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Role of Meningioma 1 for maintaining the transformed state in MLL-rearranged acute myeloid leukemia: potential for therapeutic intervention?

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Meningioma 1 (MN1) was cloned from a balanced chromosomal translocation in a meningioma as open reading frame encoding for a protein of 1,319 amino acids containing several proline and histidine-rich domains, acting as a transcriptional activator necessary for normal development of the bones of the skull.[1,2] Several studies found mutations or aberrant expression of MN1 in various hematologic malignancies. Characterization of a t(12;22)(p13;q11) chromosomal translocation associated with myeloproliferative disorders revealed a fusion between MN1 and the ETS-family transcription factor ETV6 (a.k.a. TEL).[3] A fusion of MN1 to Friend leukemia virus integration 1 (FLI1) has been shown to be a rare transforming oncogene in acute megakaryoblastic leukemia (AMKL).[4] Aberrant high expression of MN1 was reported in acute myeloid leukemia (AML) with inv(16) leading to the core-binding factor fusion CBFB-MYH11.[5] Clinical studies proposed that high MN1 transcript levels could be used as prognostic marker in cytogenetically-normal (CN) AML.[6] Functional studies in mice demonstrated the oncogenic potential of aberrant MN1 expression. Retroviral MN1 overexpression in murine bone marrow (BM) hematopoietic stem and progenitor cells (HSPC) followed by transplantation rapidly induced a lethal AML in mice.[7] Aberrant MN1 expression due to retroviral insertion was shown to act as collaborative oncogenic event in acute leukemia induction by the MLL-ENL or the MLL-AF9 fusion gene, respectively.[8,9] More recent work suggested that gene expression programs associated with MN1-mediated transformation of hematopoietic cells are controlled by the H3K4 and H3K79 histone methyltransferases MLL1 and DOT1L, respectively.[10] Collectively, these studies indicated that aberrant MN1 expression contributes to malignant transformation of hematopoietic cells towards AML; however, its role in the maintenance of the transformed state remained poorly understood.

In a study published in this issue of Haematologica, Sharma *et al.* functionally addressed the role of MN1 in the maintenance of AML induced by MLL fusion oncogenes.[11] They used Crisper/CAS9 to ablate MN1 in several murine AML lines, including cells transformed by retroviral overexpression of the MLL-AF9 fusion (rMLL-AF9), and in human THP1 and MV4;11 AML cell lines carrying the MLL-AF9 and MLL-AF4 fusions, respectively. They found that inactivation of MN1 impaired the clonogenic activity and proliferation associated with impaired cell cycle progression, and increased differentiation and apoptosis of murine rMLL-AF9 AML cells. Loss of MN1 also

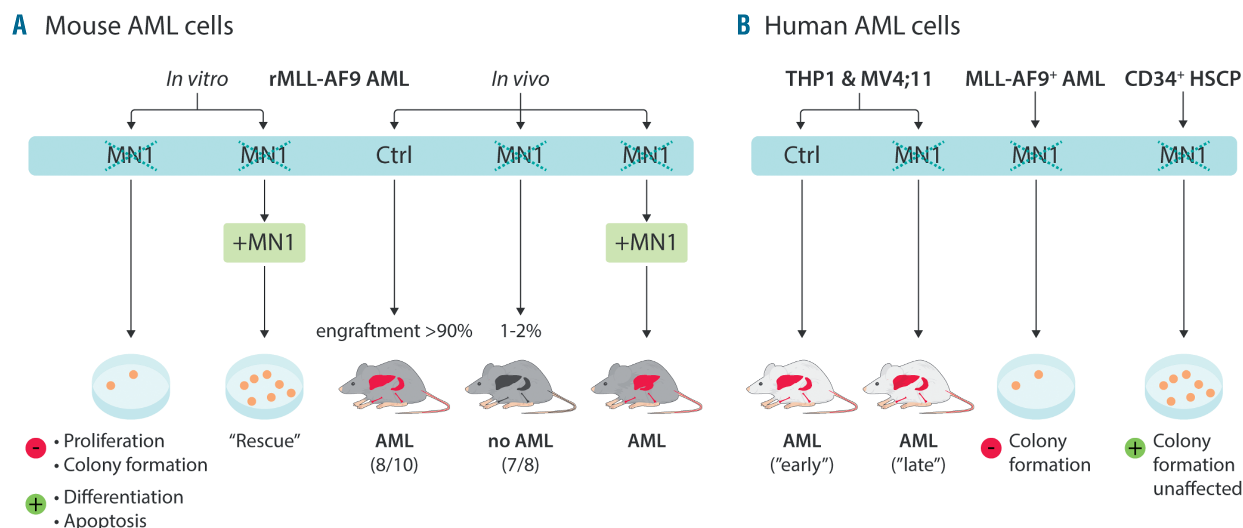

Figure 1. Schematic illustration of the findings by Sharma *et al.* in mouse (A) and human (B) acute myeloid leukemia (AML) cells expressing high levels of Meningioma 1 (MN1) and in CD34+ hematopoietic stem and progenitor cell (HSPC) controls (Ctrl).

reduced *in vivo* leukemia induction after transplanting the cells into syngenic mice. MLL-AF9+ AML cells lacking MN1 were significantly impaired in engrafting upon intravenous injection resulting in AML after long latency or no disease at all (Figure 1A). Notably, MN1 inactivation did not impair homing of transplanted cells to the BM. Most importantly, overexpression of exogenous MN1 'rescued' the anti-leukemic effects of ablation of endogenous MN1 in mouse rMLL-AF9 AML cells resulting in increased proliferation and clonogenic activity *in vitro* and increased engraftment and disease induction *in vivo.* Similar to murine cells, MN1 inactivation also delayed leukemia induction by transplantation of human THP1 or MV4;11 cells into immune deficient NSG mice. As in mouse rMLL-AF9 AML cells, reduced MN1 expression (by siRNA) also impaired clonogenic activity of primary MLL-AF9 human AML cells, whereas colony formation by normal CD34+ hematopoietic stem and progenitor cells (HSPC) was not affected (Figure 1B).

