HOXA9/MEIS1 targets in leukemia: reinforced signaling networks and therapeutic opportunities

Xinyue Zhou[1,2] and Rui Lu[1,2]

[1]Department of Medicine, Division of Hematology/Oncology, University of Alabama at Birmingham Heersink School of Medicine and [2]O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL, USA

Correspondence: R. Lu
ruilu1@uabmc.edu

Received: August 17, 2022.
Accepted: August 19, 2022.
Early view: August 25, 2022.

https://doi.org/10.3324/haematol.2022.281779

In this issue of *Haematologica*, Sahoo *et al.* demonstrate that a novel target of HOXA9 and MEIS1, *SCUBE1*, is critically involved in the development of MLL-rearranged (MLL-r) acute myeloid leukemia (AML).[1] The MLL fusion protein predominantly activates the oncogenic transcription factor HOXA9 and its cofactor MEIS1 to drive leukemogenesis.[2] A deeper understanding of the gene regulatory networks governed by HOXA9 and MEIS1 will improve our knowledge of MLL-r leukemia pathobiology and offer new therapeutic strategies. The study by Sahoo *et al.* revealed that SCUBE1 is required for both initiation and maintenance of MLL-r AML by promoting activation of the FLT3-LYN signaling axis. The authors also developed an antibody-drug conjugation-based strategy to target SCUBE1-expressing leukemic cells for specific and effective inhibition of leukemia growth.

Overexpression of transcription factor HOXA9 and its cofactor MEIS1 is a hallmark of MLL-r AML and many other subtypes of AML.[2] *MLL* gene translocation is found in approximately 10% of AML patients and is associated with poor response to treatment and reduced overall survival.[3] The MLL fusion protein drives leukemia development through direct activation of pro-leukemic transcription factors such as HOXA9 and MEIS1.[2] While progresses have been made in inhibiting *HOXA9* and *MEIS1* transcription by targeting the MLL complex proteins,[4] it is equally important to identify the transcriptional targets controlled by HOXA9 and MEIS1 to identify novel therapeutic strategies. Previous genomics and transcriptomics studies identified several transcriptional targets regulated by HOXA9 and MEIS1, including genes encoding transcription factor *LMO2*, anti-apoptotic factor *BCL2*, and receptor tyrosine kinase FLT3.[5-8] These HOXA9 and MEIS1 targets and their associated signaling pathways have been linked to leukemia transformation and expansion through various mechanisms (Figure 1). To date, the potential interactions and crosstalk among HOXA9 and MEIS1 targets remain largely unexplored.

By analyzing *SCUBE1* expression in AML cell lines and primary AML samples, Sahoo and colleagues found that *SCUBE1* is highly expressed in MLL-r AML cells, but not in normal hematopoietic stem and progenitor cells, peripheral blood cells, or leukemic cells that lack the *MLL* gene rearrangement. High *SCUBE1* expression was associated with shorter survival of AML patients, implying a potentially oncogenic role of SCUBE1. To understand the mechanism by which *SCUBE1* is upregulated in MLL-r AML, the authors examined whether *SCUBE1* is activated directly by the MLL fusion protein or indirectly by MLL downstream factors such as HOXA9 and MEIS1. While the authors did not find significant enrichment of MLL fusion protein at the *SCUBE1* locus by interrogating previously published MLL-AF9 chromatin immunoprecipitation sequencing data, they identified two putative HOXA9/MEIS1 co-bound sites located at distal regulatory regions of the *SCUBE1* gene. Using chromatin immunoprecipitation assay, luciferase reporter assay, and shRNA-mediated knockdown of *HOXA9* and *MEIS1*, the authors further confirmed that *SCUBE1* is a target that is transcriptionally activated by HOXA9 and MEIS1. Their data collectively suggest that *SCUBE1* is a novel target of HOXA9 and MEIS1 with elevated expression in MLL-r AML.

