Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia

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T cell acute lymphoblastic leukemia (T-ALL) is an immature hematopoietic malignancy driven mainly by oncogenic activation of NOTCH1 signaling1. In this study we report the presence of loss-of-function mutations and deletions of the EZH2 and SUZ12 genes, which encode crucial components of the Polycomb repressive complex 2 (PRC2)2,3, in 25% of T-ALLs. To further study the role of PRC2 in T-ALL, we used NOTCH1-dependent mouse models of the disease, as well as human T-ALL samples, and combined locus-specific and global analysis of NOTCH1-driven epigenetic changes. These studies demonstrated that activation of NOTCH1 specifically induces loss of the repressive mark Lys27 trimethylation of histone 3 (H3K27me3)4 by antagonizing the activity of PRC2. These studies suggest a tumor suppressor role for PRC2 in human leukemia and suggest a hitherto unrecognized dynamic interplay between oncogenic NOTCH1 and PRC2 function for the regulation of gene expression and cell transformation.

T-ALL is a hematologic malignancy5–7 characterized by activating mutations in the NOTCH1 (ref. 8) gene and alterations in the FBRW7 (ref. 9) ligase gene resulting in activation of Notch signaling. Although the importance of NOTCH activation in T-ALL is well established, the detailed molecular mechanisms mediating NOTCH1-induced transformation remain unknown. We hypothesize that NOTCH1 interacts with epigenetic modulators to control gene expression. In addition, we propose that genetic alterations in key components of the epigenetic machinery could amplify oncogenic signals. To test this notion, we analyzed an extensive series of array comparative genomic hybridization (aCGH) data on 68 adult T-ALL primary samples for the presence of recurrent deletions in genes involved in epigenetic regulation. This analysis revealed the presence of recurrent deletions involving genes encoding core components of PRC2. This multisubunit complex is the ‘writer’ of a major repressive chromatin modification, H3K27me3, and consists of Suz12, Eed, Ezh2 (SET domain with histone methyltransferase activity) and RbAp48 (histone binding domain). We found recurrent deletions encompassing the EZH2 (refs. 10–12) and SUZ12 (refs. 13,14) loci (Supplementary Fig. 1 and Supplementary Table 1).

Next, we screened primary tumor DNA samples for the presence of somatic mutations affecting the genes encoding the EZH2 and SUZ12 core components of PRC2 (ref. 15). This analysis revealed the presence of truncating or missense mutations in both EZH2 (11/68) and SUZ12 (3/68). EZH2 mutations included four nonsynonymous single-nucleotide substitutions, one nonsense mutation and six frameshift-creating insertions and deletions. Some samples (see below) contained both a mutation and a deletion in the same locus (Fig. 1a,b, Supplementary Fig. 1 and Supplementary Table 1). SUZ12 mutations identified in T-ALL included two missense and one frameshift mutation (Fig. 1c,d). Loss-of-function mutations and deletions in EZH2 have been previously associated with myeloid leukemias10–12. In contrast, gain-of-function EZH2 mutations involved in B cell lymphomas are typically single amino acid substitutions involving Tyr641 (refs. 16,17). Nonsense and frameshift mutations in EZH2 and SUZ12 in T-ALL

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The PRC2 complex as a tumor suppressor in T-ALL.

(a) Structure of the EZH2 protein including two SWI3, ADA2, N-CoR and TFIIB (SANT) DNA binding domains, the cysteine-rich CXC domain and the catalytic SET domain. Overview of all EZH2 mutations identified in primary T-ALL samples. Filled circles: nonsense (X) and frameshift (fs) mutations; open circles: missense mutations.

(b) Representative chromatograms of paired diagnosis and remission genomic DNA samples showing somatic mutations in exon 8 and exon 10 of EZH2. (c) Structure of the SUZ12 protein including a zinc finger domain and the SET domain. Overview of all SUZ12 mutations, including somatic mutations in exon 8 and genomic DNA samples showing paired diagnosis and remission genomic DNA samples showing a somatic point mutation in exon 14 of this gene. (d) Pie chart summarizing the frequencies of homozygous and heterozygous mutations of EZH2 and SUZ12 in adults with T-ALL. (e) EZH2 protein levels in adult T-ALL samples with mutations and deletions in the EZH2 gene (subjects 1 and 4) compared to adult T-ALL samples with a wild-type EZH2 locus. (f) Silencing of EZH2 and SUZ12 in the Jurkat human T-ALL line, HES1 and DTX1 mRNA expression levels were increased upon silencing of either SUZ12 or EZH2. Knockdown of the luciferase (LUC) gene was used as a control. Error bars indicate s.d., and experiments were performed in triplicate.

