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-remodeling protein that is overexpressed in AML and may contribute to leukemogenesis. We aimed to determine whether HMGN1 overexpression disrupts myeloid differentiation, alters chromatin structure, and blocks myeloid differentiation.

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 and in vitro.

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 specific up- and down-regulation in 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 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 genes (p<0.0001). At all differentiation time points analyzed (Fig B, lower panel), Competitive trans-plantation 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 and lineage markers confirmed increased levels of H3K27ac 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 Cs46 rescued the differentiation block in OE-HMGN1 cells (93% Cd11b+Gr1+ in WT vs 89% in OE-HMGN1, p<NS).

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.

Figure 1.