Choreography of methylation: KDM4C histone demethylation enables ALKBH5 m6A RNA demethylation to sustain AML stem cells

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Acute myeloid leukemia (AML) arises when hematopoietic stem or progenitor cells accumulate sufficient cancer driver events to confer a transformed phenotype, resulting in an accumulation of myeloid blast cells in the bone marrow and periphery. Prognostic stratification of AML based on karyotypic and driver mutation load has become increasingly accurate in predicting risk of relapse.[1] In therapeutic design, the utility of genomic structure and mutation data is beginning to inform the selection of targeted agents for use during induction and consolidation therapy regimens, but the number of approved drugs is far from complete when compared to the catalog of AML genomic driver events.[2,3] In parallel to efforts to identify new targeted therapeutics in AML, investigation to identify shared oncogenic properties of AMLs that are not necessarily exclusive to particular driver events may yield novel therapeutic opportunities.

One shared property in AMLs is the manifestation of leukemia stem cell (LSC) populations. LSCs contribute to leukemogenesis and therapy resistance in AML, and targeting LSC populations is thought to be critical for inducing long-term remission. LSC populations are characterized by the ability to sustain self-renewal through asymmetric cell division, coupled with oxidative metabolism that depends at least in part on the apoptosis resistance protein Bcl2.[4] Each of these cellular programs is potentially targetable to eliminate LSCs, as recently reviewed by Vetrie et al.[5]

A key question is how could AMLs arising due to heterogenous chromosomal and gene mutation driver events acquire the shared cellular functions that sustain LSCs? Studying multiple forms of AML, Wang et al and Shen et al have reported in complementary studies in *Cell Stem Cell* that AMLs collectively experience an increase in the expression of AlkB homolog 5 (ALKBH5).[6,7]

ALKBH5 catalyzes the removal of the methyl group from 6-methyl-adenosine (m6A), a modification that regulates multiple aspects of the global transcriptome, including mRNA stability and Splicing.[8] ALKBH5 together with the fat mass and obesity-associated protein (FTO) are known as m6A "erasers," reflecting their activity in catalyzing the removal of m6A marks from mRNAs. Additional enzymes known as m6A "writers" and "readers" collaborate with the erasers to regulate fundamental cellular programs.[9] In several cancers, the eraser FTO has been shown to be required for stem cell maintenance and oncogenesis, including in AML.[10,11] Building on these previous studies, new data from Wang et al and Shen et al indicate that among the enzymes known to regulate m6A, ALKBH5 expression was consistently increased in AML.[6,7]

Examining genomic methylation patterns, Wang et al found that chromatin opening associated with AML is frequently observed at the ALKBH5 locus. In AML cells, histone 3 lysine 9 (H3K9) demethylation of the ALKBH5 locus required the activity of KDM4C, and ectopic expression of ALKBH5 in KDM4C-deficient cells was sufficient to restore colony formation in AML cells, establishing a key role for ALKBH5 in mediating the leukemogenic functions downstream of KDM4C. ALKBH5 expression was elevated specifically in LSCs compared to nontransformed hematopoietic populations, and hematopoiesis of non-transformed cells was not significantly impaired in competitive transplantation and colony formation assays. Importantly, Shen et al showed a requirement for ALKBH5 in serial replating assays using AML mouse models driven by MLL-AF9, MLL-AF10, AML1-ETO, and FLT3-ITD/NPM1 mutant. Altogether the data establish a broad functional requirement for ALKBH5 in leukemic cells but not for normal hematopoiesis. Consistent with increased expression in LSCs, ALKBH5 expression was positively associated with a significantly increased frequency of relapse in AML patients.

Analysis of total RNA abundance and m6A fluctuations in response to ALKBH5 inactivation across the transcriptome, both groups identified gene response patterns indicative of alterations in signal transduction, metabolism, and apoptosis control. Wang et al uncovered intriguing links between ALKBH5 activity and mRNAs coding for the PSAT1 and PHGHD enzymes that mediate serine biosynthesis from glycolytic precursors. In ALKBH5-deficient leukemia cells, re-expression of PHGDH partially restored clonogenic potential. The partial effect of PHGDH restoration on clonogenicity may simply indicate a need for coordinated restoration of PSAT1, which functions downstream of PHGDH in serine biosynthesis. Serine metabolism can contribute to oncogenesis through many potential metabolic fates, including the production of phospholipids, purine nucleotides, and proteins.[12] Further key experiments using metabolic complementation and tracing will be needed to definitively establish the functional role of serine metabolism in AML LSCs.

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Shen et al focused on TACC3 as its expression was prognostic for relapse in AML, consistent with the use of ALKBH5 as a prognostic marker. Indeed, ALKBH5 knockdown decreased TACC3 mRNA levels, TACC3 deficiency induced AML apoptosis, and TACC3 reexpression partially restored AML growth in ALKBH5-deficient cells. A recurring oncogenic fusion gene involving TACC3 (FGFR3-TACC3) has been identified to mediate increased mitochondrial biogenesis and oxidative phosphorylation in glioblastoma.[13] It will be interesting to determine whether the increased TACC3 expression found by Shen et al in AML drives similar increases in mitochondrial oxidative phosphorylation, sustaining LSC populations as previously established.[4]

Finally, among the ALKBH5-regulated mRNAs, Wang et al found that increased mRNA expression of the receptor tyrosine kinase AXL in AML is dependent on the activity of ALKBH5, and that reexpression of AXL in cells lacking ALKBH5 is sufficient to partially restore leukemia cell clonogenicity, which is indicative of LSC function. AXL, a member of the TYRO3-AXL-MERTK receptor tyrosine kinase subgroup (TAM-RTKs) that was initially cloned from a chronic myelogenous leukmia cell line, can participate in both oncogenesis and therapy resistance in leukemia, glioblastoma, and other tumors.[14] Importantly, the FLT3 inhibitor gilteritinib that was recently approved for treatment of FLT3-mutant AMLs is also an effective inhibitor of AXL.[15,16] Thus the present findings indicate a broader potential application of gilteritinib and other AXL-inhibitors in AML.

Overall the reports from Shen et al and Wang et al support a leukemia-specific function of ALKBH5 in maintenance of AML stem cells through the activation of oncogenic metabolism and signaling. Therapeutic opportunities to counteract the oncogenic function of ALKBH5-supported pathways include inhibitors of KDM4C histone demethylation, ALKBH5 mRNA demethylation, PHGDH and PSAT1 biosynthesis of serine, mitochondrial function, and AXL tyrosine kinase activity. Investigating epigenomic and transcriptomic methylation in AML, the reports by Wang et al and Shen et al have revealed multiple new strategies for reducing LSC burden and the risk of relapse in AML.

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