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Loss of p300 Accelerates MDS-associated Leukemogenesis

Guoyan Cheng¹, Fan Liu¹, Takashi Asai¹, Fan Lai², Na Man¹, Haiming Xu⁴, Shi Chen¹, Sarah Greenblatt¹, Pierre-Jacques Hamard¹, Koji Ando¹, Conception Martinez¹, Madhavi Tadi¹, Lan Wang¹, Mingjiang Xu¹, Feng-Chun Yang¹, Ramin Shiekhhattar², and Stephen D. Nimer^{1,3,*}

¹Department of Biochemistry and Molecular Biology, Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL 33136

²Department of Human Genetics, Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL 33136

³Department of Medicine, Sylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL 33136

⁴Memorial Sloan-Kettering Cancer Center, Cancer Biology and Genetics Program, New York, NY 10065

Abstract

The role that changes in DNA methylation and histone modifications play in human malignancies is poorly understood. p300 and CBP, two distinct but highly homologous lysine acetyltransferases (KATs), are mutated in several cancers, suggesting their role as tumor suppressors. In the current study, we found that deletion of p300, but not CBP, markedly accelerated the leukemogenesis of *Nup98-HoxD13* (*NHD13*) transgenic mice, an animal model that phenotypically copies human myelodysplastic syndrome (MDS). p300 deletion restored the ability of *NHD13* expressing hematopoietic stem and progenitor cells (HSPCs) to self-renew *in vitro*, and to expand *in vivo*, with an increase in stem cell symmetric self-renewal divisions and a decrease in apoptosis. Furthermore, loss of p300, but not CBP, promoted cytokine signaling, including enhanced activation of the MAPK and JAK/STAT pathways in the HSPC compartment. Altogether, our data indicate that p300 plays a pivotal role in blocking the transformation of MDS to acute myeloid leukemia (AML), a role distinct from that of CBP.

*Corresponding author: Stephen D Nimer; Address: 1120 NW 14th St Miami FL 33136; snimer@med.miami.edu; Phone number: 305-243-1775, Fax number: 305-243-4435.

Authorship contributions:

S.D.N. supervised the project. G.C. designed and performed most of the experiments, analyzed the data and wrote the manuscript; F.Liu analyzed the data and revised the manuscript; T.A., M.N., and F.Lai, performed some of the experiments and analyzed the data; H.X. generated the mouse colonies. S.C., S.G., P.J.H., K.A., C.M., M.T. and L.W. helped with some of the experiments.

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Introduction

Myelodysplastic syndrome (MDS) is a clonal disorder of hematopoiesis, characterized by inefficient hematopoiesis with bone marrow dysplasia, peripheral blood cytopenias, and a predisposition to progress to acute myeloid leukemia (AML)¹. Considering the therapeutic response of MDS to DNA hypomethylating agents, and the recurrent somatic mutations found in epigenetic modifiers, including ASXL1, TET2 and DNMT3A^{2,3}, many cases of MDS appear to represent an epigenetic disease.

The histone lysine acetyltransferases (KATs), p300 and its homologue CBP (CREB binding protein), function primarily as transcriptional co-factors for a number of nuclear proteins, including MYC, p53, and E2F⁴. Both p300 and CBP have been implicated in tumorigenesis. For example, germline mutations that disrupt p300 and CBP are responsible for some cases of Rubinstein–Taybi syndrome (RTS), a genetic disorder with increased predisposition to develop tumors of neural and hematopoietic origins⁵. Somatic, loss-of-function mutations in p300 and CBP are found in lymphomas and some solid tumors, including colorectal cancer and gastric cancer⁶. Although rare, chromosomal translocations, t(8;16) and t(8;22), that fuse amino terminus of *CREBBP* (the gene encoding CBP) or *EP300* (the gene encoding p300) to *MOZ* (the gene encoding monocytic leukemia zinc-finger protein) are found in the M4/M5 subtype of AML^{7,8}. These translocations generate haploinsufficiency for CBP and p300, in addition to the gain-of-functions intrinsic to the fusion proteins they generate.

