Transcriptional Regulation of JARID1B/KDM5B Histone Demethylase by Ikaros, Histone Deacetylase 1 (HDAC1), and Casein Kinase 2 (CK2) in B-cell Acute Lymphoblastic Leukemia*

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Impaired function of the Ikaros (IKZF1) protein is associated with the development of high-risk B-cell precursor acute lymphoblastic leukemia (B-ALL). The mechanisms of Ikaros tumor suppressor activity in leukemia are unknown. Ikaros binds to the upstream regulatory elements of its target genes and regulates their transcription via chromatin remodeling. Here, we report that Ikaros represses transcription of the histone H3K4 demethylase, JARID1B (KDM5B). Transcriptional repression of JARID1B is associated with increased global levels of H3K4 trimethylation. Ikaros-mediated repression of JARID1B is dependent on the activity of the histone deacetylase, HDAC1, which binds to the upstream regulatory element of JARID1B in complex with Ikaros. In leukemia, JARID1B is overexpressed, and its inhibition results in cellular growth arrest. Ikaros-mediated repression of JARID1B in leukemia is impaired by pro-oncogenic casein kinase 2 (CK2). Inhibition of CK2 results in increased binding of the Ikaros-HDAC1 complex to the promoter of JARID1B, with increased formation of trimethylated histone H3 lysine 27 and decreased histone H3 Lys-9 acetylation. In cases of high-risk B-ALL that carry deletion of one Ikaros (IKZF1) allele, targeted inhibition of CK2 restores Ikaros binding to the JARID1B promoter and repression of JARID1B. In summary, the presented data suggest a mechanism through which Ikaros and HDAC1 regulate the epigenetic signature in leukemia: via regulation of JARID1B transcription. The presented data identify JARID1B as a novel therapeutic target in B-ALL and provide a rationale for the use of CK2 inhibitors in the treatment of high-risk B-ALL.

IKZF1 encodes the Ikaros DNA-binding zinc finger protein (1–4). Ikaros is essential for normal hematopoiesis and acts as a tumor suppressor (5, 6). In humans, deletion of a single Ikaros allele is associated with the development of high-risk B-cell precursor acute lymphoblastic leukemia (B-ALL)3 that is characterized by resistance to chemotherapy and poor prognosis (7–9). Alterations in the Ikaros have also been associated with T cell ALL (10, 11) and myeloid leukemias (12–16). Ikaros regulates transcription of its target genes via chromatin remodeling (9). Ikaros has been shown to directly bind histone deacetylases HDAC1 and HDAC2 and to associate with the chromatin remodeling complex NuRD through interaction with the Mi-2 protein (9, 17).

Ikaros is hypothesized to recruit chromatin remodeling complexes to the regulatory elements of its target genes, resulting in chromatin modifications (primarily histone deacetylation) and transcriptional repression or activation of its target genes (18–20). Mechanisms of Ikaros-mediated repression that are independent of histone deacetylation have also been described (18, 21).
This hypothesis is supported by reports that describe Ikaros activity during differentiation of murine thymocytes. Ikaros binds to upstream regulatory elements (UREs) of dntt, the gene encoding terminal deoxynucleotidyl transferase (4), and regulates its transcription (21). The binding of Ikaros to dntt is associated with the induction of epigenetic changes that are characteristic of transcriptionally repressed chromatin.

The function of Ikaros is regulated by posttranslational modifications (22–24). Phosphorylation of Ikaros by oncogenic casein kinase II (CK2) and the role of this phosphorylation in regulating Ikaros activity have been studied most extensively (22, 25). Data from these studies suggest that Ikaros is phosphorylated by CK2 at multiple residues and that CK2-mediated phosphorylation reduces the DNA binding affinity of Ikaros and abolishes its localization to pericentromeric heterochromatin (26, 27). These data led to a model of leukemogenesis in which CK2 promotes malignancy by inhibiting Ikaros function as a transcriptional regulator (28).

Despite extensive studies of Ikaros function in murine hematopoiesis, the molecular mechanisms by which Ikaros regulates transcription of its target genes and its role in the epigenetic control of gene expression in human leukemia remain unknown. Here, we report that Ikaros directly represses transcription of JARID1B (KDM5B) (lysine-specific demethylase 5B) histone demethylase, which specifically demethylates histone H3 at lysine 4. We show that Ikaros represses expression of JARID1B by recruiting the histone deacetylase, HDAC1, to the JARID1B promoter, resulting in epigenetic alterations that lead to the formation of a repressive chromatin environment. Our data suggest that in leukemia, CK2-mediated phosphorylation interferes with Ikaros-mediated repression of JARID1B. We demonstrate that inhibition of CK2 results in the down-regulation of JARID1B and a global increase in trimethylation of histone H3 at lysine 4 (H3K4me3). We propose a model whereby CK2, Ikaros, and HDAC1 regulate H3K4me3 in leukemia via transcriptional control of JARID1B.

Experimental Procedures

Cells, Cell Culture, and Reagents—Nalm6, CCRF-CEM, U937, and MOLT4 leukemia cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and cultured in RPMI 1640 medium (Cellgro) supplemented with 10% fetal bovine serum (Hyclone). HEK293T cells were cultured in DMEM (Cellgro) supplemented with 10% fetal calf serum and 1% l-glutamine (Cellgro). DN3 cells have been described previously (29). MS275 (Cayman), quinalizarin (Calbiochem), PH009215 (2–4(4-methylphenyl)-1,2-benzothiazol-3(2H)-one) (PBIT), and 4,5,6,7-tetrabromobenzotriazole (TBB) were purchased from Sigma. CX-4945 was a gift from Cylene Pharmaceuticals (San Diego, CA). Cells were incubated at 37 °C in a humidified atmosphere of 5% CO2. Primary leukemia and normal cells were obtained from Loma Linda University (Loma Linda, CA) and Children’s Hospital Los Angeles, and their use was approved by the institutional review boards at the respective institutions and at Penn State Hershey College of Medicine. The wild type full-length Ikaros or Ikaros deletion in (IKZ1haploid) cells were cultured briefly (1 day) in the same conditions as Nalm6.

ChIP-seq Experiments—ChIP-seq assays for Ikaros and HDAC1 in leukemia cells were performed as reported previously (30, 31). For the ChIP-seq library, affinity-purified anti-Ikaros antibody (24) or anti-HDAC1 antibody (ab7028) was incubated with chromatin. ChIP-seq libraries were created using the ChIP-seq DNA sample preparation kit (Illumina) and size-selected, and the 200–400-bp fraction was extracted and purified. Libraries were sequenced at the High Throughput Genomics Center at the University of Washington (Seattle, WA). ChIP-seq sequences were generated by an Illumina Hiseq 2000 or Genome Analyzer II system and then were mapped onto HG19 (Human Genome version 19 from NCBI) using the ELAND algorithm at the Genome Sequencing Center, University of Washington (Seattle, WA). CisGenome version 2.0 (32) was used to detect binding peaks on HG19. Parameters lpcut = 1e − 3 and c = 2 were selected. Other parameters remained at default settings for the program run.

