CANCER THERAPY

Epigenetic Achilles' heel of AML

Mutations in genes encoding epigenetic modifiers are frequent in acute myelogenous leukemia (AML) and have been proposed to cause AML via activation of oncogenes and repression of tumor suppressors. Two studies now identify unexpected oncogenic mechanisms and therapeutic vulnerabilities in AML arising from mutations in genes encoding the epigenetic regulators DNMT3A and ASXL1.

Koki Ueda and Ulrich Steidl

Epigenetic dysregulation is a common feature found across cancer types. In particular, patients with myeloid malignancies such as acute myeloid leukemia (AML), those with myelodysplastic syndromes, and asymptomatic people with clonal expansion of 'pre-leukemic' precursor lesions (clonal hematopoiesis (CH)) frequently harbor mutations in genes encoding epigenetic regulators. Mutations of *DNMT3A* (which encodes a DNA methyltransferase) and *ASXL1* (which encodes a chromatin remodeler) are among the most common alterations in AML and typically display relatively high allele frequency[1,2]. The therapeutic targeting of such mutant clones has been proposed not only as a strategy for the treatment of AML but also as a potential 'precision prevention' strategy in people with CH[3,4]. However, in-depth understanding of the precise oncogenic mechanisms set in motion by such mutations, including those in *DNMT3A* and *ASXL1*, is a prerequisite for the clinical implementation of these paradigm-shifting approaches. Previous studies have largely postulated direct epigenetic effects caused by the assumed loss-of-function consequences of DNMT3A or ASXL1 mutants. In this issue of *Nature Cancer*, studies by Scheller et al.[5] and Wang et al.[6] report unexpected oncogenic functions of the products of the highly frequent *DNMT3A*-R882H and *ASXL1*-Y591*/fs mutations in AML, and provide potential targeted therapeutic strategies for cancers bearing either of these mutations.

DNMT3A is one of the DNA methyltransferases that newly methylates unmethylated DNA. As most of the mutations in *DNMT3A* occur in the region encoding the catalytic domain, they are usually considered loss-of-function mutations[7]. Moreover, the hot-spot mutation product of the *DNMT3A* mutation, DNMT3A-R882H, acts in a dominant negative way to inhibit DNA methylation by wild-type DNMT3A[8]. As the result, DNA methylation in hematopoietic stem cells (HSCs) with mutated *DNMT3A* is altered globally, including methylation of the *HOX* gene cluster and other HSC-associated signature genes, whose increased expression has been described among the major consequences of *DNMT3A* mutations in HSCs[7]. In general, oncogene de-repression has been recognized as a major alteration