The function of p300/CBP in normal and malignant hematopoiesis has been studied in genetic mouse models. Both *p300*^{-/-} and *CBP*^{-/-} mice exhibit embryonic lethality due to similar defects in growth and neural tube closure⁹. p300 or CBP deficiency impairs the self-renewal and the differentiation of hematopoietic stem cells in mice^{10,11}. In addition, heterozygous CBP knockout mice, but not heterozygous p300 knockout mice, develop hematopoietic malignancies¹², indicating the non-redundant function of these two KATs in leukemogenesis.

While the above evidence suggests a tumor suppressor role of p300/CBP, we previously reported that p300 acetylates the AML1-ETO leukemogenic fusion protein and is required for AML1-ETO-driven AML development¹³, suggesting an oncogene-specific and cell context-dependent function of p300 in tumorigenesis. Genetic studies on MDS and MDS-related AML patients revealed various mutations, deletions and translocations in *Ep300* gene^{14–17}. The mutation rate of *Ep300* ranges from 2 to 7% in different cohorts of patients in these studies. Here, we examined the role of p300 and CBP in the pathogenesis of MDS-derived AML; using *Mx1Cre* induced deletion of *Ep300* or *CREBBP* in *Nup98-HoxD13* (NHD13) transgenic mice. We found that loss of p300, but not CBP, significantly accelerated the onset of AML and triggered hematopoietic stem and progenitor cell (HSPC) expansion. The early onset of transformation in p300 deleted *NHD13* mice is associated with an enhanced “cytokine signaling profile”, affecting both MAPK and JAK/STAT pathways. Overall, our results indicate that p300, but not CBP, functions as a powerful tumor suppressor in the progression of NHD13-driven MDS to AML.

Materials and Methods

Mice

CD45.1 B6SJL mice and *Mx1Cre* mice were purchased from Jackson laboratory. *NHD13* transgenic mice (kindly provided by Perter Aplan, National Institute of Health), *p300^{flx/flx}* mice and *CBP^{flx/flx}* mice (kindly provided by Paul K. Brindle, St. Jude Children's Research Hospital) were previously described¹⁸. All mice used in the experiments are in pure C57BL/6 genetic background. Mice were maintained in the University of Miami animal facility under virus-antibody-free (VAF) conditions. Animal studies were approved by the Institutional Animal Care and Use Committee at the University of Miami.

Flow analysis and cell sorting

Single cell suspensions were prepared from bone marrow and spleen cells as previously described¹⁹. APC-c-kit, PE-Cy7-Sca-1 and Pacblue-Ki67 antibodies were purchased from eBioscience. APC-Cy7-Streptavidin, FITC-CD45.1 and APC-CD45.2 antibodies were purchased from Biolegend. The bone marrow biotin lineage panel and 7AAD/annexin-V staining kit were purchased from BD Pharmingen. All flow samples were analyzed by Canto-II or Fortessa flow cytometer and sorted by Aria-II cell sorter (Becton Dickinson).

For phospho-Flow, bone marrow cells were stimulated with or without a cytokine cocktail, containing murine IL-3 (PeproTech) 10ng/mL, murine IL-6 (PeproTech) 10ng/mL, murine SCF (PeproTech) 100ng/mL, and murine GM-CSF (BioLegend) 200 ng/mL for 15 minutes. Cells were then fixed in 1.5% paraformaldehyde (Electron Microscopy Sciences) at 37 °C for 10 minutes and permeabilized on ice with Methanol for 15 minutes. Subsequently, cells were stained with antibodies to cell surface markers as well as to pERK1/2 (T202/Y204), pSTAT3 (Y705), pSTAT5 (Y694), pAKT (S473) or IgG isotope control for 30 minutes at room temperature, before being subjected to FACS analysis. All phospho-specific antibodies were purchased from eBiosciences and are PE-conjugated.

Bone marrow transplantation and induced deletion of p300/CBP

8-week old B6SJL recipient mice were lethally irradiated (total body irradiation at 950 rads), and 6 hours later, 2×10^6 donor bone marrow cells were injected intravenously (i.v.) into each recipient mouse. Four weeks after transplantation, *Mx1Cre*-directed deletion of *p300/CBP* was induced by three intraperitoneal (i.p.) injections of poly(I:C) (10ug/G body weight, Invivogen), given every other day.