Antibodies—The antibodies used to immunoprecipitate and detect the C terminus of Ikaros (Ikaros-CTS) have been described previously (33). The antibodies used for quantitative chromatin immunoprecipitation (qChIP) for epigenetic changes and Western blotting were as follows: anti-H3K9ac (ab4441, Abcam), anti-H3K27me3 (07–449, Millipore), anti-HDAC1 (ab7028, Abcam), anti-HDAC2 (39934, active motif), anti-HDAC3 (3945P, Cell Signaling), anti-HDAC8 (ab137474, Abcam), anti-H3K4me3 (ab8580, Abcam), JARID1B (ab56759, Abcam), proliferating cell nuclear antigen (FL–261, Santa Cruz Biotechnology, Inc.), histone H3 (ab1791, Abcam), actin (SC–1616-R, Santa Cruz Biotechnology), casein kinase II (made by ProSci Inc. against CK2a catalytic subunit-GST), anti-rabbit IgG (ab46540, Abcam), and secondary anti-rabbit IgG (HRP) (ab6721, Abcam).

Cell Proliferation Assay—The cell proliferation assay was performed as described previously (34). Briefly, the colorimetric assay (WST-1 reagent) from Roche Applied Science was performed in 96-well white clear bottom plates (3603, Costar). 104 cells were seeded per well with no treatment or with PBIT treatment at the indicated concentration and cultured for 72 h. The WST-1 reagent was added (10 μl/well) for 4 h, and absorbance at 440 nm was measured using the BioTek Synergy Mx plate reader.

Plasmid Construction—Wild-type human HA-tagged Ikaros (IKZF1) cDNA was cloned into BgilI and EcoRI sites in the pMSCV bicistronic retroviral vector (MIG vector), which contains a 5’ long terminal repeat, internal ribosome entry site, and enhanced green fluorescent protein (35). For construction of the pGL4.15 promoter of JARID1B (pGL4.15/JARID1B), the promoter region of JARID1B (−2.5 kb from the transcriptional start site (TSS)) was amplified by PCR from the genomic DNA of Nalm6 cells by using primers CCCAGGCTCGGCCTAGAGCAGACAAATGCG (forward) and CCAAGACTTAGGCTGGCAGGCGCC (reverse). The PCR product was cloned into the pGL4.15 vector by ScaI and HindIII restriction enzymes (New England Biolabs).

Retroviral Transfer and Cell Sorting—Retroviruses were produced by transient transfection in amphotropic pack-
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aging 293 cell lines as described previously (26). Nalm6 cells were plated in 24-well plates at $4 \times 10^5$ cells/well and suspended in retroviral supernatants with 12 μg/ml Polybrene and centrifuged at 1,400 x g, at 32 °C, for 2 h. The cells were then suspended in fresh 10% FBS RPMI 1640 and cultured at 37 °C, 5% CO₂, for 3 days. The cells were isolated by Ficoll separation, and the GFP(+) cells were sorted using a FACSAria high speed sorter (BD Biosciences). Sorted cells were further cultured followed by RNA isolation.

Luciferase Assay—HEK293T cells were seeded into 24-well plates and transiently transfected with 150 ng of the indicated promoter reporter constructs and 150 ng of pcDNA3.1-Ikaros or pcDNA3.1 vector as a control using Lipofectamine 2000 protocol as above. Luciferase assays were performed following the same protocol as above.

Western blotting, and the optimal gene knockdown shRNA plasmid was selected for further studies. HEK293 cells were carried out with 20 μl of lysate on the dual luciferase reporter assay system (Promega GloMax 20/20 Luminometer). The activity of firefly luciferase was normalized to the activity of Renilla luciferase. Luciferase activities were expressed as -fold change relative to values obtained from pGL4.74 (hRLuc/TK) vector only control cells. Each experiment was performed in triplicate, and all transfection and reporter assays were performed independently at least three times.

HDAC1 siRNA Knockdown in Luciferase Reporter Assay—Four unique 29-mer shRNA constructs for human HDAC1 in GFP vector (pGFP-V-RS) were purchased from Origene. The set of four shRNA plasmids for HDAC1 was tested first by Western blotting, and the optimal gene knockdown shRNA plasmid was selected for further studies. HEK293 cells were transiently transfected with 0.2 μg of scrambled shRNA (siCTL) or HDAC1 shRNA (siHDAC1) plasmids together with 150 ng of the indicated promoter reporter constructs and 150 ng of pcDNA3.1-Ikaros or pcDNA3.1 vector as a control using Lipofectamine 2000 transfection reagent (Invitrogen). The luciferase reporter assays were performed following the same protocol as above.

qChIP—qChIP assays were performed as reported previously (31). Briefly, cells were collected by centrifugation at 2,000 x g for 5 min and cross-linked in cross-link solution containing 1% formaldehyde for 10 min on ice. The reaction was stopped by adding glycine to a final concentration of 0.125 M. Ikaros ChIP samples were prepared as follows: 2 x 10⁷ Nalm6 cells or primary leukemia cells (4–10 x 10⁶) per condition were treated with solution I (50 mM Hepes/KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% Nonidet P-40, 0.25% Triton X-100, protease inhibitor) with rotation at 4 °C for 10 min. The cells were pelleted and treated with solution II (0.2 M NaCl, 1 mM EDTA, pH 8.0, 0.5 mM EGTA, pH 8.0, 10 mM Tris, pH 8.0, protease inhibitor) with rotation for 10 min at room temperature. The chromatin was fragmented in solution III (1 mM EDTA, pH 8.0, 0.5 mM EGTA, pH 8.0, 10 mM Tris, pH 8.0, protease inhibitor) with a Bioruptor (Diagenode) for 14 min (30-s pulses, 90-s pauses) to obtain an average size of 400 bp, and the chromatin was centrifuged at 4,000 rpm for 10 min at 4 °C and added with 10% glycerol. ChIP assays were performed by incubation of the chromatin in buffer (1% Triton X-100, 0.1% deoxycholate, 1× TE, and protease inhibitor) with 20 μl of rabbit polyclonal anti-Ikaros sera or normal rabbit IgG (ab46540, Abcam) or mouse IgG (5415S, Cell Signaling) as control, which was precoated onto goat anti-rabbit IgG or sheep anti-mouse IgG Dynabeads (Invitrogen). Following overnight incubation at 4 °C, protein-DNA complexes were captured with a magnetic particle concentrator (Invitrogen). Beads were washed extensively with radioimmunoprecipitation assay buffer (50 mM Hepes, pH 8.0, 1 mM EDTA, pH 8.0, 1% Nonidet P-40, 0.7% deoxycholate, 0.5 M LiCl) and TE. chromatin-immunoprecipitated DNA was eluted with 50 μl of elution buffer (10 mM Tris, pH 8.0, 1 mM EDTA, 1% SDS). Cross-links were reversed in the presence of 0.6 M NaCl at 65 °C overnight. Samples were treated with proteinase K, extracted with phenol/chloroform, and then treated with RNase A. Finally, the DNA was purified using the QIAquick PCR purification kit (Qiagen).

