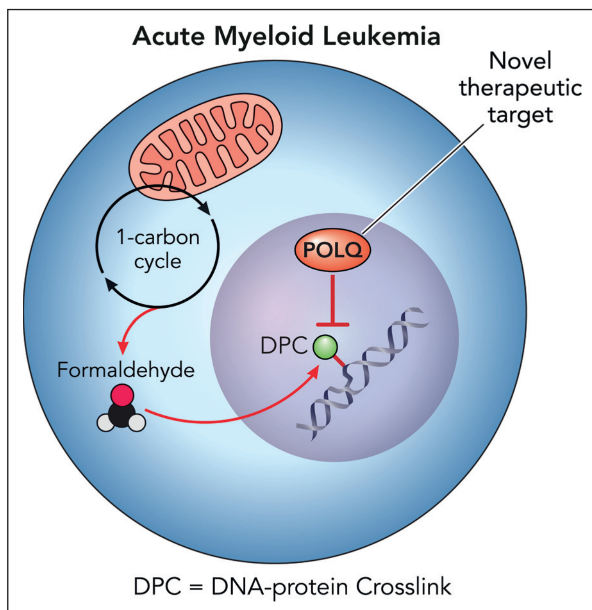
MYELOID NEOPLASIA

Comment on Vekariya et al, page 2372

Targeting leukemia with a metabolic genotoxin

Meng Wang | Cornell University

In this issue of *Blood*, Vekariya et al[1] find increased DNA damage in acute myeloid leukemia (AML) arising from an elevated burden of a metabolic genotoxin, formaldehyde. To protect itself, AML cells rely on DNA repair mediated by DNA polymerase θ (POLQ), which can be targeted as a novel therapeutic strategy against AML (see figure).



Increased 1-carbon cycle turnover in AML results in elevated production of genotoxic formaldehyde. AML upregulates POLQ to repair formaldehyde-DNA-protein cross-links (DPCs). Deficiency of POLQ DNA repair in AML results in accumulation of DPCs and resultant cytotoxicity. Inhibition of POLQ presents an opportunity to harness endogenous formaldehyde as a novel therapeutic strategy against AML. Professional illustration by Patrick Lane, ScEYEnce Studios.

In 1946, Goodman et al[2] showed that the nitrogen mustard compound used in poison gas was an effective treatment for blood cancers. Nitrogen mustard is a potent genotoxin through alkylation of DNA, thus inhibiting DNA replication and limiting the growth of highly proliferative cells. Almost 80 years later, genotoxic chemotherapy remains the backbone of cancer treatment, proving that DNA damage is a reliable mechanism to kill cancer cells. However, the adverse effects are considerable because of the lack of specificity against normal proliferative tissue. AML therapy is no exception, with genotoxic daunorubicin and cytarabine remaining front-line therapy for many patients. To develop more cancer-specific genotoxic treatments, we must better understand how DNA repair operates in cancer cells and target cancer cells' vulnerability to specific types of DNA damage.

Both normal and cancer cells rely on complex and diverse DNA repair pathways to protect their DNA against the constant attack by reactive chemicals found in the environment and within the body.[3] Of these reactive genotoxins, the most fundamental are oxygen and water, essential molecules for life. Another class of reactive genotoxins produced in the body are aldehydes, which can attack DNA to produce a range of toxic adducts and cross-links. Mammals produce toxic levels of a particular aldehyde called formaldehyde[4] and rely on detoxification by acetaldehyde dehydrogenase 2 (ALDH2) and alcohol dehydrogenase 5 (ADH5) enzymes. Furthermore, DNA repair mechanisms such as the Fanconi anemia (FA) pathway are necessary to prevent accumulation of toxic formaldehyde-DNA lesions.[5] In humans with an inborn deficiency in ALDH2 and ADH5, the increased formaldehyde-DNA damage in blood cells results in bone marrow failure and increased frequency of leukemia. Given the ubiquitous nature of aldehydes in the body, important questions are whether these metabolic genotoxins and their respective DNA repair pathways are altered in cancer cells, and whether this could provide more specific anticancer treatments.

Vekariya et al provide proof in principle that metabolism-derived genotoxins, such as formaldehyde, can be therapeutically exploited. An important discovery is that leukemic cells harbor elevated levels of formaldehyde compared with normal blood cells. The excess formaldehyde burden in leukemic cells originates from their increased serine/1-carbon utilization. Restriction of 1-carbon metabolism decreased the formaldehyde level in leukemic cells. How do leukemic cells protect themselves from the cytotoxic formaldehyde? The authors find a key DNA repair enzyme, POLQ, is upregulated in leukemic cells to repair formaldehyde-derived DNA-protein adducts through microhomology-mediated end joining. Most strikingly, genetic and pharmacologic inhibition of POLQ resulted in cytotoxicity only in leukemic cells, while sparing normal blood stem cells. This finding was validated in xenograft models, where treatment of leukemia-engrafted mice with the POLQ inhibitor novobiocin and the FLT3 inhibitor quizartinib resulted in synergistic restriction of leukemic growth, translating to significantly prolonged survival.