Paired daughter cell assay

CD34⁺ LSK cells were sorted individually into 96-well plates with 150μl of StemSpan Serum-Free Expansion Medium (Stem Cell Technology) per well, supplemented with 100ng/ml murine SCF and TPO. After overnight culture, if the single cell divided into 2 cells, the medium was vigorously mixed to separate the daughter cells. IMDM containing 10% FBS plus murine SCF (100ng/ml), TPO (100ng/ml), G-CSF (20ng/ml each), IL3 (10ng/ml) and EPO (2U/ml) was added to these wells to make the final volume to 200μl/well. Plates were incubated for another 2 weeks (37°C/5% CO₂). Cytospins were prepared from the clones formed by each daughter pair. After Diff-Quick staining, symmetric self-

renewal divisions (SS) were determined by the presence of all 4 lineages (granulocyte, erythroid, megakaryocyte, monocyte) from each of the paired daughter cells. Symmetric commitment divisions (SD) were determined by the presence of 3 or less lineages from each of the paired daughter cells. Asymmetric self-renewal divisions (AS) were determined by the presence of 3 or less lineages from one daughter cell but 4 lineages from the other daughter cell.

Serial replating assay

1×10^4 BM Lin⁻c-kit⁺ HSPCs were plated as duplicates in methylcellulose media (MethoCult™ GF M3434, Stem Cell Technologies). Colonies were scored 7 days after seeding; replating was conducted weekly.

RNA-sequencing and data analyses

Sorted Lin⁻c-kit⁺ HSPCs were lysed in Trizol (Invitrogen) and RNA was isolated and treated with DNase as manufacturer's instruction. The quality of RNA was evaluated on an Agilent Bioanalyzer, and samples with RNA integrity numbers > 8.0 were prepared for sequencing. Poly(A)-tailed RNA was prepared by using mRNA Sequencing Sample Preparation kit (Illumina) and libraries for the deep sequencing studies were generated by the Genomic Core Facility at the University of Miami. Sequencing and analysis were performed with three individual samples per group. Reads were aligned to the GRCm38/mm10 build of the *Mus musculus* genome with Subread aligner. We tested differentially expressed genes for enrichment in KEGG gene sets²⁰ in the Molecular Signature Database (MSigDB <http://www.broadinstitute.org/gsea/msigdb/index.jsp>; version 5.0). Gene set enrichment analysis (GSEA) was conducted using the pre-ranked tool in GSEA software²¹.

RT-PCR

RNA was extracted from bone marrow cells and spleen cells using the RNeasy Mini or RNeasy Micro kit (Qiagen). cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad). Mouse *Ep300* primers (Mm01310115_m1), *Crebbp* primers (Mm01342452_m1), *Il3ra* primers (Mm00434273_m1), *Il4ra* primers (Mm01275139_m1), *Il6ra* primers (Mm00439653_m1), *Csf2rb* primers (Mm00655745_m1) and *Gapdh* PCR primers (Mm99999915_g1) were purchased from Invitrogen. qPCR was performed on an ABI 7500 real-time cycler. Data were analyzed by comparative CT method as 2^{-CT} .

Statistical Analysis

All data were analyzed by GraphPad Prism 6. For survival analysis, we plotted Kaplan-Meier curves and performed the log-rank (Mantel-Cox) test to assess the significance. The comparison of two groups or multiple groups was performed by unpaired t-test or ANOVA with Tukey's multiple comparisons test. Significant differences are designated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Results

Loss of p300 greatly accelerates the leukemogenesis of NHD13 BM cells

To evaluate the role of p300 and CBP in the progression of MDS disease, we utilized the well-studied, *NHD13* transgenic mouse model of MDS²². Initially identified in therapy-related MDS, the NHD13 fusion protein induces murine MDS that recapitulates many key clinical features of human MDS, including peripheral blood cytopenias, bone marrow dysplasia with increased apoptosis, and a significant risk of transformation to acute leukemia.

