Supplementary Methods

Flow cytometry

Sorting of myeloid progenitors was accomplished by staining bone marrow cells with purified rat anti-IL-7Rα chain monoclonal antibodies (A7R34) (e-Bioscience, San Diego, CA) and purified or PE-Cy5-conjugated rat antibodies specific for the following lineage markers: CD3 (CT-CD3), CD4 (RM4-5), CD8 (5H10), B220 (6B2), Gr-1 (8C5), Ter119, and CD19 (6D5) (Caltag, Burlingame, CA), followed by PE-Cy5-conjugated goat anti-rat IgG (Caltag). After blocking with rat IgG (Sigma), cells were stained with PE-conjugated anti-FcγRII/III (2.4G2), FITC-conjugated anti-CD34 (RAM34), APC-conjugated anti-c-Kit (2B8) and biotinylated anti-Sca-1 (E13-161-7) monoclonal antibodies (Pharmingen, San Diego, CA), followed by avidin-APC-Cy7 (Caltag). Myeloid progenitors were sorted as IL-7Rα$^-$Lin$^-$Sca-1$^-$c-Kit$^+$CD34$^+$FcγRII/III^{lo} (CMPs), IL-7Rα$^-$Lin$^-$Sca-1$^-$c-Kit$^+$CD34$^+$FcγRII/III^{hi} (GMPs) and as IL-7Rα$^-$Lin$^-$Sca-1$^-$c-Kit$^+$CD34$^-$FcγRII/III^{lo} (MEPs) as described previously (Akashi et al., 2000). HSCs and CLPs were sorted as IL-7Rα$^-$Lin$^-$Sca-1^{hi}c-Kit^{hi} and IL-7Rα$^+$Lin$^-$Sca-1^{lo}c-Kit^{lo} populations, respectively (Kondo et al., 1997). Cells were sorted using a highly modified double laser (488nm/350nm Enterprise II + 647nm Spectrum) high-speed FACS (Moflo-MLS, Cytomation, Fort Collins, CO, USA). Data were analyzed with FlowJo software (Treestar, Inc., San Carlos, CA).

Southern blot analysis and RT-PCR analysis

Total RNA was isolated from tissues using TRIzol (Invitrogen) according to manufacturer's instructions. RNA was reverse transcribed using the Retroscript kit (Ambion, Austin, TX) and 1ul of the cDNA was subjected to PCR amplification.

Detection of the *Mll-Cbp* transcript was performed using primers to murine *Mll* exon 7 (5'-CTCGAATGGCATCAGTTCTAAGCAG-3') and Cbp primer (5'-GCAACTGGGACATATTTGGCACAG-3). Amplification was carried out with 10 min at 95°C for denaturation, followed by 30 PCR cycles (1 min at 95°C, 1 min at 55°C, 1 min at 72°C) and ending with 10 min at 72°C.

Histopathology

Murine tissues were fixed for 24 hours in 10% neutral buffered formalin (Sigma) and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE) for histological analysis. Smears of peripheral blood cells were stained with May-Grünwald-Giemsa stains.

cDNA microarrays

Total RNA was isolated using the Absolutely RNA Nanoprep Kit (Stratagene) as described by the manufacturer, eluted into a final volume of 10 µl, and used in its entirety for RNA amplification. Two serial rounds of double stranded cDNA synthesis and in vitro transcription according to a modified protocol (Febbo et al, manuscript in preparation) created by combining previously published methods (Baugh et al., 2001; Golub et al., 1999) were used to obtain sufficient cRNA for microarray analysis. Briefly, first-strand cDNA is synthesized using a T7-(dT_{24}) and SuperScript III reverse transcriptase (Invitrogen). After second strand cDNA synthesis, the double-stranded cDNA is phenol-chloroform extracted and subjected to IVT using a commercially available kit (Ambion). The resultant cRNA was purified using the Absolutely RNA Nanoprep Kit (Stratagene) and quantified with UV spectrophotometer; 600 ng of cRNA in 10 ul DEPC water are