Enrichment of the ChIP sample over input was evaluated by qPCR with specific primers in the promoter region of target genes. Three or more technical replicates were performed for most sites. -Fold enrichment was calculated by the formula, $2^{-\Delta \Delta CT}$ (sample)/$2^{-\Delta \Delta CT}$ (input), where CT represents threshold cycle number of sample and input. Relative concentration of ChIP-qPCR product is presented as the -fold change of DNA-Ikaros complex level of samples relative to control. qChIP assays of HDAC1 and histone modification markers were performed according to the same protocol as Ikaros qChIP except using 1 x 10⁶ cells and 1–5 μg of antibody for the histone modification markers.

DNA Sequence Analysis—Sequence analysis of the URE of mouse and human JARID1B was performed using the DNA BLAT program.

qChIP Primers—Primers for JARID1B URE were TCCGCCCATGTAACATCATA (sense) and AACCTAGGGACCGGGAGACTCT (antisense). Primers for JARID1B promoter and location relative to TSS were as follows: P1 (bp -507 to -423), AATTAGAACGTGCTAGTATGAAAGT (sense) and CATGTTCATGAGGAGAATGTAGTA (antisense); P2 (bp -354 to -290), CCCCTCGTGGTTTTACTG (sense) and CCCATAAGCAATCATTCAATCA (antisense); P3 (bp -259 to -183), CACCGGCAAGAATCTCTAGTACATAGT (sense) and GGATGGAGTTATACCTTAGGGTTT (antisense); P4 (bp -39 to -22), CGTCGGAGAGCTGTCGAAAAGC (sense) and GCCAAGCGAATCCTGGAGTTG (antisense); P5 (bp +242 to +296), GCCGACCCCTGGCCTT (sense) and TGGCAGTCTGATCGGCTAT (antisense). Primer sequences for qChIP to detect Ikaros binding across the JARID1B start site in parenthesis: URE1 (bp -2,818 to -2,756), AGGATGGAGACACCTAGGGATGT (sense) and CAAACCGCCCTATTTGGTG (antisense); URE2 (bp -2,412 to -2,350), CGCGCGTGTGGCCTGCA (sense) and CTCCTGCCCTTATGATTTTACAT (antisense); URE3 (bp -2,157 to -2,098), CCGGACTGGAGACCTTGA (sense) and TTCCTCGATCGTGCTC (antisense); URE4 (bp -1,943 to -1,880), GCAATGACACTAGAATTTCTG (sense) and GGCTCTTTGTTGCCATT (antisense); URE5 (bp -1,672 to
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Ikaros Binds the URE of JARID1B—To identify Ikaros target genes in leukemia, we determined the genome-wide occupancy of Ikaros in the human B-ALL cell line, Nalm6, using chromatin immunoprecipitation followed by deep sequencing (ChIP-seq). Results show that Ikaros bound to the DNA sequence upstream of the promoter of the JARID1B (KDM5B) gene in Nalm6 cells (Fig. 1A).

Ikaros binding at the JARID1B URE was confirmed by qChIP of leukemia cell lines representing multiple blood lineages: the B-ALL cell line, the U937 acute myeloid leukemia cell line, and the CCRF-CEM early T-cell acute lymphoblastic leukemia (T-ALL) cell line (Fig. 1B), as well as in primary B-ALL, acute myeloid leukemia, and T-ALL cells from leukemia patients (Fig. 1C). Ikaros binding to the URE of JARID1B was not detected in primary B-ALL with haploinsufficiency of Ikaros due to deletion of one allele (Fig. 1C, bar 4). The strong binding of Ikaros at the JARID1B URE in cells from a range of hematopoietic lineages supports the functional relevance of Ikaros in regulating JARID1B expression.

Sequence analysis of the URE of human and mouse JARID1B (KDM5B) revealed a domain where the Ikaros consensus core binding element (5’-GGGA-3’) (35) occurs at high frequency and in close proximity (Fig. 1D). The frequency of core binding elements was 7-fold (human) and 5-fold (mouse) greater than that expected based on a random nucleotide distribution. Comparative analysis of human and mouse genomes within the URE of JARID1B identified the presence of multiple evolutionarily conserved Ikaros consensus binding sites in close proximity to each other, along with several evolutionarily conserved Ikaros core binding elements surrounding the transcriptional start site (TSS) of JARID1B (data not shown). This suggests that the binding of Ikaros to the URE of JARID1B is functionally important.

Ikaros Represses Transcription of JARID1B—The effect of Ikaros binding on transcriptional regulation of JARID1B was determined using gain-of-function and loss-of-function experiments. We used the luciferase reporter assay to analyze the direct effect of Ikaros binding to the JARID1B upstream regulatory region on its transcription. The URE containing the Ikaros binding sites was cloned into a luciferase reporter plasmid. HEK293T cells were each co-transfected with the JARID1B-luciferase constructs and either a control plasmid pcDNA3.1-HA or a plasmid encoding human or mouse Ikaros (pcDNA3.1-HA-hIkaros or pcDNA3.1-HA-mIkaros, respectively). The effect of co-transfection with the murine or human
full-length Ikaros isoform was compared with the negative control with empty plasmid. Results showed that expression from the JARID1B promoter was 2-fold higher without Ikaros as compared with cells that expressed murine or human Ikaros (Fig. 2A). These data suggest that Ikaros can directly repress transcription of JARID1B.

Next, we tested the effect of Ikaros on JARID1B transcription in leukemia. First we used the DN3 cell line, a T cell leukemia derived from the Ikaros knock-out mouse (29). These cells do not express Ikaros. Retroviral transduction of DN3 cells with Ikaros resulted in reduced transcription of JARID1B, as indicated by qRT-PCR (Fig. 2B), when compared with control cells transduced with empty retroviral vector. These data suggest that Ikaros represses JARID1B transcription in leukemia.

Next, we compared JARID1B expression in human Nalm6 B-ALL cells transduced with retrovirus containing wild type Ikaros or with empty retroviral vector (negative control). The effect of Ikaros overexpression on JARID1B transcription was studied using qRT-PCR and at the protein level by Western blotting. Results show that cells with increased Ikaros expression have 2.5-fold reduced transcription of JARID1B, as measured by qRT-PCR (Fig. 2C). Western blotting shows that increased Ikaros protein in Nalm6 cells correlates with reduced JARID1B protein levels (reduced to one-third that observed in control cells (Fig. 2, D and E (left))).
JARID1B acts as a histone H3 demethylase, targeting the histone H3K4me3 epigenetic modification, and thus is one of the primary enzymes controlling the global level of H3K4me3 modifications in the nucleus (36). To determine whether Ikaros overexpression results in changes in the level of H3K4me3 modifications, we performed Western blotting analyses with antibodies specific to Ikaros and to H3K4me3. Our results show that overexpression of Ikaros leads to reduced levels of the JARID1B protein and a 2-fold increase in H3K4me3 histone modifications in Nalm6 cells (Fig. 2, D and E (right)).

To study the effect of reduced Ikaros activity on JARID1B transcription, we targeted Ikaros with shRNA (Fig. 2 F, left). Nalm6 cells transfected with Ikaros shRNA showed increased transcription of JARID1B, as measured by qRT-PCR, when compared with controls (Fig. 2 F, right).