We crossed *NHD13* transgenic mice with *p300*- or *CBP*-floxed mice; these mice were further crossed with *Mx1Cre* transgenic mice to generate *NHD13*-positive; *p300* or *CBP* conditional KO mice. To determine p300/CBP function in MDS, we first performed BM transplantation using 2-month old mice with the following genotypes as donors: *NHD13;p300^{flox/flox};Mx1Cre⁻* (referred to as NHD13 in figures), *NHD13;p300^{flox/+};Mx1Cre⁺*, *NHD13;p300^{flox/flox};Mx1Cre⁺*, *NHD13;CBP^{flox/flox};Mx1Cre⁺* and *p300^{flox/flox};Mx1Cre⁺*. Deletion of *p300* or *CBP* was induced in the transplant recipient mice 4 weeks later by three i.p. injections of poly(I:C), and qPCR analysis confirmed the efficient deletion of these genes (Fig S1A). To our surprise, the mice that received *NHD13;p300^{flox/flox};Mx1Cre⁺* BM cells developed increased WBC counts and decreased platelet counts as early as 4 weeks post poly(I:C) injections (Fig S1B). While all of the *NHD13* mice developed MDS, with one-third of them died from MDS and one-third of them died from MDS-derived AML by week 40, all of the *NHD13;p300^{-/-}* recipient mice died from AML by week 20, without a typical period of cytopenias (Fig 1A and Table). In contrast, loss of *CBP* in *NHD13*-expressing BM cells did not affect disease progression in the recipient mice. Furthermore, *p300* deletion in wild-type (WT) BM cells had no obvious effect on the survival of recipient mice (Fig 1A and Table), as all mice were disease-free 40 weeks post poly(I:C) injection.

The leukemic *NHD13;p300^{-/-}* mice had markedly enlarged spleens, increased white blood cell (WBC) counts, and decreased red blood cell (RBC) and platelet (PLT) counts compared to age-matched *NHD13* mice (Fig 1B). Immunostaining and flow cytometry showed an expanded c-kit⁺ population, while Giemsa staining of BM cytopsin showed a significant increase in leukemic blasts (Fig S1C).

p300 loss expands the HSPC population in NHD13 mice

To better understand the acceleration of leukemogenesis observed in *p300* deleted *NHD13* mice, we analyzed the BM HSPC compartment in all recipient mice two weeks after the poly(I:C) injection, when there was no obvious sign of MDS or leukemia. Loss of *p300* rapidly altered the BM compartment of the *NHD13* mice with a marked expansion of both Lin⁻ c-kit⁺ Sca-1⁻ (LK) cells and Lin⁻ c-kit⁺ Sca-1⁺ (LSK) cells at this early time point (Fig 1C). In contrast, deletion of *p300* in WT BM cells had only modest effect on the HSPC compartment, identifying the context-dependent regulation of hematopoiesis by p300. Furthermore, deletion of *CBP* in *NHD13* mice did not significantly change the LK/LSK cell profile compared to *CBP* intact *NHD13* mice (Fig S1D).

To exclude any potential influence of the BM ablation that follows lethal irradiation on HSPC behavior and disease progression, we performed similar experiments using primary mice, and the same genotypes as used for the transplantation experiments. Mice were injected with poly(I:C) at the pre-MDS stage (2 months of age) and followed for 40 weeks. We confirmed the survival pattern seen in the BM transplantation model, as loss of *p300* accelerated leukemogenesis and impaired survival, while the loss of *CBP* did not alter disease progression or the survival of the *NHD13* mice (Fig S2A). The expansion of both LK and LSK population was similar to that seen in the transplanted models (Fig S2B), including the absence of any change in the HSPC compartment when *CBP* was deleted in the primary *NHD13* mice (Fig S2C). In addition, we have also performed the bone marrow transplantation experiments using leukemia cells isolated from primary *NHD13*, *NHD13;p300*^{-/-} and *NHD13;CBP*^{-/-} mice, and found that leukemias from all 3 genotypes are all transplantable to secondary recipient mice (data not shown).

Loss of p300 enhances NHD13 HSPC self-renewal

To understand the basis for the *p300* deleted *NHD13* HSPC expansion, we performed serial replating assays using HSPCs isolated from transplant recipient mice (Fig 2A) or primary mice (Fig 2B) 2 weeks after poly (I:C) injection. In both cases, the *NHD13;p300*^{-/-} HSPCs showed increased self-renewal, which was particularly evident after the third replating. As expected, HSPCs from the *NHD13* mice had little ability to form colonies in semi-solid culture media. Loss of *p300* only minimally affected colony formation of WT cells, confirming the unique role of *p300* in *NHD13*-expressing HSPCs.