are prototypical loss-of-function truncating alleles consistent with a tumor suppressor role for PRC2 in T cell transformation. Notably, seven EZH2 and two SUZ12 mutations were heterozygous, but 4 out of 11 EZH2 and one out of three SUZ12 mutations were homozygous18. In the eight cases (six EZH2 and two SUZ12 variants) with available matched bone marrow remission genomic DNA (out of 14 adult T-ALL cases that showed a mutation in EZH2 or SUZ12), we confirmed the somatic origin of the EZH2 and SUZ12 mutations (Supplementary Table 1). The convergent findings of our resequencing effort and copy number analysis thus identified EZH2 and SUZ12 as previously unknown tumor suppressor genes mutated and deleted in T-ALL. Overall, we identified genetic lesions targeting EZH2 or SUZ12 in 17/68 (25%) of primary T-ALL samples (Fig. 1e). The complete absence of EZH2 protein expression in two cases with combined deletion and mutation of the EZH2 gene (Fig. 1f) revealed that these are indeed loss-of-function mutations and suggested that inactivation of the PRC2 complex may constitute an important pathogenetic event in human T-ALL. Targeted resequencing revealed that PRC2 genetic alterations were frequently (in 65% of the cases) associated with oncogenic NOTCH1 mutations (Supplementary Table 1). This frequency suggested that the two events could cooperate. We analyzed the effects of PRC2 inactivation on the expression of prototypical NOTCH1 target genes such as HES1 and DTX1 in human T-ALL cell lines harboring NOTCH1 mutations9,19. These experiments showed that silencing of both EZH2 and SUZ12 resulted in transcriptional upregulation of the HES1 and DTX1 target genes both in basal conditions (Fig. 1g) and after inhibition of NOTCH signaling using γSI (Supplementary Fig. 2 and data not shown), suggesting that loss of PRC2 could potentiate the NOTCH1 transcriptional program.

To further explore the role of PRC2 in Notch target expression and T-ALL induction and progression, we aimed to dissect the epigenetic changes associated with transformation in T-ALL. Chromatin immunoprecipitation (ChIP) studies using CUTL1 cells15, a human T-ALL line20 characterized by a NOTCH1 translocation, showed that NOTCH1 binding on the promoter of HES1, one of the canonical targets required for NOTCH1-induced transformation21 (Supplementary Fig. 3a and Supplementary Table 2), peaks at ~50 to ~100 base pairs relative to the transcriptional start site (TSS) followed by enrichment of RNA polymerase II (POL II) (Supplementary Fig. 3). We observed no binding for NOTCH1 or POLII in a NOTCH1-negative T-ALL cell line, Loucy. (Supplementary Fig. 3b). Inhibition of NOTCH1 signaling with a γ-secretase inhibitor (γSI)20 (Supplementary Fig. 4a) abrogated NOTCH1 binding on the HES1 promoter and led to decreased levels of HES1 mRNA expression (Supplementary Fig. 4b,c). Subsequent γSI removal restored high levels of NOTCH1, POL II and the activating mark acetylation of Lys9 of H3 (H3K9ac) on the HES1 promoter as well as HES1 expression (Supplementary Fig. 4d–e).

To further test the interplay between activation of NOTCH1 and epigenetic regulation we used an intracellular Notch1 (Notch1-IC)-induced T-ALL mouse model23, which recapitulates most of the features of human T-ALL (Fig. 2a and Supplementary Fig. 5a–c). Most Notch1-induced leukemias show a double-positive phenotype characterized by the expression of both CD4 and CD8 T cell receptors (Fig. 2b). To study the transcriptional and epigenetic changes in Hes1 during Notch1-driven leukemogenesis, we compared FACS-sorted double-positive Notch1-transformed cells (T-ALL) to normal double-positive thymocytes, which are known to show low levels of Notch1 and Hes1 activation, reflecting activity of the pathway (Fig. 2b–d). PCR-mediated scanning of the mouse Hes1 promoter revealed marked enrichment for Notch1 binding in T-ALL double-positive cells compared to normal double-positive cells (Fig. 2c) accompanied by...
enrichment of PolIII on the TSS (Fig. 2f). Moreover, ChIP experiments showed enrichment of the activating H3K9ac23 and trimethylation of Lys36 on H3 (H3K36me3) on the gene body (Fig. 2i). We obtained similar results for Dtx1, which is also a direct Notch1 target gene (Supplementary Fig. 6), but not on the Gapdh locus, used as a control. These results demonstrate that Notch1-mediated oncogenic transformation is coupled to epigenetic changes, including the loss of the H3K27me3 histone mark from Notch1 target gene promoters.