Taken together, gain-of-function and loss-of-function experiments provide evidence that Ikaros acts as a transcriptional repressor of JARID1B and that increased Ikaros activity results in reduced JARID1B, leading to global changes in the histone methylation status of H3K4me3.

Repression of JARID1B by Ikaros Occurs via HDAC1—Next, we studied the mechanism by which Ikaros represses JARID1B. Previous reports show that although Ikaros can directly activate or repress gene transcription, it often regulates gene expression by recruiting chromatin remodeling complexes to the regulatory regions of its target genes (20, 37). In vivo, Ikaros associates with HDAC1, a component of the NuRD histone deacetylase complex (17). We tested whether HDAC1 binds to the URE of JARID1B at the same site as Ikaros in leukemia cells. ChIP-seq experiments demonstrate that HDAC1 binds to the URE of JARID1B in close proximity to Ikaros binding (Fig. 3 A). To confirm HDAC1 occupancy at JARID1B upstream regulatory regions, qChIP assays were performed using HDAC1 antibody on the leukemia cell lines and samples and with the qChIP primers used to determine Ikaros occupancy in Fig. 1. Strong HDAC1 binding was detected at the JARID1B URE in all leukemia cell lines as well as in primary leukemia cells from multiple blood lineages (Fig. 3, B and C). In contrast, no binding of other class I histone deacetylases (HDAC2, HDAC3, and HDAC8) at the JARID1B URE was detected in B-ALL or T-ALL cell lines.
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FIGURE 3. Repression of JARID1B by Ikaros occurs via HDAC1. A, representative ChIP-seq signal map for HDAC1 binding to the JARID1B locus in Nalm6 cells. Arrows, ChIP-seq locations of HDAC1 binding sites that are in close proximity to the TSS of JARID1B. B and C, qChIP analysis of HDAC1, HDAC2, HDAC3, and HDAC8 occupancy at the JARID1B URE (URE) in human leukemia cell lines (B) and in primary human leukemia cells originating from a range of blood lineages (C). D, expression from the URE of JARID1B as measured by a luciferase reporter assay following transfection with human Ikaros (hIK) in the absence (bar 2) or presence (bar 3) of the HDAC inhibitor trichostatin (TSA) or HDAC1-specific inhibitor MS-275 (bar 4) normalized to control vector. Western blots of Ikaros and actin under each condition are shown at the top. Graphed data are the mean ± S.D. (error bars) of triplicate determinations representative of one of three independent experiments. E, additional luciferase reporter assays measured expression from the URE of JARID1B following co-transfection with human Ikaros (hIK) and scrambled siRNA (bar 3) or HDAC1-specific siRNA (bar 4), normalized to control vector. *** p < 0.001.

(Fig. 3B, middle and right) or in primary B-ALL and T-ALL cells (Fig. 3C, middle and right). This suggested that Ikaros binds the URE of JARID1B in complex with HDAC1. Next, we evaluated the functional significance of HDAC1 binding by determining whether histone deacetylase activity is essential for Ikaros-mediated repression of JARID1B. As shown previously in luciferase reporter assays (Fig. 2A), co-transfection of Ikarosdownregulated transcription of JARID1B in 293T cells. However, treatment of 293T cells with the general histone deacetylase inhibitor, trichostatin, abolished Ikaros-mediated repression of JARID1B (Fig. 3D, bar 3). These results suggest that histone deacetylase activity is essential for Ikaros-mediated repression of JARID1B. To determine specifically whether HDAC1 is essential for Ikaros-mediated repression of JARID1B, 293T cells were treated with the HDAC1 inhibitor MS-275, at a concentration that is specific for HDAC1 and does not inhibit other class I histone deacetylases (38–40). Treatment with MS-275 abolished Ikaros-mediated repression of JARID1B (Fig. 3D, bar 4) in a manner similar to that observed when cells were treated with trichostatin. In addition, transfection of 293T cells with the HDAC1-specific siRNA abolished Ikaros-mediated repression of JARID1B (Fig. 3E, bar 4 compared with bar 3). These results suggest that the activity of HDAC1 is essential for Ikaros-mediated repression of JARID1B.
CK2 Inhibits Ikaros-mediated Repression of JARID1B—Our next goal was to determine the signal transduction pathway that regulates Ikaros-mediated repression of JARID1B transcription. Previously published data show that Ikaros is a direct phosphorylation target of CK2 and that phosphorylation of Ikaros by CK2 at its N-terminal serines/threonines reduces its DNA binding ability and alters its subcellular localization (26).

The role of CK2 in Ikaros-mediated repression of JARID1B was studied by a luciferase reporter assay in 293T cells (Fig. 4A). Results showed that co-transfection of Ikaros exhibits stronger repressor activity in the presence of the CK2-specific inhibitor TBB (Fig. 4A, bar 3 compared with bar 2). Next, we used phosphomimetic and phosphoresistant Ikaros mutants to further test whether phosphorylation by CK2 kinase affects Ikaros-mediated repression of JARID1B. Known CK2 phosphorylation sites in Ikaros (serine or threonine) were mutated to alanine (to produce Ikaros phosphoresistant mutants) or to aspartate (to produce Ikaros phosphomimetic mutants). The ability of Ikaros...
phosphomimetic and phosphoresistant mutants to repress transcription from the URE of JARID1B was tested by luciferase reporter assay. Results show that an Ikaros phosphomimetic mutant (with mutations at all 11 amino acids phosphorylated by CK2 (positions 13, 21, 23, 63, 101, 294, 389, 393, 394, 396, and 398, as described previously), mutant IK-A11 (27)) acts as a transcriptional repressor of JARID1B in luciferase assays and represses gene expression at least as well as wild type Ikaros (Fig. 4A, bars 2 and 5). However, the phosphomimetic Ikaros mutant with mutations that mimic phosphorylation at five N-terminal amino acids phosphorylated by CK2 (mutant IK-D5) (26) was unable to repress transcription of JARID1B in the luciferase reporter assay (Fig. 4A, bar 4 compared with bar 2). Treatment with the CK2-specific kinase inhibitor TBB did not result in transcriptional repression of JARID1B (Fig. 4A, bar 6) unless cells were transfected with wild-type Ikaros (Fig. 4A, bar 3 as compared with bar 6). These results suggest that phosphorylation of Ikaros at N-terminal CK2 phosphosites inhibits its ability to repress JARID1B transcription. These data also provide evidence that the inhibition of CK2 does not repress JARID1B transcription in the absence of Ikaros expression.