To further our understanding of the improved self-renewal of the *NHD13;p300*^{-/-} HSPCs, we performed paired daughter cell assays *in vitro* using sorted CD34⁻ LSK cells obtained 2 weeks after the poly(I:C) injection. Cytospins were prepared from the clones formed by each daughter pair, and the frequency of symmetric vs. asymmetric division was quantified (Fig 2C). Symmetric commitment divisions (SD) were dominant in *NHD13* CD34⁻ LSK cells (50%) compared to WT (28%), *p300*^{-/-} (22%) or *NHD13;p300*^{-/-} (14%) CD34⁻ LSK cells, while symmetric self-renewal divisions (SS) were over-represented in the *NHD13;p300*^{-/-} HSCs (68%) comparing to the WT (31%), *p300*^{-/-} (48%) or *NHD13* (28%) cells. Altogether, these data indicate a crucial role for *p300* in controlling the balance of symmetric stem cell self-renewing divisions vs. stem cell depleting divisions in *NHD13* mice.

Deletion of p300 decreases apoptosis in NHD13 BM cells

To further clarify the effect of *p300* deletion on *NHD13* expressing BM cells, we determined their proliferation and apoptosis 2 weeks after poly(I:C) injection. Using BM cells isolated from primary mice, we found that loss of *p300* reduced the elevated apoptotic rate in *NHD13*-transduced BM cells, especially in the stem cell enriched LSK cells (Fig 2D). In contrast, *p300* deletion had little if any effect on the proliferation of WT or *NHD13*-positive BM cells (Fig S3). Thus, loss of *p300* led to unique alterations in the survival of *NHD13*-expressing BM cells, which would be expected to contribute to leukemia progression.

RNA-sequencing analysis reveals a unique gene signature in p300-null NHD13 HSPCs

To further understand how loss of p300 promotes leukemogenesis, we also purified HSPCs from WT, *NHD13*, *p300*^{-/-} and *NHD13;p300*^{-/-} primary mice two weeks after the poly(I:C) injection and from *NHD13;p300*^{-/-} mice at the leukemia stage, and prepared RNA samples for comparative RNA-sequencing analysis. We initially normalized the expression profiles of the top 100 genes with the largest standard deviations across all samples by comparing each to the WT HSPCs and noticed many similarities between the WT, *p300*^{-/-} and *NHD13* samples (Fig 3A). In contrast, the *NHD13;p300*^{-/-} HSPCs exhibited a unique profile at the pre-AML, but also at the leukemic stage, indicating a specific interaction between *p300* and *NHD13* in HSPCs. Consistent with this notion, we found that p300 deletion affected significantly more genes in the *NHD13* HSPCs than in the WT HSPCs (2612 vs. 545), with only 148 genes being commonly affected by p300 deletion in both genotypes (Fig 3B). Using unbiased, gene set enrichment analysis (GSEA), we identified three up-regulated pathways that were directly related to hematopoiesis, including the cytokine/cytokine receptor interaction pathway (Fig 3C). Down-regulated pathways included several pathways thought to be involved in malignant transformation, such as RNA splicing, oxidative phosphorylation, and mismatch repair^{23–25}.

Given the flurry of papers reporting the abnormal expression of cytokine receptors on MDS/AML initiating stem cells^{26–29}, we performed qPCR assays for several genes, including *Il3ra*, *Il4ra*, *Il6ra* and *Csf2rb* to validate the RNA-seq data, and we observed highly correlated data showing the up-regulation of these cytokine receptors (Fig 3D and S4A), which could help drive leukemogenesis in the p300-deleted *NHD13* mice. In contrast, loss of *CBP* had no obvious impact on the expression of these cytokine receptors, except for *Il4ra* (Fig S4B).

p300 deletion enhances cytokine signaling in NHD13 HSPCs

Aberrant cytokine signaling occurs in a variety of malignancies³⁰, with activation of JAK/STAT signaling commonly underlying hematopoietic cell transformation³¹. To further explore cytokine signaling, we performed the phospho-Flow analyses, using BM cells isolated from mice 2 weeks post poly(I:C) injection. As shown in Fig 4A, B, and C, loss of p300, but not CBP, enhanced phosphorylation of ERK1/2 and STAT5 in response to cytokine stimulation in both WT and NHD13-transformed LK and LSK cells. This suggests that p300 can function to repress cytokine signaling in HSPCs. Given the importance of STAT3 signaling in HSC self-renewal³², it is intriguing to see that p300 deletion markedly up-regulated the STAT3 signaling pathway specifically in NHD13-transformed, but not WT, LSK cells. In contrast, we failed to see any change in AKT phosphorylation after p300 deletion in HSPCs of all genotypes we tested (Fig S5).

