Background: Acute myeloid leukemia (AML) is characterized by rapid growth and block in differentiation of myeloid progenitors. The AML blast is defined by having "open" chromatin. We hypothesized that alterations of chromatin compaction may promote AML. Reversing those changes could represent a novel therapeutic approach.
Aims: Gain of chr21q22 is the most common focal amplification in complex karyotype AML. HMGN1 is a chromatin-regulatory protein on 21q22 known to affect lymphoid development, and our preliminary data suggested that HMGN1 could directly mediate a myeloid differentiation block. Since HMGN1 is known to decompact chromatin and alter histone marks, our goal was to *define and therapeutically target the mechanisms by which HMGN1 overexpression disrupts myeloid differentiation and promotes clonal dominance.*
Methods: We immortalized bone marrow progenitors from wild-type (WT) or OE-HMGN1 mice (transgenic overexpressing HMGN1) with an estrogen receptor-HoxB8 fusion protein. Using exogenous estrogen to control nuclear translocation of HoxB8, we analyzed synchronized myeloid differentiation by flow cytometry, RNAseq, and TMT proteomic analysis. We performed MINT-ChIP-seq (MNase Indexed T7-chromatin IP) to measure the histone marks H3K27ac, H3K27me3, H3K4me3 and total Histone H3. We also measured histone marks in hematopoietic stem and progenitor subpopulations *in vivo*. We performed competitive bone marrow transplantation with CD45.1 WT and CD45.2 OE-HMGN1 donors and measured the relative contribution to hematopoiesis over time.
Results: Synchronized differentiation in WT cells progressed over 6 days from myeloid progenitors to mature neutrophils and monocytes, analyzed by cell surface markers, morphology, and gene and protein expression. OE-HMGN1 cells proliferated faster and remained as undifferentiated myeloblasts (84% Cd11b+Gr1+ in WT *vs* 4% in OE-HMGN1, p=0.002; **Fig A**). Gene set enrichment analysis revealed more similarity to undifferentiated hematopoiesis and leukemia signatures in OE-HMGN1 cells. MINT-ChIP indicated higher global and locus-specific levels of H3K27ac in OE-HMGN1 cells (**Fig B**, upper panel), consistent with an increase in gene transcription, confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, highest at HoxA7 and HoxA9, genes known to be important in AML pathogenesis. In agreement with gene expression, among the most differentially measured histone peaks genome-wide were higher H3K27ac at HoxA gene promoters at all differentiation time points analyzed (**Fig B**, lower panel). Competitive transplantation demonstrated an advantage to OE-HMGN1 stem and progenitor cells. The clonal dominance of OE-HMGN1 over WT cells extended to all populations analyzed (long- and short-term HSCs, multipotent progenitors, CMP, GMP and MEP; **Fig C**) and to mature lineages (myeloid, B and T cells). MINT-ChIP in LK and LKS stem and progenitor cells revealed an increase in H3K27ac peaks at cell cycle and leukemia-related genes in the context of OE-HMGN1. H3K27 acetylation is catalyzed by the CBP/p300 histone acetyltransferase (HAT), suggesting that HAT inhibition could target leukemias with HMGN1 overexpression. Indeed, treatment of myeloid progenitors with the CBP/p300 inhibitor C646 rescued the differentiation block in OE-HMGN1 cells (93% CD11b+Gr1+ in WT *vs* 80% in OE-HMGN1, p=NS).

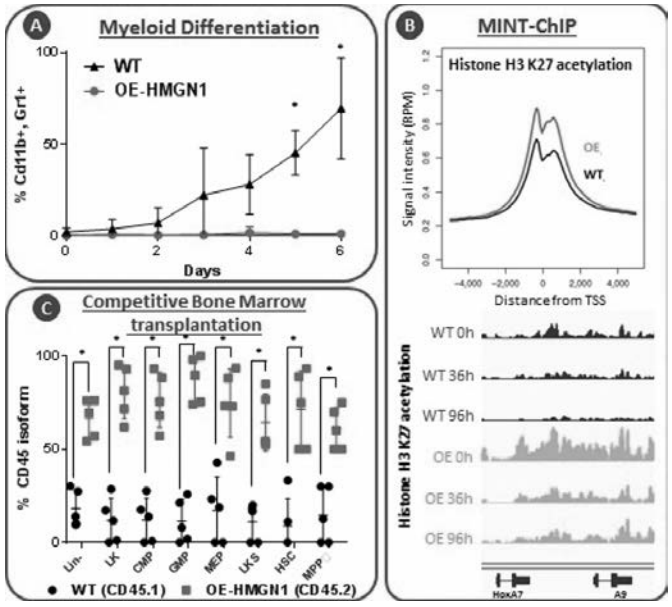

Figure 1.