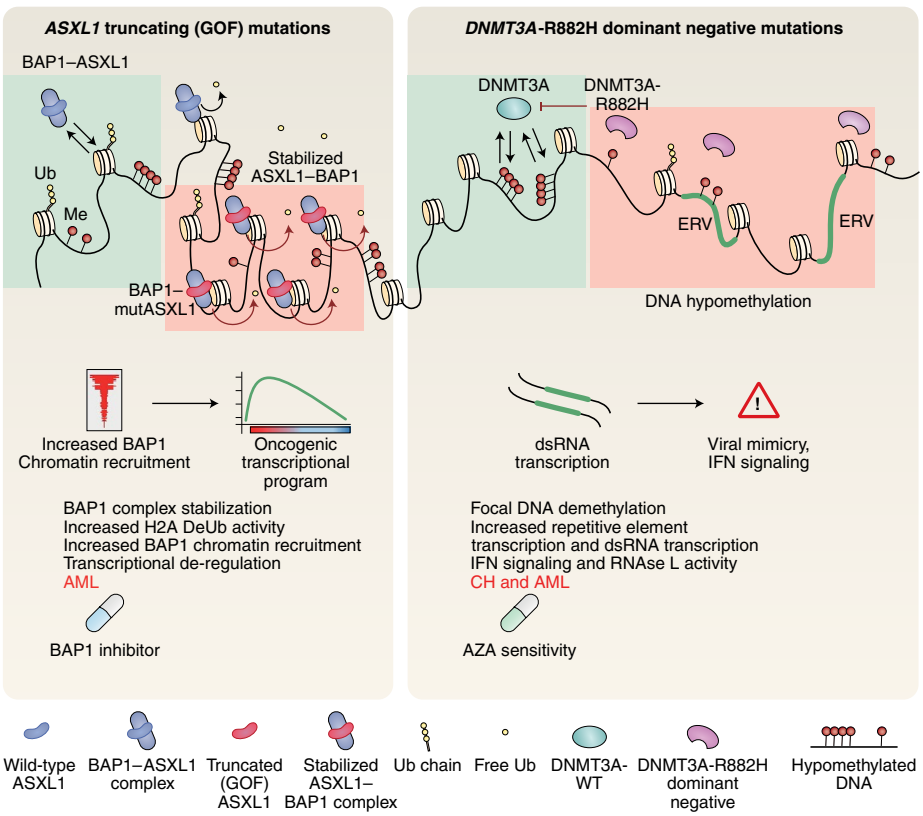

Fig. 1 | Novel oncogenic mechanisms of mutant epigenetic modifiers. Wang et al. described *ASXL1* mutations that resulted in increased stabilization of BAP1 and its recruitment to chromatin and the induction of an oncogenic transcriptional program[6]. This created a reliance on BAP1–ASXL1 signaling to sustain AML proliferation and thus posed a vulnerability to specific inhibitors of BAP1 in ASXL1-mutant AML. Scheller et al. showed that DNMT3A-R882H mutant acted in a dominant negative way to diminish DNMT3A-dependent methylation of CpG islands of endogenous retroviral lesions (ERVs)[5]; they found that AZA treatment further hypomethylated DNA (Me, methylated cytosine), which induced a subsequent increase in double-stranded RNA (dsRNA); this led to an interferon (IFN) response to activate Rnase L–mediated RNA degradation, which resulted in translational inhibition that led to apoptosis. GOF, gain of function; Ub, ubiquitin; mutASXL1, mutant ASXL1; DeUb, deubituitinase.

caused by mutated *DNMT3A*, although these findings alone cannot fully explain the leukemogenic effects of *DNMT3A* mutations.

Hypomethylating agents (HMAs) such as azacytidine (AZA) are used therapeutically in patients with AML or high-risk myelodysplastic syndrome who are ineligible for aggressive chemotherapy and/or stem-cell transplantation. HMA treatment has been shown to counteract the abnormal hypermethylation of genes encoding tumor suppressors, which eventually leads to anti-leukemic effects[9]. However, changes in the methylation status of these genes and the biological responses to HMA do not necessarily correlate[10]. This suggests that there might be alternative mechanisms that mediate the anti-leukemic effects of HMAs.