To test whether the Hes1 behavior is part of a wider Notch1-driven epigenetic reprogramming in leukemia cells, we performed whole-transcriptome profiling (Supplementary Tables 3 and 4) and ChIP-sequencing (ChIP-seq, Supplementary Table 5) for H3K4me3, H3K9ac and H3K27me3 (Fig. 3a,b and Supplementary Fig. 7). Computational validation of ChIP-seq results showed high correlation among biological replicates (Supplementary Fig. 7a) and consistency with gene expression (Supplementary Figs. 8 and 9). Enrichment analyses (Supplementary Tables 6–9) revealed that the regulated (and marked by histone mark modifications) genes belong in functional categories related to normal T cell differentiation and T cell transformation (Fig. 3c and Supplementary Fig. 10). Genes upregulated in Notch1-driven leukemic cells compared to normal double-positive thymocytes were primarily characterized by loss of H3K27me3 ($P = 6.91 \times 10^{-22}$). Unexpectedly, gain of H3K9ac in these genes was much less significant ($P = 1.52 \times 10^{-4}$) (Fig. 3a,b and Supplementary Tables 10 and 11), suggesting that loss of H3K27me3 is the most prominent epigenetic change coupled to gene activation. This, in turn, provided evidence for a central role of PRC2 in T-ALL. In contrast, downregulated genes showed primarily loss of H3K9ac ($P = 2.79 \times 10^{-19}$, Supplementary Tables 11 and 12). Most notably, the loss of H3K27me3 from the TSS region of T-ALL upregulated transcripts was not due to lower total levels of the H3K27me3 in T-ALL (Supplementary Fig. 11 and Supplementary Table 5). Changes in H3K4me3 seemed to have a lesser role related to context-dependent and fine-tuning regulation of gene expression (Fig. 3a,b and Supplementary Table 11). Targets of Notch1, such as Hes1, Dtx1, Ptcra (encoding pre-T cell receptor-β) and Myc, showed loss of H3K27me3, accompanied by less striking changes in the H3K4me3 and H3K9ac marks, whereas the changes of H3K4me3 and H3K9ac histone marks were less universal and were gene specific (Fig. 3d and Supplementary Fig. 12), suggesting a functional interaction between Notch1 expression and promoter binding and loss of H3K27me3, a histone mark modification generally associated with decreased activity of PRC2.

To further explore the role of Notch1 in driving the loss of H3K27me3, we performed ChIP-seq for Notch1 (Fig. 4a and Supplementary Fig. 13). Whereas we detected no peaks in normal double-positive-cells, analysis of Notch1-transformed T-ALL lymphoblasts revealed a large number of direct Notch1 chromatin binding events. Notably, H3K27me3 loss in T-ALL was broadly overlapping with direct Notch1 binding in TSS regions (Fig. 4a,b). The lack of substantial changes for H3K9ac (enrichment for H3K9ac gain or loss) suggested that Notch1 binding is highly specific to H3K27me3 loss (Fig. 4a). The observed loss of H3K27me3 in Notch1 targets was mainly localized in a narrow region around TSSs (Fig. 4b). We observed loss of H3K27me3 specifically on Notch1 targets and not in the whole T-ALL genome (Fig. 4a and Supplementary Figs. 14 and 15). These combined data suggested that marked loss of H3K27me3 is a hallmark of the oncogenic function of Notch1 in T-ALL.

The rapid increase of Notch1-IC protein amounts in human T-ALL lines upon γS removal (Supplementary Fig. 4) resulted in a dynamic and rapid loss of H3K27 (Fig. 4c and Supplementary Fig. 16), further proving the inverse correlation of the two events. This led us to further investigate this relationship in additional human T-ALL cell lines and primary T-ALL samples. Initially, we screened additional T-ALL lines (DND41 and CEM) showing high Notch1-IC and HES1 expression and normal (HES1 nonexpressing) human thymocytes (Fig. 4d). The levels of H3K27me3 were once more inversely correlated with HES1 expression (Fig. 4d). To exclude the possibility that these results were due to cell line artifacts, we studied primary samples whose high leukemic potential we evaluated using transplantation (Fig. 4e and Supplementary Fig. 17). The primary T-ALL leukemic blasts had higher HES1 mRNA levels compared to normal human thymocytes, and the degree of H3K27me3 was inversely correlated with HES1 expression (Fig. 4f). These studies demonstrated that the correlation between oncogenic NOTCH1 binding and loss of H3K27me3 is a general characteristic of T-ALL.