To assess the functional role of SCUBE1 in leukemia development, Sahoo and colleagues then performed a series of *in vitro* and *in vivo* experiments using both human and murine MLL-r AML models. In human MLL-r AML, knockdown of *SCUBE1* resulted in decreased cell survival *in vitro* and reduced leukemic cell engraftment *in vivo*. In mice, while *SCUBE1* overexpression was not sufficient to drive oncogenic transformation of hematopoietic progenitor cells, depletion of murine *Scube1* in hematopoietic progenitor cells significantly impaired the initiation of MLL-AF9-mediated leukemia. By using a *Scube1* conditional knockout mouse model, the authors further assessed the role of *Scube1* in maintaining MLL-r AML development *in vivo*. Tamoxifen-mediated acute depletion of *Scube1* significantly delayed leukemic progression and prolonged the survival of recipient mice bearing MLL-AF9 leukemia. Together,

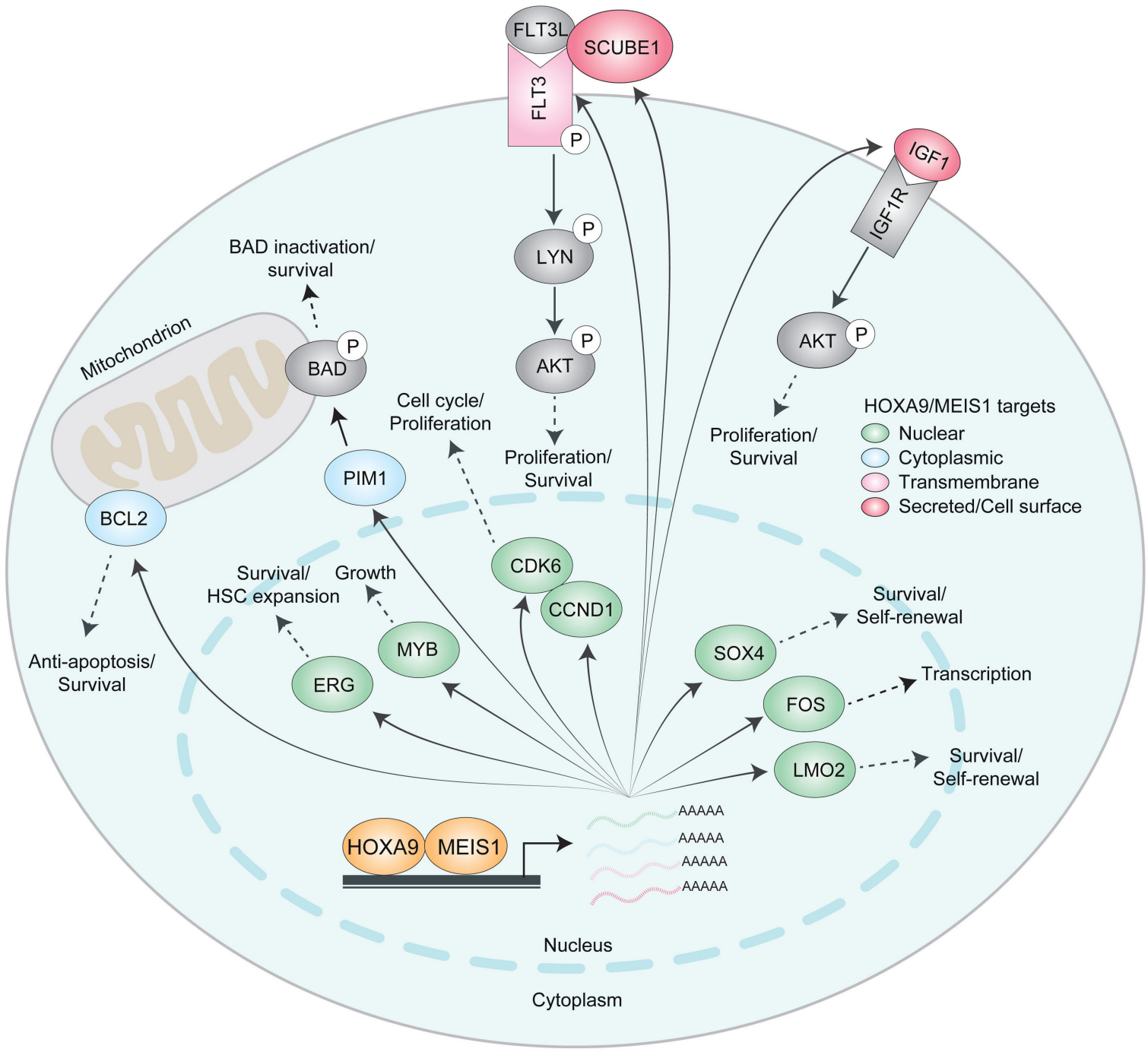

Figure 1. Transcription targets of HOXA9 and MEIS1 and their associated pathways in leukemia. P: phosphorylation; HSC: hematopoietic stem cells.

these data strongly suggest that SCUBE1 plays an essential role in both the initiation and maintenance of MLL-r leukemia.