Discussion

We report here that loss of *p300* significantly accelerated the transformation of *NHD13* BM cells, identifying p300 as a tumor suppressor in *NHD13* driven leukemogenesis, which is quite distinct from its role in promoting AML1-ETO associated leukemogenesis³³. Mechanistically, loss of *p300* enhanced self-renewal and reduced apoptosis in *NHD13* BM

cells; in contrast, loss of *p300* did not significantly alter WT hematopoiesis, indicating the cell context-dependent role of *p300*. An earlier study of NUP98-HOXA9-associated blast crisis chronic myeloid leukemia (CML) and AML reported a shift from asymmetric stem cell divisions to symmetric self-renewing divisions and proposed that this shift could lead to disease progression³⁴. Similarly, the increase in symmetric self-renewal divisions (68% vs. 28%) that follows the deletion of *p300* in *NHD13*-expressing HSCs may underlie the profoundly increased risk for leukemogenesis in these mice.

Loss of *p300* triggered a unique gene signature in both *NHD13*-expressing and *NHD13*-transformed HSPCs, including the up-regulation of the cytokine/cytokine receptor interaction pathway. While this pathway is crucial for normal HSPC biology, it is co-opted in myeloid leukemia cells with inappropriate activation of cytokine signaling resulting from the overexpression or constitutive activation of receptor tyrosine kinases, such as c-KIT, FLT3, and IL-3Rα³⁵. The *NHD13;p300* / HSPCs have increased expression of several cytokine receptors, including IL-3Rα, IL-4Rα, IL-6Rα and CSFRβ, which activate the MAPK, PI3K/AKT, and JAK/STAT pathways in response to cytokines³⁶. We found significantly increased MAPK and JAK/STAT activation in *NHD13;p300* / BM cells. Although PI3K/AKT pathway is also downstream of these receptors, it is not affected by *p300* deletion, indicating that other mechanism(s) play a more important role in regulating this signaling pathway. It appears that the elevated cytokine signaling that follows the loss of *p300* in *NHD13* BM confers a growth advantage to pre-leukemic blasts.

Both *p300* and CBP were recently shown to facilitate leukemogenesis in cooperation with MOZ-TIF2 and NUP98-HOXA9³⁷. In the *NHD13* MDS model, *p300* acts to prevent the transformation to AML, while loss of CBP has no effect on disease progression. Even though these two proteins are highly homologous, *p300* and CBP appear to have non-redundant functions in *NHD13*-mediated leukemogenesis, and it will likely take a combined proteomics and expression profiling approach to better understand how *p300* differs from CBP in modifying the epigenetic landscape and controlling this transition.

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Figure 1. Lack of p300 accelerates the leukemogenesis of NHD13 BM cells in a transplantation model

(A) BM cells from $p300^{flox/flox}; Mx1Cre^{+}$, NHD13; $p300^{flox/flox}; Mx1Cre^{-}$ (NHD13), NHD13; $p300^{flox/+}; Mx1Cre^{+}$, NHD13; $p300^{flox/flox}; Mx1Cre^{+}$ and NHD13; CBP^{flox/flox}; $Mx1Cre^{+}$ mice were injected into 8-week old lethally irradiated B6SJL recipient mice (n=12–18). Poly(I:C) administrations were performed 4 weeks post transplantation. Shown is the Kaplan-Meier survival curve of recipient mice of each genotype post poly(I:C) injections.

(B) Top panels show BM and spleen cellularity of leukemic NHD13; $p300^{-/-}$ mice, compared to that of age-matched NHD13 mice. Bottom panels show WBC, RBC and platelet counts from peripheral blood of leukemic NHD13; $p300^{-/-}$ and age-matched NHD13 mice (n=4–5).