We then focused on the relationship between oncogenic NOTCH1 and PRC2. Initially, the analysis revealed that Notch1 binding sites are enriched for PRC2 targets (4.3-fold enrichment and $P = 8.45 \times 10^{-110}$,
Characterization of T-ALL epigenetic landscape using ChIP-Seq for H3K9ac, H3K4me3 and H3K27me3. (a) Cluster of the major gene expression changes between T-ALL and DP and the accompanied epigenetic changes. Left, expression heatmap representing up (red)- and down (blue)-regulated genes with significant epigenetic changes at 5% false discovery rate. Right, heatmap representation of the epigenetic marks in TSSs of selected genes in T-ALL and DP cell populations. Plus sign indicates gain and minus sign indicates loss in the levels of epigenetic mark in T-ALL versus DP. Loss of H3K27me3 (P = 6.91 × 10⁻²², blue bar) and gain of H3K9ac (P = 1.52 × 10⁻⁴, green bar) are enriched in upregulated genes, whereas loss of H3K9ac (P = 2.79 × 10⁻¹⁹, red bar) is enriched in downregulated genes. Nrarp, Heyl and Mrpl12 are canonical Notch1 targets. (b) Bar graphs indicating the percentage of genes characterized by each modification in T-ALL cells. The plus and minus signs are used as above. Slashes indicate ‘and’. Pink and blue boxes indicate prevalent epigenetic clusters in downregulated and upregulated genes, respectively. (c) Functional annotation of epigenetic changes (T-ALL versus DP) in H3K9ac and H3K4me3 showing enrichment in specific biological processes. Error bars represent s.d. (Supplementary Methods). (d) ChIP-seq results for two well-characterized Notch1 targets, Hes1 and Dtx1 (arrows denote the TSS).

Supplementary Table 9). Moreover, we analyzed the effects of Notch1 activation on the occupancy of Notch1 target genes by the EZH2 catalytic subunit of PRC2. These studies demonstrated that Notch1 binding led to considerable Ezh2 eviction from the Hes1 promoter (Fig. 4g). This could not be attributed to lower EZH2 expression in the cancer cells (Supplementary Fig. 18a). ChIP analysis for SUZ12 binding yielded results similar to those in the Ezh2 studies (Fig. 4h). EZH2 or SUZ12 eviction and H3K27 loss were not only features of the Notch1-IC model used, as we obtained identical results with ‘weaker’ human Notch1 alleles (including mutants of the heterodimerization and PEST domains) using in vivo disease models (Supplementary Fig. 19). We observed these epigenetic effects even at the very early stages of the disease (Supplementary Fig. 20). Moreover, downregulation of Notch1-IC by γSIL treatment led to a marked decrease of EZH2 binding on the HES1 and DTX1 promoters (Supplementary Fig. 18). The binding of JARID2 (refs. 26,27), one of the recruiters of PRC2 to DNA, on the HES1 promoter also inversely correlated with Notch1 binding (Fig. 4i). These responses were rapid, as we detected marked changes in Notch1 binding and PRC2 recruitment as early as 30 min after γSIL removal (Supplementary Fig. 21). The inverse correlation between Notch1-PRC2 binding and H3K27me3 levels was found in all T-ALL lines studied (Supplementary Fig. 22). We also observed identical epigenetic changes when we inhibited the Notch pathway with a dominant-negative form of Mastermind-like 1, a transcriptional partner of Notch1 (Supplementary Fig. 23).