The study by Scheller et al. identifies novel mechanisms of specific susceptibility to AZA treatment in DNMT3A-R882H-mutant AML[5]. The authors first analyzed data from a clinical trial studying the addition of AZA to standard chemotherapy in patients with AML[11]. The initial purpose of the clinical trial had been to test whether adding AZA to standard chemotherapy with AraC and anthracycline enhanced the effects of chemotherapy, following the assumption that AZA upregulates genes encoding tumor suppressors via promoter hypomethylation, which leads to enhanced sensitivity to cytotoxic drugs. However, this trial failed to show overall clinical benefits. Through a subgroup analysis that took into account mutation status, the authors now found that the addition of AZA did prolong survival specifically in patients with mutated *DNMT3A*, while it had a negative impact on the survival of patients with wild-type *DNMT3A* (*DNMT3A*-WT). Through the use of xenotransplantation models in immunodeficient mice, the authors demonstrated that HSCs from patients with CH in DNMT3A-mutant contexts were highly sensitive to AZA monotherapy. These unexpected findings suggest that the hypomethylation targets of AZA might be context dependent, and that specific targets in cells with mutated *DNMT3A* could be mediating the increased sensitivity to AZA. To delineate the mechanisms of the increased sensitivity to AZA in DNMT3A-mutant cells specifically, the authors generated a genetically engineered mouse model carrying the human *DNMT3A*-R882H mutation. Their model readily recapitulated the features of human CH, similar to other genetically engineered mouse models of mutant DNMT3A, and showed that administration of AZA, with or without additional AraC, specifically eradicated DNMT3A-R882H-mutant cells in competitive transplantation models. Leveraging that model, Scheller et al. set out to study the mechanism of how AZA sensitizes cells with mutated *DNMT3A* to chemotherapy[5]. First, they used whole-genome bisulfite sequencing to analyze the genome-wide methylation changes in mouse HSCs expressing *DNMT3A*-WT or *DNMT3A*-R882H that were treated with AZA or saline in vivo. Both *DNMT3A*-R882H and AZA focally increased differentially methylated regions (DMRs), although some of the DMRs were more substantially hypomethylated in cells expressing *DNMT3A*-R882H than in those expressing *DNMT3A*-WT, and these were further hypomethylated after AZA treatment. Regions showing this methylation pattern showed enrichment for retrotransposons such as LTR-ERV1, LTR-ERVK, LTR-ERVL-MALR and LINE-L1, as well as pro-inflammatory signaling gene sets such as genes encoding molecules involved in DNA repair, in the response to interferon-α and interferon-γ, and in signaling via IL-2–Stat5. The authors demonstrated upregulation of these genes by RNA sequencing, and also confirmed hypomethylation of similar regions in human AML, as well as in colorectal cancer cells treated with AZA. Hypomethylation of endogenous retroviral regions has previously been reported to result in the production of double-stranded RNA[12,13], and Scheller at al. indeed found an increase in double-stranded RNA molecules in DNMT3A-R882H bone marrow cells, many of which encoded anti-viral and pro-inflammatory products[5]. This series of reactions is considered a mimicry of viral infection, which has been reported to induce antitumor effects[12,13]. The authors confirmed this mimicry response in additional mouse and human samples.

To further substantiate their findings functionally, Scheller at al. assessed the oligoadenlyate synthetase–RNase L pathway[5], which induces arrest of protein synthesis in response to interferon signaling. They found that this pathway was upregulated after AZA treatment, and that this lasted more than 10 days in *DNMT3A*-R882H cells, whereas protein synthesis was rapidly restored in cells expressing *DNMT3A*-WT.

These findings strongly suggest that the effect of AZA depends on the DNA methylation status before treatment. Even though AZA hypomethylates endogenous retroviral regions and genes encoding pro-inflammatory molecules and enhances their expression, as previously reported[14], the findings of Scheller et al.[5] suggest that AZA alone is insufficient, and that viral-infection mimicry primed by mutations in *DNMT3A* is needed to induce robust antitumor effects through further methylation by AZA (Fig. 1).

Another frequently mutated gene encoding an epigenetic modifier in AML, as well as in healthy people with CH, is *ASXL1*. ASXL1 is an interaction partner of the polycomb repressive complex PRC2, which downregulates various genes such as the *HOXA* cluster, and also one of the main components of the BAP1 complex, which activates various pathways through deubiquitination by histone H2Ak119. Thus, ASXL1 modulates both gene suppression and gene activation, and wild-type ASXL1 is crucial for normal HSC maintainance[15]. Numerous studies have focused on the abnormal functions of mutant ASXL1. Variant forms of ASXL1 with C-terminal truncation, which are the protein products of the majority of *ASXL1* mutations, interact with both PRC2 and BAP1–PRC1[15]. These aberrant interactions are considered a key contributor to the myeloid transformation of HSCs; however, the precise molecular epigenetic changes induced by mutant ASXL1 have not been fully elucidated.