(C) HSPCs were isolated from recipient mice 2 weeks post poly(I:C) injections. Shown are the representative FACS profiles of Lin⁻ (upper panel), LK and LSK cells (middle panel) of each genotype indicated in the plots. The absolute number of Lin⁻, LK and LSK BM cells (from 2 femurs and 2 tibias of each mouse) is plotted in the bottom panel.

Figure 2. Increased stem cell self-renewal and reduced cell death are intrinsic to p300 deleted NHD13 BM cells

(A) HSPCs were sorted from the indicated recipient mice 2 weeks post poly(I:C) injection. Serial replating assays were performed and the resulting colonies were counted weekly before being re-plated. Shown is the data from 2 independent experiments.

(B) Serial replating assays were performed using HSPCs isolated from the indicated primary mice 2 weeks post poly(I:C) injection. Data plotted here is from 2 separate experiments.

(C) Two weeks post poly(I:C) injection, CD34⁻ LSK cells were isolated from each indicate primary mice and sorted individually into 96-well plate for the paired daughter cell assays. Data are presented as mean frequency from one representative assay out of 2 independent experiments in total.

(D) Shown are the representative FACS profiles of Annexin-V and 7AAD staining of LK (upper panel) and LSK cells (bottom panel) from the indicated mice.

Figure 3. Differentially expressed genes in p300-null NHD13 HSPCs

HSPCs were isolated from the indicated primary mice and sorted 2 weeks after poly(I:C) injection. Lin- and kkit+ cells were also isolated from *NHD13;p300* Δ/Δ mice at leukemia stage. RNA samples were then prepared for RNA-sequencing analyses.

(A) Normalized expression profiles of the top 100 genes with the largest standard deviations across all samples, including samples from the leukemic *NHD13;p300* Δ/Δ mice. Data represented are variance-stabilizing transformation (VST) counts from the DESeq2 package. The dendrogram shows samples in all groups clustered as expected.

(B) Venn diagram shows the overlap of differentially expressed genes in p300 deleted WT vs NHD13 mice. Genes were considered to be differentially expressed if they had a P-value of less than 0.05 and absolute log fold change of more than 1.5.

(C) Up- and down-regulated pathways identified using Gene Set Enrichment Analysis (GSEA) after *p300* deletion in *NHD13* HSPCs.

(D) qPCR validation for several differentially expressed genes. Data were normalized to the expression of GAPDH.

Figure 4. Activated MAPK and JAK/STAT signaling in response to cytokine stimulation in *NHD13;p300*^{-/-} HSPCs

BM cells isolated from mice 2 weeks post poly(I:C) injection from wt, *NHD13*, *p300*^{-/-}, *NHD13;p300*^{-/-}, *CBP*^{-/-}, and *NHD13;CBP*^{-/-} were cultured in DMEM supplemented with 20% FBS. Phosphorylation of (A) ERK1/2, (B) STAT3 and (C) STAT5 in LK and LSK cells were determined by flow cytometry after cells were cytokine starved for 2 hours, and stimulated or un-stimulated with a cytokine cocktail (GM-CSF 200ng/ml, SCF 100ng/ml, IL-3 10ng/ml and IL-6 10ng/ml) for 15 minutes. Left panels show representative FACS profiles. Right panels plot the frequency of pERK1/2, pSTAT3, and pSTAT5 positive cells in LK and LSK population (n=4). 1-Wt; 2-*NHD13*; 3-*p300*^{-/-}; 4-*NHD13;p300*^{-/-}; 5-*CBP*^{-/-} and 6- *NHD13;CBP*^{-/-}.

The cause of death by 40 weeks post poly(I:C) injections for each genotype in the transplanted experiment.

Table

Cause of death	NHD13	NHD13 p300 +/-	NHD13 p300 /	NHD13 CBP /	p300 /
AML	4 (33.3%)	6 (50.0%)	18 (100%)	4 (33.3%)	0
MDS	4 (33.3%)	4 (33.3%)	0	4 (33.3%)	0
Others	0	0	0	1 (16.6%)	0
# of mice alive	4 (33.3%)	2 (16.6%)	0	3 (25.0%)	12 (100%)