The presented mechanistic interaction between NOTCH1 and PRC2 suggested a potential role for PRC2 mutations in NOTCH-induced transformation, although Notch-independent effects are also possible. To start addressing pathway interaction, we used a Drosophila Notch-driven tumor model (see Supplementary Data) to evaluate the impact of knockdown of the Drosophila ortholog of the human EZH2 gene (E(z)) in cells that express weak activating alleles of Notch (Supplementary Fig. 24). In this case, we found that the combination of Notch activation and E(z) loss resulted in eye tumor overgrowth in approximately 50% of the progeny (n = 64). In agreement with such a notion of cooperation between Notch and PRC2 loss, we were able to show that EZH2 silencing resulted in decreased apoptosis triggered by γSIL inhibition of Notch signaling in human T-ALL lines (Supplementary Fig. 25). Moreover, EZH2 silencing increased the in vivo tumorigenic potential of human T-ALL cells transplanted into immunodeficient mice, as they led to enhanced mortality (Supplementary Fig. 26). These studies suggest a striking conservation of the Notch-PRC2 pathway interaction in tumorigenesis and further establish the role of PRC2 as a tumor suppressor in T-ALL, although the exact mechanisms of PRC2 function in this disease have
Figure 4 Notch1 binding mediates loss of H3K27me3 and evocation of PRC2 in T-ALL.
(a) The enrichment of Notch1 binding sites around TSSs characterized by each indicated histone mark. (b) H3K27me3 average signal profiles around TSS areas. (c) ChiP for H3K27me3 in a T-ALL cell line (CUTLL1) treated with γSi for the inhibition of the Notch1 pathway (+γSi), followed by release from the inhibitor (post γSi). The Loucy T-ALL line is used as a negative control. (d) Left, HES1 expression in the indicated leukemic cell lines and normal human thymocytes. Right, ChiP for H3K27me3 in the indicated leukemic cell lines and primary human thymocytes. (e) High leukemogenic potential of the human T-ALL samples (sample M105 is shown here) in xenograft models. Spleen sections of recipient mice stained with an antibody specific for HES1 in primary human T-ALL samples and normal human thymocytes (P < 0.0001 between one of the primary T-ALL samples (M69) and the human thymus). (g) ChiP experiments for Ezh2 on the Hes1 promoter in DP and T-ALL. (h) Suz12 binding on the Hes1 promoter. (i) γSi-mediated changes of the Notch1-IC levels modulate JARID2 recruitment to the HES1 promoter (P = 0.059). Error bars indicate s.d., and experiments were performed in triplicate.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturemedicine/

Accession codes. Microarray data are available in the Gene Expression Omnibus under accession code GSE34554. ChiP-seq data can be accessed under accession code GSE34954.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

I.A. and P.N. conceived of the studies, directed research, analyzed the results and wrote the manuscript. P.N. performed xenograft experiments, isolated and characterized mouse samples and performed and analyzed the biochemical experiments, helped by T.T. and J.S. A.T. directed research, analyzed data,
developed computational methods and wrote the manuscript. J.N. isolated and characterized mouse samples, helped to project design and wrote the manuscript. S.B., Z.T. and T.H. helped with the analysis of the genome-wide data. P.A. helped with the design and execution of the biochemical experiments. F.U. created the resource website. P.V.V., M.H., L.R., J.B.S. and J.P. performed mutation analysis of SUZ12, EZH2 and EED. R.L.L. designed and supervised sequence analysis. P.V.V. performed xenograft experiments. P.V.V. and K.D.K. performed aCGH analysis. R.R. analyzed aCGH data. M.S.F. performed the genetic silencing studies of PRC2. E.P., J.R. and J.M.R. provided samples and correlative clinical data from the Eastern Cooperative Oncology Group. S.P. and F.P. performed and supervised experiments related to NOTCH activation into primary T-ALL samples and the Eastern Cooperative Oncology Group. S.P. and F.P. performed and supervised tumor experiments.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Cell culture and primary cell samples. The human T-ALL cell lines CUTLL1, Loucy, Jurkat, P12-ICHIKAWA, DND41 and CEM36 were cultured in RPMI-1640 supplemented with 20% FBS and penicillin-streptomycin. Tissue culture reagents are purchased from Gibco.

Mouse models and samples preparation. To generate leukemic T cells expressing high amounts of Notch1-IC (T-ALL), we used a Notch1 overexpression mouse model22, which consists of the Cre recombinase C-terminally fused to the estrogen receptor knocked in the ROSA26 locus. All mouse procedures were carried out in compliance with the guidelines of the Institutional Animal Care and Use Committee of the New York University School of Medicine, and the mice were kept in specific pathogen-free animal facilities.

Antibodies and reagents. Compound E from Alexis Bioscience was used as a γ secretase inhibitor at a 500 nM final concentration. The protein G Dynal beads were purchased from Invitrogen. All antibodies have been widely used in published studies13,35.

We purchased the antibodies from Santa Cruz (Notch1, PolII), Abcam (S2-P-PoII, H3K36me3, H3K9ac, Suz12, Jarid2, total H3), Millipore (H3K27me3, Hes1), Bethyl (Suz12), Cell Signaling (Ezh2, Notch1-IC), Active Motif (H3K4me3, H3K27me3, H3K27ac, Ezh2). A second Ezh2-specific antibody was provided by D. Reiner. FACS antibodies were purchased from eBioscience. The reagents for the annexin V staining were purchased from BD Biosciences. The shRNAs lentiviral vectors (PLKO.1-puro) for the EZH2 and Suz12 knock-out were purchased from Sigma.