CK2 activity is increased in leukemia as compared with normal bone marrow (41, 42). Because the results in Fig. 4A suggest that phosphorylation by CK2 can regulate the ability of Ikaros to repress transcription of JARID1B, we hypothesized that increased CK2 activity interferes with Ikaros-mediated repression of JARID1B in leukemia. To test this hypothesis, the effect of CK2 inhibition on Ikaros regulation of JARID1B transcription in leukemia was determined. Molecular inhibition of CK2 function using shRNA against the CK2 catalytic subunit CK2 α resulted in reduced transcription of JARID1B (as measured by qRT-PCR) (Fig. 4B). The effect of pharmacological inhibition of CK2 on JARID1B transcription was tested using three different CK2 inhibitors: TBB, quinalizarin, and CX-4945. TBB has been used as a standard CK2-specific inhibitor for many years; however, quinalizarin exhibited a higher specificity toward CK2 when tested against a panel of 80 kinases (43). CX-4945 is the inhibitor that has the highest known specificity against CK2 (44, 45) and is currently being tested in a Phase 1 clinical trial. The effect of these CK2-specific inhibitors on JARID1B transcription was measured by qRT-PCR in a panel of leukemia cells. Results showed that CK2 inhibition with TBB had a profound repressive effect on JARID1B transcription in all types of leukemia cells tested, except in DN3 cells, a T cell leukemia that is derived from Ikaros knock-out mice and thus lacks the Ikaros (Ikaros) gene (Fig. 4C, column 5). Treatment with quinalizarin and CX-4945 had similar effects on JARID1B transcription in leukemia cell lines (Fig. 4C, middle and right, respectively). Western blotting analysis demonstrated that the inhibition of CK2 also results in reduced expression of the JARID1B protein in various types of leukemia cells (Fig. 4D). Decreased expression of JARID1B was associated with increased levels of the histone H3K4me3 modification as documented by Western blotting (Fig. 4D). Next, we determined whether the presence of Ikaros is necessary for the transcriptional repression of JARID1B following CK2 inhibition. Nalm6 cells were transfected with scrambled or Ikaros shRNA. The effect of CK2 inhibition on the transcription of JARID1B was compared in cells with reduced Ikaros expression and in control cells. Expression analysis shows that the CK2 inhibitor CX-4945 represses JARID1B transcription in cells treated with scrambled shRNA (Fig. 4E, bar 3 versus bar 1). However, the ability of CX-4945 to repress JARID1B is abolished in cells with shRNA knockdown of Ikaros as compared with control cells with scrambled shRNA (Fig. 4E, bar 4, as compared with bar 3, versus bar 1). These results, along with the inability of CK2 inhibitors to repress JARID1B transcription in cells that do not express Ikaros (293T cells (Fig. 4A, bar 6) and DN3 cells (Fig. 4C, column 5)), suggest that the JARID1B repression following CK2 inhibition requires Ikaros function as a transcriptional repressor.

Inhibition of CK2 Enhances Ikaros Binding and Recruitment of HDAC1 to the JARID1B URE—The effect of CK2 inhibition on Ikaros DNA binding affinity toward the JARID1B URE was studied by qChIP. Results show that CK2 inhibition enhances Ikaros DNA binding affinity toward the JARID1B URE in Nalm6 cells (Fig. 5A). In addition, CK2 inhibition is associated with enhanced HDAC1 recruitment, as indicated by the much stronger HDAC1 binding at the regulatory region upstream of JARID1B that spans the area of Ikaros binding (Fig. 5B). These results suggest that CK2 activity regulates Ikaros-mediated repression of JARID1B via direct phosphorylation of Ikaros and possibly by affecting HDAC1 recruitment to the JARID1B URE.

Inhibition of CK2 Kinase Represses JARID1B Expression via Chromatin Remodeling—Both Ikaros and HDAC1 regulate transcription of their target genes via chromatin remodeling by inducing epigenetic changes around the TSS. Because inhibition of CK2 activity results in repression of JARID1B via Ikaros and HDAC1, we tested whether repression of JARID1B is achieved through an epigenetic mechanism. Changes in the epigenetic markings around the TSS of JARID1B were measured by serial qChIP following CK2 inhibition. The five sets of primers used in qChIP spanned the promoter region from bp −500 to +300 relative to the JARID1B TSS (Fig. 5C). Results show that the inhibition of CK2 induces a strong enrichment in H3K27me3 throughout the promoter region of JARID1B (Fig. 5D). In addition, the acetylation of lysine 9 of histone H3 at the JARID1B promoter was diminished following CK2 inhibition (Fig. 5E).

These results demonstrate that in leukemia cells, chromatin at the JARID1B promoter is in an open configuration, permissive to transcription, with strong H3K9 acetylation and the absence of H3K27me3 and high-level expression of JARID1B. CK2 inhibition results in increased Ikaros binding and enhanced recruitment of HDAC1 to the URE of JARID1B. This is associated with the formation of repressive chromatin, characterized by the loss of H3K9ac and the emergence of H3K27me3 at the JARID1B promoter and transcriptional repression of JARID1B.

Inhibition of CK2 Restores Ikaros-mediated Repression of JARID1B in High-risk B-ALL—Deletion of a single Ikaros allele leads to the development of high-risk B-ALL that is characterized by poor prognosis and increased chance of relapse (8). Because CK2 activity interferes with Ikaros function, it has been
hypothesized that the inhibition of CK2 might restore or enhance activity of the remaining Ikaros allele (28). To address this question, we tested whether CK2 regulates Ikaros-mediated repression of JARID1B in primary high-risk B-ALL cells. These cells were obtained from a high-risk B-ALL patient with a deletion in one Ikaros allele resulting in IKhaploid. qChIP analysis of IKhaploid B-ALL cells showed a lack of Ikaros binding at the URE of JARID1B. Following inhibition of CK2 by TBB for 24 h, Ikaros binding to the same URE was detected by qChIP (Fig. 6A, left columns). Similarly, binding of HDAC1 was undetectable in untreated IKhaploid B-ALL cells, but following CK2 inhibition with TBB, HDAC1 binding to the URE of JARID1B in IKhaploid B-ALL cells was demonstrated by qChIP (Fig. 6A, right columns). Both Ikaros and HDAC1 occupancy of the URE of JARID1B were detected by qChIP using two different sets of primers that span a 200-bp region (Fig. 6A) (data not shown). These data suggest that CK2-mediated phosphorylation of Ikaros can abolish the ability of Ikaros to bind the URE of JARID1B in high-risk leukemia. Inhibition of CK2 restores the ability of Ikaros to bind the URE of JARID1B and results in simultaneous recruitment of HDAC1 at this site.

We tested whether restoration of Ikaros binding to the URE of JARID1B following CK2 inhibition affects HDAC1 occupancy at the promoter region of JARID1B in high-risk leukemia. Results showed that HDAC1 did not bind to the JARID1B promoter in untreated high-risk leukemia cells, but inhibition of CK2 (TBB) resulted in HDAC1 occupancy at the JARID1B promoter (Fig. 6B).

These results suggest that recruitment of HDAC1 to the URE and promoter of JARID1B is directly related to Ikaros binding at this site and, thus, that Ikaros binding is the critical step responsible for the regulation of JARID1B transcription following CK2 inhibition.

Expression analysis shows that TBB treatment of IKhaploid B-ALL cells resulted in transcriptional repression of JARID1B, suggesting that the restoration of Ikaros binding and the recruitment of HDAC1 to the URE and promoter of JARID1B leads to transcriptional repression of JARID1B (Fig. 6C).