To determine the potential signaling pathways associated with SCUBE1 in leukemia, the authors performed an unbiased proteomic proximity labeling and mass spectrometry analysis, through which they identified that the cell surface SCUBE1 protein is associated with receptor tyrosine kinase FLT3 and its direct signaling component LYN. More specifically, the authors found that the spacer region and the CUB domain of SCUBE1 primarily interact with the ligand-binding extracellular Ig-like domains of FLT3 and FLT3L. Gain-of-function and loss-of-function studies further demonstrated that SCUBE1 plays a role in activating FLT3-LYN signaling, potentially through acting as a co-receptor to facilitate FLT3L binding to FLT3. Lastly, Sahoo *et al.* generated a SCUBE1-targeting antibody-drug conjugate which links an internalizable anti-SCUBE1 monoclonal antibody to a proteolytically cleavable valine-citrulline linker and an anti-microtubule cytotoxic agent. This antibody-drug conjugate was able to selectively kill SCUBE1-expressing MLL-r leukemia cells but not the SCUBE1-negative leukemic cells *in vitro*, as well as inhibit MLL-r leukemia growth in xenograft models. Together, their results highlight SCUBE1 as a novel activator of FLT3 signaling pathway and a potential therapeutic target in MLL-r AML. Taken together, the findings of Sahoo and colleagues revealed important roles of SCUBE1, a new transcriptional target of HOXA9/MEIS1, in the initiation and maintenance of MLL-r leukemia. Intriguingly, SCUBE1 binds to another HOXA9/MEIS1 target FLT3 and facilitates activation of FLT3 signaling, implying a reinforced signaling network downstream of HOXA9 and MEIS1. A recent discovery that HOXA9 directly activates cyclin-dependent kinase CDK6 and its cognate cyclin CCND1 further supports this possibility.[7] Further studies are needed to systematically identify HOXA9 and MEIS1 targets and to investigate the

potential crosstalk among their associated signaling pathways. Recent advances in targeted inducible protein degradation and CRISPR screens may offer opportunities to discover immediate HOXA9/MEIS1 target genes and to perform unbiased functional evaluations, respectively.[9,10] In addition, because HOXA9 and MEIS1 are highly expressed in many other non-MLL-r leukemia subtypes,[2] understanding the role of SCUBE1 and other HOXA9/MEIS1 targets may have broader implications in other hematologic malignancies.

Disclosures

No conflicts of interest to disclose.

Contributions

XZ and RL both contributed to this editorial.

References

1. Sahoo BK, Lin Y-C, Tu C-F, et al. Signal peptide-CUB-EGF-like repeat-containing protein 1-promoted FLT3 signaling is critical for the initiation and maintenance of MLL-rearranged acute leukemia. Haematologica. 2023;108(5):1288-1299.
2. Collins CT, Hess JL. Deregulation of the HOXA9/MEIS1 axis in acute leukemia. Curr Opin Hematol. 2016;23(4):354-361.
3. Meyer C, Kowarz E, Hofmann J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009;23(8):1490-1499.
4. Aryal S, Zhang Y, Wren S, Li C, Lu R. Molecular regulators of HOXA9 in acute myeloid leukemia. FEBS J. 2023;290(2):321-339.
5. Collins CT, Hess JL. Role of HOXA9 in leukemia: dysregulation, cofactors and essential targets. Oncogene. 2016;35(9):1090-1098.
6. Huang Y, Sitwala K, Bronstein J, et al. Identification and characterization of Hoxa9 binding sites in hematopoietic cells. Blood. 2012;119(2):388-398.
7. Zhong X, Prinz A, Steger J, et al. HoxA9 transforms murine myeloid cells by a feedback loop driving expression of key oncogenes and cell cycle control genes. Blood Adv. 2018;2(22):3137-3148.
8. de Bock CE, Demeyer S, Degryse S, et al. HOXA9 cooperates with activated JAK/STAT signaling to drive leukemia development. Cancer Discov. 2018;8(5):616-631.
9. Röth S, Fulcher LJ, Sapkota GP. Advances in targeted degradation of endogenous proteins. Cell Mol Life Sci. 2019;76(14):2761-2777.
10. Shalem O, Sanjana NE, Zhang F. High-throughput functional genomics using CRISPR-Cas9. Nat Rev Genet. 2015;16(5):299-311.