Wang et al. demonstrate that the common *ASXL1* mutations *ASXL1*-Y591* and *ASXL1*-Y591fs encode a truncated ASXL1 protein that is more stable than wild-type ASXL1[6]. *ASXL1*-Y591*/fs was originally reported as a loss-of-function type of mutation, and was shown to alter trimethylation of histone H3K27 by PRC2[16]. Wang et al. found that a prominent feature of cells carrying the *ASXL1*-Y591*/fs mutation was a substantial alteration in the targets of BAP1[6]. Mechanistically, they found that truncated ASXL1 bound to and stabilized BAP1 better than wild-type ASXL1 did, which would suggest that the E3 ligase activity of wild-type ASXL1 (which degrades BAP1) is lost in the truncated ASXL1. *ASXL1* mutations and stabilized BAP1 led to the upregulation of genes encoding regulators of metabolism and development (Fig. 1). Although *BAP1* mutations are frequent in solid tumors such as renal carcinoma and have been shown to upregulate the transcription of oncogenes, *BAP1* is rarely mutated in AML[15]. The findings of Wang et al.[6] suggest that *ASXL1* mutations serve a role in leukemia analogous to that of *BAP1* mutations in solid tumors, and that they induce similar cellular and transcriptional responses.

In addition, Wang et al. performed a systematic small-molecule screen and identified a potent inhibitor of BAP1 (iBAP)[6]. iBAP inhibited the deubiquitinase activity of BAP1 with reasonable specificity. As predicted, AML cell lines expressing mutated *ASXL1* were more sensitive to iBAP

treatment than were cell lines expressing wild-type *ASXL1*. The authors further confirmed the efficacy and specificity of iBAP at a molecular level and found that iBAP reversed the expression of ASXL1–BAP1 target genes, such as the *HOXA* cluster, that are upregulated by mutant ASXL1. Finally, Wang et al. tested their new compound in xenograft models of an AML cell line and patient-derived cells expressing mutated *ASXL1* and demonstrated that iBAP treatment prolonged the survival of recipient mice without substantial toxicity[6].

In summary, the work by Scheller et al.[5] and Wang et al.[6] has identified novel mechanisms and functions of mutant DNMT3A and mutant ASXL1, respectively, products of two of the most frequently mutated genes in both myeloid malignancies as well as healthy people with CH. Their results highlight the finding that mutations in genes encoding epigenetic modifiers not only lead to alterations of their canonical targets but also result in broader changes to the epigenetic status of cells, which can in turn provide new entry points for targeted therapy. Hypomethylation of endogenous retroviral regions by DNMT3A-R882H sensitized cells to further hypomethylation by AZA, and stabilization of BAP1 by ASXL1-Y591*/fs sensitized cells to catalytic inhibition of BAP1. Both mutations frequently occur early in disease pathogenesis and are considered 'ancestral' mutations and thus continue to be shared by numerous subclones that appear later during the course of the disease. Thus, targeting specific molecular vulnerabilities caused by mutations in *DNMT3A* or *ASXL1* could potentially lead to more-specific and therefore less-toxic targeting of disease-driving clones and could also lead to longer-lasting remissions in patients with AML. These advances also further propel the provocative idea of possible therapeutic strategies of 'precision prevention' in people with DNMT3A- or ASXL1-mutant CH. In this context, the potential side effects of the treatment constitute an important caveat to balancing its efficacy in the prevention setting. For example, the sustained pro-inflammatory reaction triggered by combination of DNMT3A-R882H and AZA may induce new oncogenic alterations, in line with data showing that chronic inflammation can alter HSC function and aid malignant transformation[17]. Likewise, the pharmacological properties of iBAP need to be developed further for comprehensive assessment of potential toxicities and improvement in therapeutic benefits. These aspects will be addressed in future studies. The identification and study of novel epigenetic mechanisms in the pathogenesis of leukemia and in the context of drug treatment, such as the advances presented here by Scheller et al.[5] and Wang et al.[6], will undoubtedly be instrumental in the quest toward fundamental improvements in clinical outcomes, and potentially in prevention settings for high-risk patients too, of the most aggressive hematological malignancies. ❐

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Competing interests

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