We tested whether the inhibition of CK2 in primary high-risk IKhaploid B-ALL affects the epigenetic signature around the TSS of JARID1B as it does in the Nalm6 B-ALL cell line. Serial qChIP experiments spanning the JARID1B promoter showed that the inhibition of CK2 induced high levels of the H3K27me3 repressive chromatin mark, along with reduced H3K9ac at the JARID1B promoter (Fig. 6D and E). Taken together, these results demonstrate that the inhibition of CK2 kinase in high-risk B-cell precursor leukemia restores...
Ikaros binding to the JARID1B URE, along with recruitment of HDAC1 and transcriptional repression of JARID1B via chromatin remodeling.

Inhibition of JARID1B Impairs Leukemia Cell Proliferation—We analyzed the impact of Ikaros-mediated repression of JARID1B on leukemia cells. First, we compared expression of JARID1B in primary cells from patients with B-ALL and from normal bone marrow using qRT-PCR. Results showed that JARID1B is strongly up-regulated in B-ALL as compared with normal bone marrow (Fig. 7A). These results are consistent with previously published results (46) and suggest that overexpression of JARID1B might play a significant role in the development of B-ALL.

Molecular inhibition of JARID1B using siRNA has shown that JARID1B is important for hematopoietic stem cell renewal (47, 48). Here, we tested the effect of pharmacological inhibition of JARID1B in primary cells from patients with B-ALL and from normal bone marrow using qRT-PCR. Results showed that JARID1B is strongly up-regulated in B-ALL as compared with normal bone marrow (Fig. 7A). These results are consistent with previously published results (46) and suggest that overexpression of JARID1B might play a significant role in the development of B-ALL.

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Molecular inhibition of JARID1B using siRNA has shown that JARID1B is important for hematopoietic stem cell renewal (47, 48). Here, we tested the effect of pharmacological inhibition of JARID1B on the proliferation of leukemia cells. Leukemia cell lines representing various lineages (as described in the legend to Fig. 1B) were treated with the specific JARID1B inhibitor PBIT (34). Our results show that the inhibition of JARID1B by PBIT leads to severely impaired cellular proliferation (Fig. 7B). This effect is more pronounced in ALL than in acute myeloid leukemia cell lines (Fig. 7B, left versus right). PBIT similarly affects cellular proliferation in primary human leukemia cells (Fig. 7C). These data provide evidence that JARID1B expression and/or activity is essential for leukemia cell growth. These data suggest that one of the mechanisms by which Ikaros exerts its tumor suppressor activity in leukemia involves transcriptional repression of JARID1B and identify JARID1B as a potential therapeutic target in leukemia.

Discussion

Ikaros acts as a tumor suppressor in human leukemia by regulating the expression of a large set of genes. Although in vivo Ikaros binding at the promoter regions of multiple genes has been shown by ChIP-seq experiments, the mechanism through which Ikaros regulates global gene expression remains unknown (49, 50). The studies presented here use gain-of-function and loss-of-function experiments to show that Ikaros represses transcription of JARID1B and regulates the global cellular level of H3K4me3. Our data suggest that Ikaros represses JARID1B by recruiting HDAC1 to the JARID1B promoter, inducing a repressive state of chromatin.

JARID1B contains a JmjC domain that functions to remove tri- and dimethyl groups from lysine 4 of histone H3 (H3K4) (51). H3K4 di- and trimethyl modifications at the TSS are a marker of actively transcribed genes (52–54). Overexpression of JARID1B is associated with breast (55, 56), melanoma (57), prostate (51), and bladder (57, 58) cancers. A search of the Oncomine database (59, 60) showed that JARID1B is up-regulated in multiple types of leukemia (46). JARID1B is important for hematopoietic stem cell renewal (47, 48) and is overex-
pressed in acute myeloid leukemia. Our data demonstrated a strong increase in expression of JARID1B in B-ALL as compared with normal bone marrow. Drug development studies of small molecule inhibitors of JARID1B and vaccine-based immunotherapy directed at JARID1B are ongoing (34, 36, 58). The strong up-regulation of JARID1B expression in leukemia coupled with growth arrest following JARID1B inhibition in various types of leukemia suggests an essential role for JARID1B in leukemia proliferation. This suggests that one mechanism of Ikaros tumor suppression involves negative regulation of JARID1B expression.

CK2 is a pro-oncogenic kinase that is up-regulated in various types of cancer, including leukemia (41). CK2 directly phosphorylates Ikaros at specific amino acids and impairs its function (26, 27). It has been hypothesized that CK2 exerts its pro-oncogenic potential by inhibiting the tumor suppressor activity of Ikaros (28). Several lines of evidence presented here suggest that CK2 regulates JARID1B transcription by impairing Ikaros function as transcriptional repressor: 1) Ikaros mutants that mimic CK2 phosphorylation lose the ability to repress JARID1B expression, whereas phosphoresistant mutants restore repression of JARID1B in luciferase reporter assays (Fig. 2A); 2) both molecular (with shRNA) and pharmacological inhibition (with three different inhibitors) of CK2 result in repression of JARID1B; and 3) CK2 inhibition has no effect on JARID1B repression in cells that do not express Ikaros (e.g. 293T and DN3 cells) or in B-ALL cells with Ikaros knockdown using shRNA. These data suggest that Ikaros has an important and potentially critical role in the regulation of JARID1B transcription in B-ALL. Overall, the data presented here reveal the role of CK2, Ikaros, and HDAC1 in regulating JARID1B transcription via epigenetic modifications and chromatin remodeling. Based on these results, we propose a model by which CK2, Ikaros, and HDAC1 regulate transcription of JARID1B in B-ALL (Fig. 8). In this model, Ikaros, in complex with HDAC1, represses JARID1B transcription by inducing the formation of repressive chromatin with H3K27me3 and loss of H3K9ac. Phosphorylation by CK2 decreases Ikaros DNA binding affinity, which also impairs the recruitment of HDAC1 to the JARID1B promoter, resulting in the loss of H3K27me3-associated repression at the JARID1B promoter.

FIGURE 7. Targeting JARID1B with a specific inhibitor has an anti-leukemia effect. A, qRT-PCR analysis of JARID1B expression in primary normal bone marrow (BM) cells (control) as compared with leukemia samples from patients with B-ALL. n = 17 normal BM and 11 primary B-ALL. B and C, WST-1 cell proliferation assays of leukemia cell lines (B) and primary leukemia cells (C) untreated or treated with various doses of the JARID1B-specific inhibitor, PBIT. Shown are the ratios of absorbance at 440 nm of day 3/day 0 (D3/D0). Graphed in B and C are means ± S.D. (error bars) of triplicate determinations representative of one of three independent experiments.