To understand the molecular mechanisms, Sharma *et al.* compared gene expression signatures of MLL-AF9+ leukemic cells before and after MN1 inactivation, and evaluated public chromatin immunoprecipitation sequencing (ChIP-seq) datasets. Enrichment of MN1 together with MLL-AF9, MEIS1 and the DOT1L-mediated H3K79me marks on the distal *Hoxa* gene cluster (Hoxa7-Hoxa10) suggested that MN1 regulates *Hoxa* gene expression. However, MN1 seemed not to co-localize with MLL-AF9, suggesting that MN1 primarily acts as a co-factor of Hoxa9 and Meis1. These observations were supported by reduced expression of the Hoxa9 target *Bcl2* (and other Hoxa9/Hoxa10 targets) upon MN1 inactivation in MLL-AF9+ AML cells. Finally, Sharma *et al.* explored the effects of reduced MN1 levels in primary cells from five AML patients and in CD34+ HSCP from healthy donors. They observed that application of MN1-targeting siRNA, either packed in lipid nanoparticles or transfected, significantly reduced the number and size of colonies formed by primary MLL-AF9+ AML cells but had no effect on colony formation by normal HSPC in methylcellulose. Sharma *et al.* concluded that MN1 is essential to maintain a transformed state of AML cells expressing MLL-AF9.[11]

The experiments by Sharma *et al.* have been performed with well-characterized mouse and human MLL-AF9+ AML cells expressing high levels of MN1 and HOXA9. However, one has to keep in mind that not all AML cells carrying MLL-AF9 or other MLL rearrangements express aberrantly high levels of MN1.[10] Although the effects of increased MN1 expression have been intensively studied, regulation of MN1 expression in hematopoietic cells still remains poorly understood. Previous reports found highest MN1 levels in hematopoietic stem cells (HSC) and early progenitor cells and very similar to HOXA genes, MN1 expression seems down-regulated during myelomonocytic differentiation.[5,12] However, not all AML patients expressing high levels of MN1 also express high levels of the *HOXA* gene cluster. Inv(16)+ AML cells often associated with aberrantly high MN1 levels mostly express very low *HOXA* levels.[13] Although Sharma *et al.* tested the efficacy of anti-MN1-siRNA in the inv(16)+ ME1 cell line, they did not show whether MN1 knockdown impaired colony formation or proliferation of these cells as efficiently as in primary MLL-AF9+ murine AML cells.

Does MN1 expression reflect the cellular origin of transformation? Highest MN1 transcript levels were found in patients with immature CD34+ AML.[14] In addition, transformation by experimental MN1 overexpression was found to depend on activation of MEIS1/AbdB-like HOX proteins present in common myeloid progenitors (CMP) but not in more differentiated granulocyte-macrophage progenitors (GMP).[15] Will the MN1 expression status change upon disease relapse? Experimental data indicated that overexpression of MN1 leads to resistance of AML cells to cytarabine and doxorubicin, suggesting selection for cells expressing highest levels during therapy.[16] However, based on a small number of patients, there seemed to be only a slight trend towards higher *MN1* mRNA expression upon disease relapse.[17] Nevertheless,

highest *MN1* transcript levels were associated with a higher incidence and shorter time to relapse.[14]

How can we then therapeutically target MN1 expression? Although useful to provide proof of concept in experimental studies, clinical siRNA-based knockdown approaches, such as those used by Sharma *et al.*, have so far been hampered by limited delivery into the target cells. However, small molecules have been generated that can interfere with certain transcription factor/co-factor protein-protein interactions or with transcription factor-DNA binding. In addition, transcription activity was successfully targeted by altering levels of ubiquitylation and subsequent proteasome degradation or by interference with regulators of transcription factor expression.[18] To target MN1 as a transcription factor, we would need to know its potential interaction partners on chromatin and/or the critical domains of MN1 that are necessary to maintain the transformed state of AML cells. Targeted genome editing screens could offer a platform to dissect structural needs of MN1 activity in AML cells.[19] Previous work that explored the transforming potential of a large number of MN1 deletion mutants suggested that 221 N-terminal amino acids are critical for induction of AML *in vivo* associated with expression of *HOXA9*, *HOXA10* and *MEIS2*.[20] Similarly, others reported that MN1 lacking amino acids 12-228 was unable to induce leukemia in the BM reconstitution assay, suggesting that overexpression of N-terminal MN1 peptides and small molecules "mimicks" might be able to compete with potentially, yet to be defined, critical protein and/or chromatin interactions.[21] Interestingly, the N-terminal region of MN1 was also shown to interact with the EP300 transcriptional co-activator, raising the question as to whether AML cells expressing high MN1 levels would be particularly sensitive to recently developed small molecule EP300 inhibitors.[22,23]

Collectively, by demonstrating a critical role for MN1 in AML maintenance, the work by Sharma *et al.* suggests that targeting the aberrantly high levels of MN1 expression would have strong anti-leukemic activity. However, the AML patients that would profit from such intervention, and the most efficient clinically applicable strategy, remain to be elucidated.

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