This study advances the field through several discoveries. Although the 1-carbon cycle, along with other metabolic pathways, is able to produce formaldehyde,[5] it was not clear which pathway is the most significant contributor to total cellular formaldehyde. Vekariya et al now present compelling evidence supporting 1-carbon metabolism as a meaningful source of formaldehyde when upregulated in leukemic cells. This finding could potentially apply to many other cancer types. This study also shows POLQ to be important in cellular defense against endogenous formaldehyde-DNA lesions and adds to several mammalian DNA repair pathways known to protect against aldehyde-DNA damage, including the FA pathway, homologous recombination (HR), nonhomologous end joining, nucleotide excision repair, and SprT-like N-terminal domain nucleases.[5] Previous studies have demonstrated synthetic lethality in cancers deficient in both POLQ and HR,[6,7] thus raising the possibility that formaldehyde could be the common genotoxic threat defended by multiple DNA repair pathways, and a prevalent genotoxin in many human cancers. The selective vulnerability of AML cells to POLQ inhibition while sparing healthy blood cells is of particular interest. Several factors are likely to contribute to the AML-specific cytotoxicity. First, the elevated formaldehyde in AML cells compared with normal cells restricts the excess burden of aldehyde toxicity to leukemic cells. Second, in support of a previous study,[8] this study shows that in AML, the formaldehyde catabolism enzyme ALDH2 is downregulated. The reduced detoxification of formaldehyde likely contributes to the elevated

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formaldehyde in leukemic cells. Third, upregulation of POLQ in AML compared with normal blood cells indicates a functional requirement for POLQ to limit the cytotoxicity of formaldehyde genotoxicity.

This study provides needed data to address several important future questions. If formaldehyde is an Achilles heel in AML, can we further synergize leukemic cells to formaldehyde toxicity by targeting POLQ in combination with other formaldehyde protective pathways (eg, ALDH2 and ADH5 detoxification enzymes) and the FA DNA repair pathway? In addition, there are ≈500 million people worldwide, predominantly in East Asia, who carry a natural polymorphism in the *ALDH2* gene that inactivates enzymatic activity.[9] Could AMLs arising in these individuals be more sensitive to aldehyde-directed therapy, or conversely be more susceptible to toxic adverse effects because of the loss of formaldehyde catabolism in normal cells? Precision medicine could play an important factor in these treatment decisions. Extending beyond formaldehyde, are there other metabolic genotoxins that can be therapeutically harnessed for leukemia therapy, such as increased levels of malondialdehydes and fatty aldehydes reported in AML?[8,10] To fully explore this question, we will need to deploy sensitive assays to measure these reactive chemicals in patient samples, and test inhibition of protective pathways respective to these other aldehydes. Overall, the findings by Vekariya et al bring into focus a fundamental question regarding the physiological sources of DNA damage in cancer cells, and how such insight can translate into novel therapeutic strategies.

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THROMBOSIS AND HEMOSTASIS

Comment on Grover et al, *page 2390*

Hereditary angioedema and thrombosis

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In this issue of *Blood*, Grover et al report that deficiency of the plasma serpin C1 inhibitor (C1-INH) enhances thrombin generation in human plasma and venous thrombus growth in mice.[1] Last year this group presented epidemiologic evidence indicating patients with hereditary angioedema (HAE), most of whom have a deficiency of C1-INH, are at moderately increased risk for venous thromboembolism (VTE).[2] In the current study, they convincingly demonstrate that C1-INH deficiency is procoagulant in vitro and prothrombotic in a rodent model. However, the proposition that C1-INH deficiency significantly increases VTE risk in humans is likely to be controversial, as it runs counter to the clinical impression that patients with HAE, despite having recurrent episodes of soft-tissue edema due to hyperactivity of the plasma kallikrein-kinin system (KKS), rarely have thrombotic events.[3-5]

The KKS comprises the protease zymogens factor XII (FXII) and prekallikrein (PK) and the cofactor/substrate high molecular weight kininogen (HK).[3-5] In plasma, FXII and PK reciprocally convert each other to the proteases FXIIa and plasma kallikrein (PKa), respectively (see figure panel A).[6] PKa cleaves HK, releasing the vasoactive peptide bradykinin (see figure panel B), which binds to the G protein–coupled bradykinin B2 receptor, promoting vasodilatation and vascular permeability, among its several effects.[3-5] C1-INH is the main regulator of FXII/PK reciprocal activation. Patients with HAE typically have 5% to 35% of the normal C1-INH concentration in plasma,[3,5] rendering them susceptible to processes that accelerate FXII and PK activation. This leads to episodes of bradykinin-induced soft-tissue edema involving the face, oropharynx, hands, genitals, and/or gastrointestinal tract that may be life threatening.

Reciprocal FXII/PK activation is enhanced by a process called contact activation, which occurs when KKS proteins bind to certain macromolecules or "surfaces" (see figure panel C).[6] Bradykinin generation increases as a consequence of the increased PKa. A variety of organic (eg, nucleic acids, glycosaminoglycans) and inorganic (eg, polyphosphates, silicates) substances support contact activation in vitro.[5-7] High local levels of bradykinin, probably driven by contact activation, contribute to tissue edema at injury sites. It is assumed that a surface-driven process

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