FIGURE 8. A working model for the regulation of JARID1B transcription in leukemia by CK2, Ikaros, and HDAC1. In leukemia, CK2 phosphorylates Ikaros, which decreases its DNA binding and recruitment of HDAC1 to the URE of JARID1B. The loss of Ikaros-mediated HDAC1 recruitment is associated with the enrichment of H3K9ac and absence of H3K27me3 at the JARID1B promoter and strong expression of JARID1B. Inhibition of CK2 results in dephosphorylation of Ikaros, which increases its DNA binding affinity for the URE of JARID1B, recruitment of HDAC1, and epigenetic changes (loss of H3K9ac and emergence of H3K27me3) at the JARID1B promoter. These changes downstream of CK2 inhibition lead to JARID1B repression and increased levels of H3K4me3 in the cell.
Regulation of JARID1B Expression by Ikaros, HDAC1, and CK2

TSS together with increased H3K9ac and consequently increased transcription of JARID1B. Inhibition of CK2 restores Ikaros-mediated repression of JARID1B via chromatin remodeling as described above. These results suggest the presence of a CK2-Ikaros-HDAC1-JARID1B pathway that is involved in global epigenetic regulation in leukemia. In this model, abrogation of Ikaros-mediated repression of JARID1B is one mechanism by which CK2 exerts its onco-
genic action in leukemia as well as a mechanism by which CK2 regulates the global epigenetic landscape, leading to increased levels of H3K4me3.

The presence of defects in a single Ikaros allele is associated with B-ALL that is at high risk for relapse (8). Defects in a single copy of Ikaros result in the presence of one wild type allele and a second allele that either 1) does not produce Ikaros proteins or 2) produces forms of Ikaros that are defective in DNA binding and which can potentially act as “dominant negative” mutants and inhibit (or impair) the function of the remaining wild type Ikaros. It is debatable whether cells with dominant negative forms of Ikaros have any remaining Ikaros function. The elevated CK2 activity present in leukemia further abolishes the activity of any Ikaros produced by the remaining wild type Ikaros allele. Because Ikaros probably binds DNA as a dimer or multimer (21), we hypothesize that the presence of short, potentially dominant negative, Ikaros isoforms that result from the deletion of one allele do not completely impair the activity of the wild type allele, but rather the loss of Ikaros activity in high-risk leukemia is the result of a combination of one defective allele and the functional inhibition of the wild type Ikaros by CK2. Experiments presented in Fig. 7 were performed on primary high-risk ALL cells that have an Ikaros deletion affecting one allele and resulting in the expression of short DNA-non-binding Ikaros proteins as well as wild type Ikaros. Data shown here suggest that in these cells, Ikaros activity as a repressor of JARID1B is lost but that the inhibition of CK2 restores Ikaros-mediated repression of JARID1B via chromatin remodeling. These data support a model by which both increased CK2 activity and the deletion of a single Ikaros allele result in the loss of Ikaros function in high-risk ALL. More importantly, this suggests that CK2 inhibition can enhance the activity of the remaining wild type Ikaros allele and restore Ikaros tumor suppressor function, at least with respect to the regulation of JARID1B transcription. Recently, CK2 inhibition has been shown to restore Ikaros-mediated repression of the cell cycle-promoting genes and genes that regulate the PI3K pathway in high-risk ALL with deletion of a single Ikaros allele, which supports our proposed model of JARID1B regulation by CK2 and Ikaros (61). CK2 is considered a target for treatment in various types of malignancies, and a phase I clinical trial with CK2 inhibitors has recently been completed for solid tumors. Further testing of this model through studies that examine the role of CK2 inhibition in restoring Ikaros tumor suppressor activity will be important.

In summary, we have presented data suggesting that Ikaros, in complex with HDAC1, regulates the transcription of JARID1B via chromatin remodeling. In leukemia, this process is impaired by the pro-oncogenic kinase, CK2. Treatment with CK2 inhibitors restores Ikaros-mediated repression of JARID1B, causing increased global levels of H3K4me3. These studies provide a mechanistic rationale for the use of CK2 inhibitors as a potential therapeutic option in leukemia and suggest the presence of a CK2-Ikaros-HDAC1-JARID1B pathway that controls global epigenetic regulation in this disease.

Author Contributions—S. D. conceived and coordinated the study and wrote the paper. H. W., C. S., and K. J. P. wrote the paper. X. P., Z. G., C. S., Y. D., O. L. F., and K. J. P. designed, performed, and analyzed the experiments shown in Fig. 1. H. W., C. G., G. D., Y. D., Z. G., M. X., and C. S. designed, performed, and analyzed the experiments shown in Fig. 2. H. W., Z. G., and B.-H. T. designed, performed, and analyzed the experiments shown in Fig. 3. H. W., Y. D., S. M., and C. S. designed, performed, and analyzed the experiments shown in Fig. 4. H. W., Z. G., and C. G. designed, performed, and analyzed the experiments shown in Fig. 5. H. W., S. M., M. S., and Z. G. designed, performed, and analyzed the experiments shown in Fig. 6. H. W., O. L. F., C. L. M., K. J. P., H. A. A., M. X., and M. S. designed, performed, and analyzed the experiments shown in Fig. 7. H. O. and L. L. analyzed and reviewed the data. All authors contributed to interpretation of data, manuscript revision, and critical discussion. All authors reviewed the results and approved the final version of the manuscript.

References

1. Molnár, A., and Georgopoulos, K. (1994) The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. Mol. Cell Biol. 14, 8292–8303
2. Georgopoulos, K., Moore, D. D., and Derfler, B. (1992) Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. Science 258, 808–812
3. Lo, K., Landau, N. R., and Smale, S. T. (1991) LyF-1, a transcriptional regulator that interacts with a novel class of promoters for lymphocyte-specific genes. Mol. Cell. Biol. 11, 5229–5243
4. Ernst, P., Hahm, K., and Smale, S. T. (1993) Both LyF-1 and an Ets protein interact with a critical promoter element in the murine terminal transferase gene. Mol. Cell. Biol. 13, 2982–2992
5. Georgopoulos, K., Bigby, M., Wang, J. H., Molnár, A., Wu, P., Winandy, S., and Sharpe, A. (1994) The Ikaros gene is required for the development of all lymphoid lineages. Cell 79, 143–156
6. Winandy, S., Wu, P., and Georgopoulos, K. (1995) A dominant mutation in the Ikaros gene leads to rapid development of leukemia and lymphoma. Cell 83, 289–299
7. Mullighan, C. G., Goorha, S., Radtke, I., Miller, C. B., Coustan-Smith, E., Dalton, D. J., Girtman, K., Mathew, S., Ma, J., Pounds, S. B., Xu, X., Pui, C. H., Relling, M. V., Evans, W. E., Shurtleff, S. A., and Downing, J. R. (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukemia. Nature 446, 758–764
8. Mullighan, C. G., Su, X., Zhang, J., Radtke, I., Phillips, L. A., Miller, C. B., Ma, J., Liu, W., Cheng, C., Schulman, B. A., Harvey, R. C., Chen, I. M., Clifford, R. J., Carroll, W. L., Reaman, G., Bowman, W. P., Devidas, M., Gerhard, D. S., Yang, W., Relling, M. V., Shurtleff, S. A., Campana, D., Borowitz, M. J., Pui, C. H., Smith, M., Hunger, S. P., Willman, C. L., Downing, J. R., and Children’s Oncology Group (2009) Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N. Engl. J. Med. 360, 470–480
9. Kim, J., Sif, S., Jones, B., Jackson, A., Koipally, J., Heller, E., Winandy, S., Viel, A., Sawyer, A., Ikeda, T., Kingston, R., and Georgopoulos, K. (1999) Ikaros DNA-binding proteins direct expression of chromatin remodeling complexes in lymphocytes. Immunity 10, 345–355
10. Zhang, J., Ding, L., Hofmefldt, L., Wu, G., Heatley, S. L., Payne-Turner, D., Easton, J., Chen, X., Wang, J., Ruscit, M., Lu, C., Chen, S. C., Wei, L., Collins-Underwood, J. R., Ma, J., Roberts, K. G., Pounds, S. B., Ulyanov, A., Becksfort, J., Gupta, P., Huetter, R., Kriwacki, R. W., Parker, M., McGoldrick, D. J., Zhao, D., Alford, D., Espy, S., Bobba, K. C., Song, G., Pei, D.,
11. Sun, L., Heerema, N., Crotty, L., Wu, X., Navara, C., Sensel, M., Reaman, G. H., and Uckun, F. M. (1999) Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. Proc. Natl. Acad. Sci. U.S.A. 96, 680–685

