First mouse model of infant acute myeloid leukemia with t(7;12)(q36;p17)

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t(7;12)(q36;p17) is the second most frequent cytogenetic lesion in infant acute myeloid leukemia (AML) leading to a translocation of the *motor neuron and pancreas homeobox protein 1* (*MNX1*) gene telomeric on 7q36 to the *Ets-variant transcription factor* (*ETV6*) gene on 12p. Although a hypothetical fusion protein containing the MNX1 N-terminus to almost the entire *ETV6* ORF is formed, expression of fusion mRNA is only found in 50% of the patients.[1]

In this issue of *Haematologica*, Waraky *et al.* provide for the first time experimental evidence that MNX1 overexpression has *in vivo* leukemogenic potential[2] (Figure 1). More precisely, overexpression of MNX1, but not the *MNX1::ETV6* fusion in fetal liver (FL)-derived (but not adult bone marrow [ABM]-derived) hematopoietic stem and progenitor cells (HSPC) resulted in a fully penetrant and transplantable immature leukemia. Notably, the disease developed in non-conditioned immune-compromised NSBGW (*NOD.Cg-Kit^{W-41}JTyr^{+}Prkdc^{scid} Il2rg^{tm1Wjl}*) mice but not in irradiated wild-type recipients.

Earlier *in vitro* studies have provided some insights into the transforming potential of aberrant MNX1 levels, but leukemogenic activity has never been reported in a mouse model.[3] The finding that MNX1 overexpressing HSPC induce AML only when transplanted into immune-compromised mice is unexpected, as in both recipients the immune system is functionally impaired. However, NBSGW mice are not only immune-compromised, but also express a mutated partially defective KIT receptor which makes them more permissive for HSC engrafting and expansion.[4] Whether impaired KIT signaling provided a particular bias for grafting and expansion of MNX1-expressing cells remains to be elucidated. The fact that the leukemogenic potential was limited to FL- but not ABM-derived HSPC could, at least in part, be the consequence of increased induction of apoptosis that Waraky *et al.* observed *in vitro*. Whether the particular transformation susceptibility by MNX1 overexpression is also the consequence of the chromatin conformation of FL HSPC, as observed for another infant AML driver, the *CBFA2T3-GLIS2* fusion, remains to be determined.[5]

To understand the molecular mechanisms of MNX1-induced AML, Waraky *et al.* determined the gene expression signatures of FL-derived MNX1-expressing AML cells indicating significant associations to multiple processes, including DNA damage repair, substantiated by increased γ*H2AX* foci, cell cycle aberrations, and hyperploidy. Chromatin analysis of MNX1-expressing AML cells revealed changes of global histone modifications (increased H3K3me1/2/3 and reduced H3K27me3) and increased accessible regions mainly affecting promoters of differentially expressed genes involved in myeloid differentiation, cell cycle progression methylation, and DNA damage response. Cross-species comparison revealed significant overlaps between the expression signatures from MNX1-overexpressing murine AML and t(7;12)(q36;p17)-positive(+) infant AML with common enriched pathways including H3K4 methylation. Further protein pulldown and/or co-immunoprecipitation experiments identified potential interactions of MNX1 with proteins involved in methylation including the methionine adenosyl-transferase 2A/B (MAT2A/B) and the adenosyl-homocysteinase (AHCY), as well as some S-adenosylmethionine (SAM)-dependent methyltransferases. Notably, MNX1 overexpression was associated with increased cellular S-adenosylhomocysteine (SAH) and reduced free methionine, and pulled-down MNX1 showed *in vitro* methyltransferase activity on recombinant histone 3 (H3).

MNX1 expression was previously shown to block hematopoietic differentiation and induce premature senescence by increased DNA damage, cell cycle arrest, and hyperploidy.[3] The MNX1 interactome analysis perfomed by Waraky *et al.* showed enrichment for the proteins involved in senescence pathway, supporting this potential connection. Interaction of MNX1 with SAM-producing enzymes MAT2A/B could imply target gene regulation by a putative mechanism that has previously been suggested for the activity of another transcription factor called MAFK, a member of the MAF on-

