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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 entire ETV6 ORF is formed, expression of fusion mRNA is only found in 50% of the patients.

In this Issue of HAEMATOLOGICA, Waraky et al. provide for the first time, experimental evidence that MNX1 overexpression has in vivo leukemogenic potential (Fig. 1). More precisely, overexpression of MNX1, but not the MNX1::ETV6 fusion in fetal liver-(FL)- 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<sup>W</sup>41 JTyrcPrkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>) but not in irradiated wildtype recipients.

Earlier in vitro studies have provided some insights into the transforming potential of aberrant MNX1 levels, but leukemogenic activity was never reported in a mouse model. 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. 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 be at least in part 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.

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 yH2AX 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)+ 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 adenosylhomocysteinase (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. Moreover, the MNX1 interactome analysis done 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 been previously suggested for the activity for another transcription factor called MAFK, a member of the MAF oncoproteins. 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 6000 MNX1-bound gene loci of which over 40% located within promoters that were highly enriched with H3K4me3 and H3K27ac. Despite the differences related to the cellular models, comparison of this ChIPseq 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 MAT2A/B and AHCY, the nature of a potentially associated HMT
remains unclear. Several HMTs 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. Notably, 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. Earlier studies have shown that MNX1 physically interacts with MENIN, a scaffold protein that recruits KMT2A to the chromatin. 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 found aberrantly expressed in MNX1-overexpressing cells.

Finally, Waraky et al. showed that a natural nucleoside SAM analog called Sinefungin rescued the aberrant histone methylation, y2HAX foci, and the myeloid differentiation block of MNX1-overexpressing cells in vitro. Moreover, Sinefungin pre-treatment of MNX1-expressing FL-derived HSPC prevented 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 to search for strategies for targeted therapeutic interference.
References:


Figure 1. Schematic illustration of the most important findings by Waraky et al.
MNX1+ AML of fetal origin

Mechanism of MNX1-driven leukemogenesis

RNA-ACE-ATAC-seq
IP-MS, in-vivo methylation

rMNX1 AML
Leukemic BM cells

Methyltransferase inhibitor pretreatment
DNA damage
G1 arrest
H3K4me↑
H3K27me3↓
Epigenetic rewiring

Loss of leukemic transformation

rMNX1 mouse model
Target AML dataset

50%