Cells cross-linking and preparation of chromatin and ChIP-seq studies. We fixed the cells with 1% formaldehyde for 10 min at 25 °C and generated mononucleosomal particles using micrococcal nuclease (from USB). We stopped the reaction with the addition of EDTA (20 mM), and the nuclei were lysed using nuclei lysis buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0) and 1% SDS) followed by sonication (2 min in total) using the bioruptor from Diagenode and addition of nine volumes of IP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA (pH 8.0), 16.7 mM Tris-HCl pH 8.0 and 167 mM NaCl) and magnetic Dynal beads (preclearing of chromatin). For the ChIP of Notch1, the general transcriptional machinery and PRC2 components, we fixed the cells with 1% formaldehyde for 10 min at 25 °C and lysed them using cell lysis buffer (5 mM HEPEs pH 8.0, 85 mM KCl and 0.5% NP-40). We lysed the nuclei using lysis buffer followed by sonication (40 min in total) using the bioruptor from Diagenode and addition of nine volumes of IP dilution buffer and magnetic Dynal beads (preclearing of chromatin). We used standard ChIP procedures adapted to our cell numbers (~1–5×10^6 cells) for ChIP. ChIP-seq libraries were generated using standard Illumina kit and protocol.

Microarray expression data. To generate sufficient sample quantities for oligonucleotide gene chip hybridization experiments, we used the GeneChip Two-Cycle cDNA Synthesis Kit (Affymetrix) for cRNA amplification and labeling. The amplified cRNA was labeled and hybridized to MOE430 Plus 2 oligonucleotide arrays (Affymetrix). Differential expression was determined using a method that evaluates the significance of gene expression fold changes36.

ChIP-seq preprocessing and validation. We aligned the sequenced reads to the genome (mouse assembly NCBI37/mm9) using Burrows-Wheeler alignment37 with maximum two mismatches. The ChIP-seq reads were validated regarding the reproducibility of the replicates, and the sensitivity and specificity of each activating and repressive histone mark in classifying genes of high versus low expression and average read profiles of genes of high expression versus genes of low expression were constructed in TSSs flanked by 10 kb.

Epigenetic and Notch1 data analysis. We determined epigenetic changes between the double-positive and T-ALL samples by evaluating sliding windows across the genome using an approach adapted from PeakSeq. We performed all ChIP-seq read data manipulations using GenomicTools38 (http://code.google.com/p/ibm-bcf-genomic-tools/), and the algorithm for identifying epigenetic changes in T-ALL compared to double-positive was implemented in R language. Notch1 ChIP-seq peaks in T-ALL were determined using MACS39. The data are available using the link http://cbcsrv.watson.ibm.com/prc2-leukemia/.

Data integration. We determined clusters of epigenetic loss or gain in the degree of histone modifications H3K9ac, H3K4me3 and H3K27me3. We used the hypergeometric distribution to compute the P value of the associations between differentially expressed genes.

Subcutaneous and intravenous xenograft studies. For subcutaneous xenograft experiments, we injected 1×10^6 shRNA control and 1×10^6 shRNA EZH2 P12-ichikawa FUW-LUC cells subcutaneously into separate flanks of nonobese diabetic severe combined immunodeficient female mice (n = 6). For intravenous xenograft studies, we injected 1×10^6 shRNA control and 1×10^6 shRNA EZH2 CUTLL1 cells in 6- to 8-week-old Rag2<sup>−/−</sup>Il2rg<sup>−/−</sup> female mice that were sublethally irradiated (n = 7).

Drosophila husbandry. The RNAi transgenic flies and controls of unrelated RNAi lines were obtained from the Bloomington Stock Center at Indiana University and the Vienna Drosophila Stock Center.

Human primary samples. We collected all samples (T-ALL and normal human thymus) with informed consent and under the supervision of local Columbia University Medical Center Institutional Review Board. The primary T-ALL human samples (M69, M106 and M105) used for ChIP experiments were cultured and treated as described previously40.

Microarray-based comparative genomic hybridization (aCGH). We performed aCGH analysis using the SurePrint G3 Human 1x1M oligonucleotide array platform (Agilent) according to the manufacturer’s instructions.

Additional methods. Detailed methodology is described in the Supplementary Methods.

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