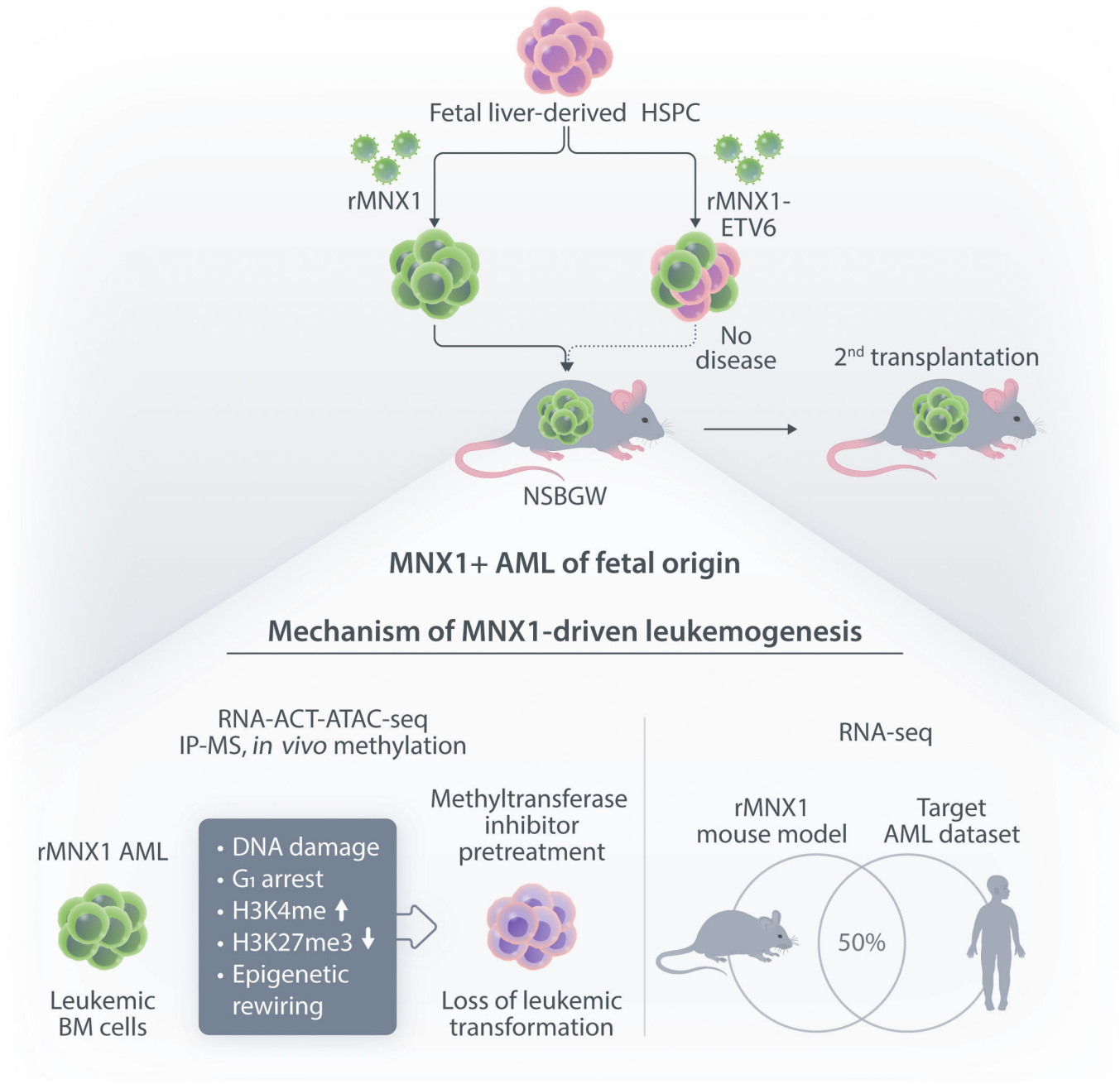

Figure 1. Schematic illustration of the most important findings by Waraky *et al.*[2] AML: acute myeloid leukemia; BM: bone marrow; HSPC: hematopoietic stem and progenitor cells.

coproteins.[6] Hereby, MAFK binds to its recognition elements and recruits MAT2A/B to provide SAM for the associated histone methyltransferase (HMT), which then modifies H3K9 and H3K4, and regulates the expression of target genes. To better understand MNX1-driven leukemogenesis, one would also like to know its genome-wide binding sites. By addressing its role in motor neuron development, MacFarlan and colleagues recently identified around 6,000 MNX1-bound gene loci of which over 40% located within promoters that were highly enriched with H3K4me3 and H3K27ac.[7] Despite the differences related to the cellular models, comparison of this chromatin immunoprecipitation with the RNA- and ATACseq datasets from Waraky *et al.* could lead to the identification of direct MNX1 targets.

