Check for updates

kinase-positive anaplastic large cell lymphoma. *Haematologica*. 2018;103(3):477-485.

8. Crescenzo R, Abate F, Lasorsa E, et al; European T-Cell Lymphoma Study Group, T-Cell Project: Prospective Collection of Data in Patients with Peripheral T-Cell Lymphoma and the AIRC 5xMille Consortium "Genetics-Driven Targeted Management of Lymphoid Malignancies". Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell*. 2015;27(4):516-532.

9. Luchtel RA, Dasari S, Oishi N, et al. Molecular profiling reveals immunogenic cues in anaplastic large cell lymphomas with *DUSP22* rearrangements. *Blood*. 2018;132(13):1386-1398.

DOI 10.1182/blood.2020007226

© 2020 by The American Society of Hematology

MYELOID NEOPLASIA

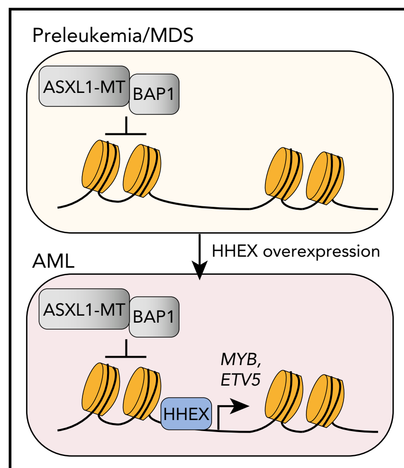
Comment on Takeda et al, page 1670

HHEX expression drives AML development

Daniel T. Starczynowski | Cincinnati Children's Hospital Medical Center

In this issue of *Blood*, Takeda and colleagues[1] reveal the importance of hematopoietically expressed homeobox (HHEX) in promoting acute myeloid leukemia (AML) transformation in cooperation with mutant additional sex combs-like 1 (ASXL1) and identify MYB and ETV5 as critical transcriptional targets shared by HHEX and mutant ASXL1.

AML results in the accumulation of immature clonal myeloid cells, driven by acquired genetic mutations in hematopoietic stem and progenitor cells (HSPCs). The largest class of recurrent AML gene mutations affect epigenetic modifiers, including DNMT3A, IDH1/2, TET2, EZH2, MLL/KMT2A, and ASXL1, which are often found as early initiating events.[2] Although mutations in any one of these epigenetic-modifying genes result in significant changes in the epigenetic landscape and gene expression in HSPCs, they are often insufficient to result in overt transformation to leukemia, suggesting that additional genetic and/or molecular changes are required for AML. For example, the cooccurrence of mutant DMNT3A and FLT3-ITD is associated with an unfavorable prognosis in patients and results in rapid and penetrant AML in mouse models.[3-5] In other cases, an initiating genetic mutation in the context of an altered cellular state coinciding with changes in the expression of critical genes is sufficient for leukemic cell transformation. Although frequently cooccurring gene mutations have been extensively studied in AML, much less is known about the relevant altered gene expression changes that cooperate with leukemia-initiating gene mutations.



ASXL1 mutant protein (ASXL1-MT) is associated with preleukemic states, including MDS. However, cooperating genetic and/or molecular changes are required for progression of ASXL1-MT–expressing preleukemic cells to AML. HHEX, a homeobox-containing DNA-binding protein, is overexpressed in AML and cooperates with ASXL1-MT to induce AML. HHEX mediates leukemia cell transformation by cooperating with ASXL1-MT to induce expression of MYB and ETV5, which are critical target genes for AML cell viability and prevention of differentiation.

ASXL1 is one of the most frequently mutated genes in myeloid malignancies, occurring in 5% to 11% of patients with AML. ASXL1 belongs to a family of chromatin-binding Polycomb proteins and is involved in controlling gene expression by interacting with epigenetic regulators, such as Polycomb repressive complex 2 (PRC2) and BAP1. Interaction of ASXL1 and BAP1 at promoters results in monoubiquitination of histone H2A at lysine 119 (H2AK119ub), catalyzed by PRC1 complexes, and subsequent repression of target gene expression. The majority of ASXL1 mutations are frameshift or nonsense mutations that disrupt the C-terminal plant homeodomain finger region resulting in C-terminally truncated ASXL1 mutant proteins (referred herein as ASXL1-MT). ASXL1-MT retains its ability to interact with several of its epigenetic binding partners and induces aberrant histone modifications in a dominant-negative or gain-of-function manner. Thus, these epigenetic changes induced by ASXL1-MT are thought to contribute to the development of myeloid neoplasms.