carried into the second round for further amplification. For the gsecond round of IVT amplification, the single stranded cDNA is primned with random hexamers while the double-stranded cDNA synthesis is primed with the T7-(dT_{24}) primer. The double stranded cDNA was extracted as described above and subjected to IVT with the addition of biotinylated CTP and UTP in a 1:4 proportion to non-biotinylated CTP and UTP. The cleanup of the labled aRNA was performed with RNeasy Mini Columns and eluted with 50 ul DEPC water. RNA was quantified by spectrophotometer and the quality assessed by running a 1% denaturing agarose gel. After fragmentation, cRNA target was hybridized to Affymetrix 430A microarrays as described previously (Golub et al., 1999).

After hybridization, the raw expression data was re-scaled as previously described to account for differences in chip intensities (Armstrong et al., 2002). Gene expression was then analyzed using the GeneCluster 2 software (available at http://www.broad.mit.edu/cancer/software/software.html). The data were preprocessed using a minimum value of 20 and a maximum of 16,000. The max/min filter was 2 and the max-min 100. After preprocessing, the 50 genes whose expression level correlated with a particular class were determined by comparing the means between the two groups using the signal-to-noise statistic (Golub et al., 1999).

Real-time PCR analysis of Hox a9 gene expression

Total RNA was extracted from 2000 target cells in 0.5 ml TRIzol (Invitrogen) adding 2 μg MS2 phage RNA (Boeringer Mannheim, Mannheim, Germany) as a carrier. First strand cDNA was synthesized from the total RNA in a 20 l reaction mixture containing oligo dT primer and Superscript II Reverse

Transcriptase (Invitrogen) according to manufacturer's protocol. Real-time PCR reactions with FAM-labeled probes were then performed to quantiitate the *HOX A9* transcripts using the Taqman 1000 Reaction PCR core reagents kit (PE Biosystems, Branchburg, NJ) in a total volume of 20 μl on an ABI prism 7700 sequence detection system. Each PCR reaction was performed in triplicates with GAPDH serving as an internal control. At least two independent experiments were carried out for HSC and the committed progenitors. The primers and probes for *HOX A9* and *GAPDH* are available upon request.

In vitro replating assays

100 sorted hematopoietic stem cells or progenitor cells were cultured in methylcellulose using MethoCult^{TM} M3434 media (StemCell Technologies, Vancouver, BC), which contains 10ng/ml murine interleukin (IL)-3, 10ng/ml human IL-6, 50ng/ml murine stem cell factor, and 3 units//ml human erythropoietin. After 4-6 days, colony number and colony type were evaluated. Single cell suspensions were made from pooled colonies, and 10^4 cells were plated in secondary or tertiary cultures. Cytospins were prepared from individual colonies and stained using May-Grünwald-Giemsa stain.

γ-Irradiation and N-ethyl-N-nitrosourea (ENU) mutagenesis

4-week-old *Mx-Cre Mll-Cbp^{STOP}* mice were treated with pI-pC as described above. One week following the last dose of pI-pC, these mice received a single dose of γ-irradiation at 4Gy. A second cohort of mice was injected intraperitoneally with a single sublethal dose of ENU at 100 mg/kg of body weight (Sigma).

Southern blot analysis and RT-PCR analysis

Genomic DNA was prepared from tissues using the PUROGENE DNA isolation kit (Gentra Systems, Minneapolis, MN) according. Fifteen micrograms of DNA were digested with EcoRI and transferred to GeneScreen Plus Hybridization Transfer membranes (NEN Life Science Products, Inc., Boston, MA). The 5' hybridization probe was an 810-bp fragment isolated using a EcoRI/SmaI double digest from pIB9 (provided by Jay L. Hess). Probes were labeled with P^{32}-dCTP using Prime-it II kit (Stratagene, La Jolla, CA).

References

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