Although Waraky *et al.* found and validated the interaction of MNX1 with the SAM producing enzymes MAF2A/B and AHCY, the nature of a potentially associated HMT remains unclear. Several HMT are well-known to play key roles in cancer, and particularly in acute leukemia of which KMT2A (also known as mixed lineage leukemia 1, MLL1) is the best characterized, as it represents a target of a large number of chromosomal aberrations, mostly leading to fusions that are the most prevalent lesions in infant AML.[8] Notably, the interactome analysis by Waraky *et al.* revealed MNX1 interaction with WDR5 (WD40 repeat domain protein 5), a member of the KMT2A complex, which is critical for KMT2A-mediated H3K4 methylation in AML.[9] Earlier studies have shown that MNX1 physically interacts with MENIN, a scaffold protein that recruits KMT2A to the chromatin.[10] These links suggest that the increase of H3K4me in MNX1 overexpressing AML cells might be mediated by WDR5/MENIN-recruited KMT2A. Although comparative gene expression profiling suggested significant differences between t(7;12)(q36;p17)$^{+}$ and 11q23/KMT2A-rearranged pediatric AML, several well-known KMT2A targets, including the *HOXA* gene cluster or *PBX3*, were also found aberrantly expressed in MNX1-overexpressing cells.[8]

Finally, Waraky *et al.* showed that a natural nucleoside SAM analog called Sinefungin rescued the aberrant histone methylation, γ2HAX foci, and the myeloid differentiation block of MNX1-overexpressing cells *in vitro*. Sinefungin pre-treatment of MNX1-expressing FL-derived HSPC pre-

vented AML induction in NBSGW mice. As Sinefungin is a pan-methyltransferase inhibitor, the observed phenotype could also be related to the inhibition of non-histone methylation. Nevertheless, this observation provides a perspective for future therapeutic interventions. Once the critical MNX1-interacting HMT is identified, combined interference with the SAM donors and the executing enzyme should result in selective and potent inhibition of AML blasts transformed by aberrant MNX1 expression. Collectively, Waraky *et al.* established the first leukemia mouse model driven by aberrant MNX1 expression phenocopying several aspects of t(7;12)(q36;p17)$^{+}$ infant AML. This model provides a platform for deeper characterization of driving molecular mechanisms and a search for strategies for targeted therapeutic interference.

Disclosures

No conflicts of interest to disclose.

Contributions

ZJ wrote, edited and conceived the illustration of the manuscript. JS conceptualized, wrote and edited the manuscript.

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References

1. Ragusa D, Dijkhuis L, Pina C, Tosi S. Mechanisms associated with t(7;12) acute myeloid leukaemia: from genetics to potential treatment targets. Biosci Rep. 2023;43(1):BSR20220489.
2. Waraky A, Östlund A, Nilsson T, et al. Aberrant MNX1 expression associated with t(7;12)(q36;p13) pediatric acute myeloid leukemia induces the disease through altering histone methylation. Haematologica. 2024;109(3):723-737.
3. Ingenhag D, Reister S, Auer F, et al. The homeobox transcription factor HB9 induces senescence and blocks differentiation in hematopoietic stem and progenitor cells. Haematologica. 2019;104(1):35-46.
4. McIntosh BE, Brown ME, Duffin BM, et al. Nonirradiated NOD,B6. SCID Il2rgamma-/- Kit(W41/W41) (NBSGW) mice support multilineage engraftment of human hematopoietic cells. Stem Cell Reports. 2015;4(2):171-180.
5. Lopez CK, Noguera E, Stavropoulou V, et al. Ontogenic changes in hematopoietic hierarchy determine pediatric specificity and disease phenotype in fusion oncogene-driven myeloid leukemia. Cancer Discov. 2019;9(12):1736-1753.
6. Katoh Y, Ikura T, Hoshikawa Y, et al. Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoprotein. Mol Cell. 2011;41(5):554-566.
7. Sun MA, Ralls S, Wu W, et al. Homeobox transcription factor MNX1 is crucial for restraining the expression of pan-neuronal genes in motor neurons. bioRxiv. 2021 Aug 07. doi: 10.1101/2021.08.07.455331 [preprint, not peer-reviewed].
8. Balgobind BV, Hollink IH, Arentsen-Peters ST, et al. Integrative analysis of type-I and type-II aberrations underscores the genetic heterogeneity of pediatric acute myeloid leukemia. Haematologica. 2011;96(10):1478-1487.
9. Yu X, Li D, Kottur J, et al. A selective WDR5 degrader inhibits acute myeloid leukemia in patient-derived mouse models. Sci Transl Med. 2021;13(613):eabj1578.
10. Scacheri PC, Davis S, Odom DT, et al. Genome-wide analysis of menin binding provides insights into MEN1 tumorigenesis. PLoS Genet. 2006;2(4):e51.