The contribution of ASXL1 mutations has been illuminated by analysis of ASXL1-MT knockin (ASXL1-MT-KI) mice, which develop mild anemia, impaired erythroid differentiation, myeloid skewing, and dysplasia.[6] Although these hematologic changes are indicative of early-stage myelodysplastic syndromes (MDS), the ASXL1-MT-KI mice never develop overt leukemia, suggesting that additional elements are required for myeloid transformation of ASXL1-mutated cells. To identify cooperating molecular events with ASXL1-MT, the team performed a retrovirus-mediated insertional mutagenesis screen and identified *HHEX* as a candidate that may cooperate with ASXL1-MT to induce myeloid leukemia.

HHEX is a homeobox-containing transcriptional repressor that is overexpressed in various subtypes of AML, including those with ASXL1 mutations.[7] In the current study, the team investigated the potential cooperation between ASXL1-MT and HHEX overexpression in myeloid leukemogenesis. Utilizing retroviral models, overexpression of HHEX expanded ASXL1-MT–expressing HSPCs by preventing apoptosis and blocking differentiation. However, overexpression of HHEX had nominal effects on normal HSPCs. Importantly, overexpression of HHEX in ASXL1-MT HSPCs accelerated the development of AML, whereas HHEX depletion attenuated the leukemic cell

Downloaded from http://ashpublications.net/blood/article-pdf/136/14/1575/1759810/bloodbld2020007049c.pdf by guest on 02 June 2024

function of human ASXL1-MT–expressing AML cells. To identify the mechanistic basis for the cooperation between HHEX and ASXL1-MT, they performed RNA-sequencing and chromatin immunoprecipitation and found that HHEX and ASXL1-MT coregulated MYB and ETV5 expression, which they demonstrated to be essential for the HHEX-induced development of AML. Mechanistically, they found that expression of ASXL1-MT enhanced the binding of HHEX to the promoter loci of *MYB* and *ETV5*. Deletion of MYB or ETV5 was sufficient to induce apoptosis or differentiation of ASXL1-MT–expressing AML cells, respectively. In contrast, expression of MYB or ETV5 was able to restore the function of HHEX-depleted ASXL1-MT–expressing AML cells. Collectively, these findings revealed a novel HHEX-*MYB*/*ETV5* axis that promotes myeloid transformation of *ASXL1*-mutated preleukemia cells (see figure).

Although the authors found that cells expressing ASXL1-MT showed binding of HHEX to MYB and ETV5 promoter loci, precisely how ASXL1-MT promotes accessibility of HHEX to the target loci requires additional investigation. It is possible that ASXL1-MT promotes accessibility of HHEX via reduction of H2AK119ub at these key loci. Alternatively, the ASXL1-MT/BAP1 complex may enhance promoter activity independent of histone modifications by recruiting transcriptional factors that enhance promoter activity, including *MYB* and *ETV5*. Future studies will need to determine the precise cooperation of target gene expression control by HHEX and ASXL1-MT.

One of the interesting and relevant findings of this work is that HHEX overexpression is not exclusive to ASXL1-MT AML, but is broadly elevated in AML as compared with normal hematopoietic cells. Based on this observation, the authors extended their analysis of HHEX and found that overexpression of HHEX in hematopoietic cells from RUNX1-ETO9a and FLT3-ITD mice similarly resulted in AML, suggesting that increased HHEX expression is critical for the progression of AML. The regulation of HHEX expression in AML remains unknown and will require future investigation. However, recent studies have described HHEX expression to be regulated by LMO2/ERG/FLI1 in T-cell leukemia and by RUNX1 in hematopoietic cells.[8,9] Given the paucity of effective treatments for AML, interfering with HHEX-mediated activity may provide new opportunities for therapeutic developments.

