haematologica
Journal of The Ferrata Storti Foundation

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Received: August 17, 2022.
Accepted: August 19, 2022.

Citation: Xinyue Zhou and Rui Lu. HOXA9/MEIS1 targets in leukemia: reinforced signaling networks and therapeutic opportunities.
Haematologica. 2022 Aug 25. doi: 10.3324/haematol.2022.281779 [Epub ahead of print]

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

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Disclosures

No conflicts of interest to disclose.

Contributions

XZ and RL both contributed to this editorial.

In this issue of *Haematologica*, Sahoo *et al*. demonstrated that a novel target of HOXA9 and MEIS1, *SCUBE1*, is critically involved in MLL-rearranged (MLL-r) acute myeloid leukemia (AML) development (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 have also developed an antibody-drug conjugation-based strategy to target SCUBE1-expression leukemic cells for specific and effective inhibition of leukemia growth.

Overexpression of transcription factor HOXA9 and its cofactor MEIS1 is a hallmark in MLL-r AML and many other AML subtypes (2). MLL gene translocation is found in approximately 10% of AML patients and is associated with poor treatment response 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 to inhibit *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 and to inform novel therapeutic strategies. Previous genomics and transcriptomics studies have 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 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 up-regulated 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 *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 *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 performed a serial *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 mouse, while *SCUBE1* overexpression was not sufficient to drive oncogenic transformation of hematopoietic progenitor cells, depletion of murine *Scube1* in hematopoietic progenitor cells significantly impaired MLL-AF9-mediated leukemic initiation. By using a *Scube1* conditional knockout mouse model, the authors further accessed 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, these data strongly suggest that SCUBE1 plays an essential role in both 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, where 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 coreceptor to facilitate FLT3L binding to FLT3. Lastly, Sahoo *et al*. generated a SCUBE1-targeting antibody-drug conjugate (ADC) which links an internalizable anti-SCUBE1 monoclonal antibody to a proteolytically cleavable valine-citrulline linker and an anti-microtubule cytotoxic agent. This ADC 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 by Sahoo and colleagues revealed important roles of SCUBE1, a new transcriptional target of HOXA9/MEIS1, in MLL-r leukemia initiation and maintenance. Intriguingly, SCUBE1 binds to another HOXA9/MEIS1 target FLT3 and facilitate 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. Recently advances in targeted inducible protein degradation

and CRISPR screens may offer opportunities to discover immediate HOXA9/MEIS1 target genes and to perform unbiased functional evaluation, 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 broader implications in other hematological malignancies.

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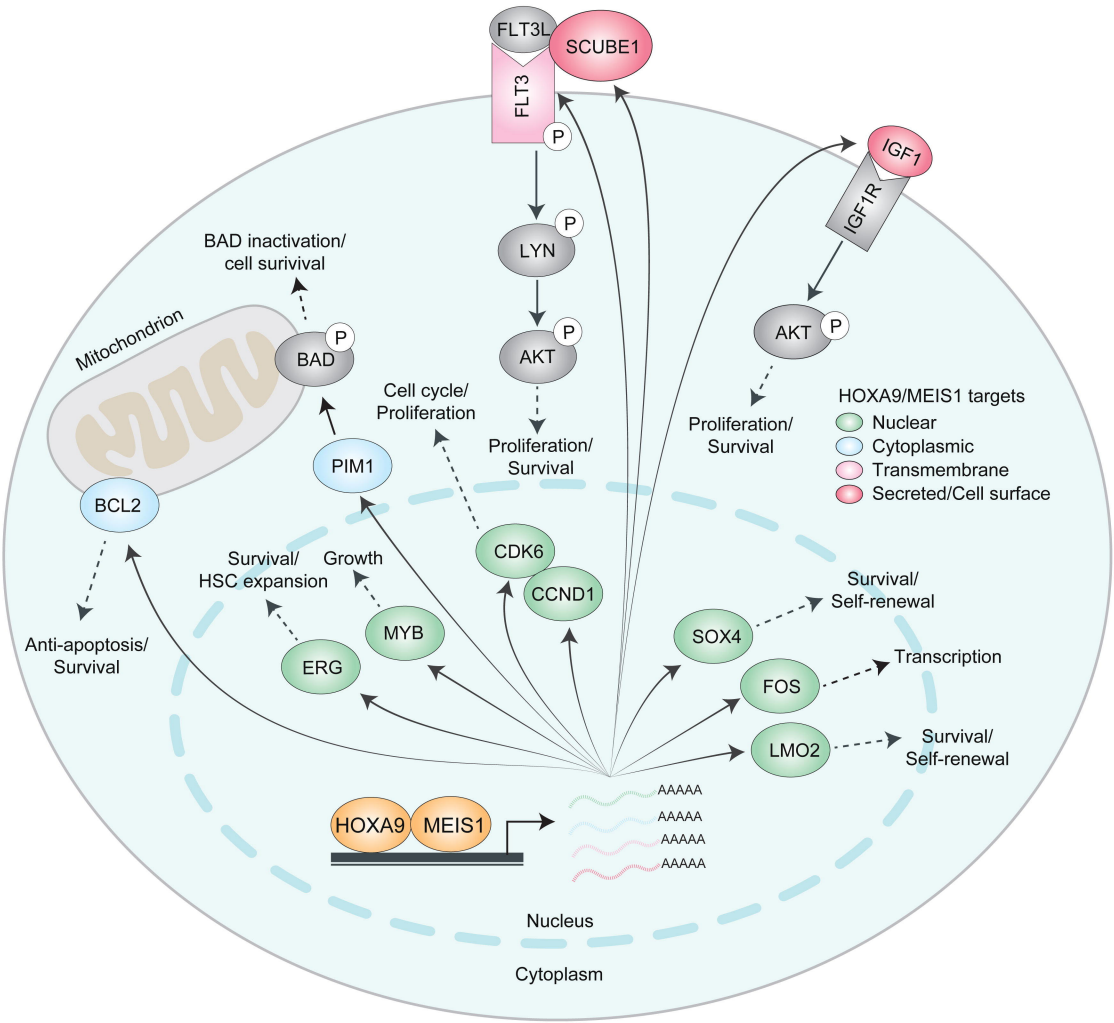
Figure Legends

Figure 1. Transcription targets of HOXA9 and MEIS1 and their associated pathways in leukemia. P, Phosphorylation.

Figure 1


FLT3L
SCUBE1
FLT3
P
LYN
AKT
IGF1
IGF1R
AKT
Proliferation/
Survival
HOXA9/MEIS1 targets
Nuclear
Cytoplasmic
Transmembrane
Secreted/Cell surface
BAD inactivation/
cell surivival
Mitochondrion
BAD
Cell cycle/
Proliferation
PIM1
BCL2
Anti-apoptosis/
Survival
Survival/
HSC expansion
Growth
ERG
MYB
CDK6
CCND1
SOX4
Survival/
Self-renewal
FOS
Transcription
LMO2
Survival/
Self-renewal
HOXA9
MEIS1
AAAAA
Nucleus
Cytoplasm