Summary/Conclusions: Our study suggests that HMGN1 overexpression blocks myeloid differentiation and promotes proliferation in hematopoietic progenitors via increased H3K27 acetylation. Targeting epigenetic changes downstream of HMGN1 or interfering with HMGN1 itself may represent a novel therapeutic strategy in AML.

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PIWIL4 ACTS AS A PIRNA BINDING, EPIGENETICALLY ACTIVE AND GROWTH REGULATORY PROTEIN IN HUMAN ACUTE MYELOID LEUKEMIA

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Background: Piwi proteins are critically important for maintaining the self-renewing stem cell population in lower organisms through epigenetic silencing of transposable elements via DNA methylation and H3K9me3 marks, in close interaction with a novel class of non-coding RNA called piwi interacting RNA (piRNA).
Aims: There are neither precise data on the function of Piwi proteins in human acute myeloid leukemia (AML), nor are there reports on expression of piRNAs in this disease. We employed functional techniques and NGS to understand the role of human PIWI-like protein, PIWIL4 and its associated piRNA in AML.
Methods: We assessed the expression of human PIWIL genes in AML and healthy bone marrow cells using qRT-PCR. Murine stem progenitors were transduced with AML specific oncogenes to evaluate the effect on Piwil4 expression. shRNA mediated knockdown (KD) of PIWIL4 was performed on AML cell lines, AML patient bone marrow (BM) cells and healthy cord blood CD34+ stem progenitors and the impact on growth was determined using *in vitro* and *in vivo* assays. Western blot, ChIP-seq for H3K9me3 and RNA-seq were performed to assess the impact of PIWIL4 KD on the epigenetic landscape and transcriptome of the AML cell line THP-1. IP for PIWIL4 followed by LC-MS was performed to determine the binding partners of PIWIL4. PAR-CLIP and microarray were performed to identify piRNAs that physically bind to PIWIL4 and to test the impact of PIWIL4 KD on piRNA expression.
Results: Among the family of human PIWIL genes, PIWIL4 showed the highest expression level and was ubiquitously expressed in healthy hematopoietic stem/progenitors, mature lymphoid and myeloid cells. Importantly, PIWIL4 was aberrantly higher expressed in more than 89% of the AML patients (n=68; p<0.0001) compared to normal CD34+ BM and total BM cells (n=3). Overexpression of AML specific oncogenes in murine stem progenitors, within 96h post-transduction, induced a 6 to 8 fold increase in Piwil4 expression compared to GFP control (n=3, p<0.0001). Knockdown (KD) of PIWIL4 in AML cell lines significantly impaired proliferation and clonogenic growth *in vitro* (n=3; p<0.001) and delayed onset of leukemia in NSG mice (n=8; p<0.0001). PIWIL4 KD in primary AML patient BM cells lead to 5-fold decrease in clonogenicity (n=3, p<0.001), but had no impact on clonogenicity of healthy stem progenitors *in vitro* (n=4). Western blot and ChIP-seq (n=2, MACS1.4, p<0.01, FDR<0.01) in THP-1 cell line revealed a marked global reduction in repressive H3K9me3 marks upon PIWIL4 KD. Over 500 promoter and 600 gene body associated loci exhibited loss of H3K9me3 marks. RNA-seq analyses revealed over 4000 differentially expressed genes upon PIWIL4 depletion. 30% of the loci that lost H3K9me3 marks at promoters and gene body were differentially expressed in RNA-seq (fold>0.05, adj. p<0.01). These genes belonged to pathways associated with RNA metabolism, transcription and cell death. Moreover, these genes were enriched for binding sites of SETDB1, an H3K9me3 establishing histone methyltransferase (ENRICHR, p<0.01, FDR<0.01). Notably, using IP/LC-MS, PIWIL4 was found to associate with SETDB1 in 293T cells. 560 unique piRNAs were found to physically bind to PIWIL4 and 981 unique piRNAs were differentially expressed upon PIWIL4 depletion in THP-1 cells.
Summary/Conclusions: Thus, collectively, we could show for the first time that PIWIL4 expression is deregulated in human AML and acts as a piRNA binding, epigenetically active growth regulatory protein in human AML.

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METTL3 CONTROLS TRANSLATION OF TARGET MRNAS BY N6 METHYLATION OF ADENOSINE RESIDUES IN THEIR CODING SEQUENCE AND CONSTITUTES A NOVEL THERAPEUTIC VULNERABILITY OF ACUTE MYELOID LEUKAEMIA

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Background: Acute myeloid leukaemia (AML) patient survival remains below 30% and there have been no major new anti-AML therapies for decades.