Conflict-of-interest disclosure: The author is a scientific cofounder and serves on the scientific advisory board of Kurome Therapeutics. ■

REFERENCES

1. Takeda R, Asada S, Park S-J, et al. HHEX promotes myeloid transformation in cooperation with mutant ASXL1. *Blood*. 2020;136(14):1670-1684.
2. DiNardo CD, Cortes JE. Mutations in AML: prognostic and therapeutic implications. *Hematology Am Soc Hematol Educ Program*. 2016;2016:348-355.
3. Yang L, Rodriguez B, Mayle A, et al. DNMT3A loss drives enhancer hypomethylation in FLT3-ITD-associated leukemias [published correction appears in *Cancer Cell*. 2016;30(2):363-365]. *Cancer Cell*. 2016;29(6):922-934.
4. Meyer SE, Qin T, Muench DE, et al. DNMT3A haploinsufficiency transforms FLT3ITD myeloproliferative disease into a rapid, spontaneous, and fully penetrant acute myeloid leukemia. *Cancer Discov*. 2016;6(5):501-515.
5. Poitras JL, Heiser D, Li L, et al. Dnmt3a deletion cooperates with the Flt3/ITD mutation to drive leukemogenesis in a murine model. *Oncotarget*. 2016;7(43):69124-69135.
6. Nagase R, Inoue D, Pastore A, et al. Expression of mutant Asxl1 perturbs hematopoiesis and promotes susceptibility to leukemic transformation. *J Exp Med*. 2018;215(6):1729-1747.
7. Shields BJ, Jackson JT, Metcalf D, et al. Acute myeloid leukemia requires Hhex to enable PRC2-mediated epigenetic repression of Cdkn2a. *Genes Dev*. 2016;30(1):78-91.
8. Oram SH, Thoms JA, Pridans C, et al. A previously unrecognized promoter of LMO2 forms part of a transcriptional regulatory circuit mediating LMO2 expression in a subset of T-acute lymphoblastic leukaemia patients. *Oncogene*. 2010;29(43):5796-5808.
9. Behrens K, Maul K, Tekin N, et al. RUNX1 cooperates with FLT3-ITD to induce leukemia. *J Exp Med*. 2017;214(3):737-752.

DOI 10.1182/blood.2020007049

© 2020 by The American Society of Hematology

Comment on Kaira et al, page 1685

Crystalizing our view of the contact system

Erik I. Tucker | Oregon Health & Science University School of Medicine

How does the contact activation system (CAS) assemble on cellular surfaces? Although researchers have been studying the contact factors prekallikrein (PK), high-molecular-weight kininogen (HK), and factor XII (FXII), and their respective roles in inflammation, immunity, and coagulation for over half a century, we now get some clarity on a quaternary structure for this fascinating multienzyme complex. In this issue of *Blood*, Kaira and colleagues describe the first crystal structure of an FXII domain in complex with a putative receptor, and they propose a model by which this binding protein, the globular complement C1q receptor (gC1qR), can act as a chaperone to cluster contact factors together prior to initiating factor XI (FXI)-dependent blood coagulation and inflammatory bradykinin (BK) liberation.[1]

Interest in the CAS has expanded significantly in recent years as contact factors are now being explored as therapeutic targets for thrombotic and inflammatory conditions. Indeed, inhibitors of and/or deficiencies in PK, HK, and FXII have been shown to limit experimental thrombosis without increased bleeding in animal models,[2] whereas early human studies have indicated that targeting FXI is antithrombotic with no signs yet of significant hemostatic compromise.[3] FXII gain-of-function mutations have also been described that trigger the rare inflammatory disease hereditary angioedema with normal C1 inhibitor.[4] Certainly, the myriad of biologic activities associated with the CAS provide opportunities for new approaches to reduce thrombosis and inflammation in diverse disease states.

Downloaded from http://ashpublications.net/blood/article-pdf/136/14/1575/1759810/bloodbld2020007049c.pdf by guest on 02 June 2024