12. Yagi, T., Hibi, S., Takashani, M., Kano, G., Tabata, Y., Imamura, T., Inaba, T., Morimoto, A., Todo, S., and Imashuku, S. (2002) High frequency of Ikaros isoform 6 expression in acute myelomonocytic and monocytic leukemias: implications for up-regulation of the antiapoptotic protein Bcl-XL in leukemogenesis. Blood 99, 1350–1355

27. Popescu, M., Gurel, Z., Ronni, T., Song, C., Hung, K. Y., Payne, K. J., and Dovat, S. (2012) Cell cycle-specific function of Ikaros in human leukemia. Pediatr. Blood Cancer 59, 69–76

14. Tefferi, A. (2010) Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms. Leukemia 24, 1290–1298

24. Gomes, A. M., Soares, M. V., Ribeiro, P., Caldas, J., Povoa, V., Martins, J. R., and Altucci, L. (2008) Novel uracil-based 2-aminooxazine and 2-aminoauinidine-like derivatives: histone deacetylation inhibitor and in-cell activities. Bioorg. Med. Chem. Lett. 18, 2530–2535

15. Theocharides, A. P., Dobson, S. M., Laurenti, E., Notta, F., Voisin, V., Cheng, P. Y., Yuan, J. S., Guidos, C. J., Minden, M. D., Mullighan, C. G., Torlakovic, E., and Dick, J. E. (2015) Dominant-negative Ikaros cooperates with BCR-ABL1 to induce human acute myeloid leukemia. Proc. Natl. Acad. Sci. U.S.A. 112, 782–796

25. Li, H., Jiang, H., Ma, W., Johnson, D. S., Myers, R. M., and Wong, W. H. (2008) An integrated software system for analyzing ChIP-chip and ChIP-seq data. Nat. Biotechnol. 26, 1293–1300

16. Dovat, S., Ronni, T., Song, C., Payne, K. J., and Li, Z. (2011) Ikaros CK2 kinase, and the road to leukemia. Mol. Cell Biochem. 356, 201–207

34. Khan, N., Jeffers, M., Kumar, S., Hackett, C., Boldog, F., Khramtsov, N., Bliesath, J., Omori, M., Huser, N., Proffitt, C., Schwaebe, M. K., Blain, Y. A., Choi, K., Farnham, P. J., and Bresnick, E. H. (2009) Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy. Mol. Cell 36, 667–681

17. Koipally, J., and Georgopoulos, K. (2000) Ikaros interactions with CtBP reveal a repression mechanism that is independent of histone deacetylase inhibitors. Oncogene 19, Koipally, J., and Georgopoulos, K. (2002) Ikaros-CtIP interactions do not regulate TdT transcription in CD4+ T-cell complexes at centromeric heterochromatin. J. Biol. Chem. 277, 7469–7474

18. Jäger, R., Gisslinger, H., Passamonti, F., Rumi, E., Berg, T., Gisslinger, B., Pietra, D., Harutyunyan, A., Klampfl, T., Olayciu, D., Cazzola, M., and Kralovics, R. (2010) Deletions of the transcription factor Ikaros in myeloproliferative neoplasms. Leukemia 24, 1128–1138

26. Bliesath, J., Omori, M., Huser, N., Proffitt, C., Schwaebe, M. K., Blain, Y. A., Choi, K., Farnham, P. J., and Bresnick, E. H. (2009) Regulation of JARID1B Expression by Ikaros, HDAC1, and CK2
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10288–10298

45. Battistutta, R., Cozza, G., Pierre, F., Papinutto, E., Lolli, G., Sarno, S., O’Brien, S. E., Siddiqui-Jain, A., Haddad, M., Anderes, K., Ryckman, D. M., Meggio, F., and Pinna, L. A. (2011) Unprecedented selectivity and structural determinants of a new class of protein kinase CK2 inhibitors in clinical trials for the treatment of cancer. Biochemistry 50, 8478–8488

46. Haferlach, T., Kohlmann, A., Wieczorek, L., Basso, G., Kronnie, G. T., Béné, M. C., De Vos, J., Hernández, J. M., Hofmann, W. K., Mills, K. I., Gilkes, A., Chiaretti, S., Shurtleff, S. A., Kipps, T. J., Rassenti, L. Z., Yeoh, A. E., Papenhausen, P. R., Liu, W. M., Williams, P. M., and Foà, R. (2010) Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. J. Clin. Oncol. 28, 2529–2537

47. Cellot, S., Hope, K. J., Chagroui, J., Sauvageau, M., Deneault, É., MacRae, T., Mayotte, N., Wilhelm, B. T., Landry, J. R., Ting, S. B., Krosl, J., Humphries, K., Thompson, A., and Sauvageau, G. (2013) RNAi screen identifies Jarid1b as a major regulator of mouse HSC activity. Blood 122, 1545–1555

48. Stewart, M. H., Albert, M., Sroczynska, P., Cruickshank, V. A., Guo, Y., Cellot, S., Hope, K. J., Chagroui, J., Sauvageau, M., Deneault, É., MacRae, T., Mayotte, N., Wilhelm, B. T., Landry, J. R., Ting, S. B., Krosl, J., Humphries, K., Thompson, A., and Sauvageau, G. (2013) RNAi screen identifies Jarid1b as a major regulator of mouse HSC activity. Blood 122, 1545–1555

49. Zhang, J., Jackson, A. F., Naito, T., Dose, M., Seavitt, J., Liu, F., Heller, E. J., Kashiwagi, M., Yoshida, T., Gounari, F., Petrie, H. T., and Georgopoulos, K. (2012) Harnessing of the nucleosome-remodeling-deacetylase complex controls lymphocyte development and prevents leukemogenesis. Nat. Immunol. 13, 86–94

50. Ferreirós-Vidal, I., Carroll, T., Taylor, B., Terry, A., Liang, Z., Bruno, L., Dharmalingam, G., Khadaye, S., Cobb, B. S., Smale, S. T., Spivakov, M., Srivastava, P., Petretto, E., Fisher, A. G., and Merkenschlager, M. (2013) Genome-wide identification of Ikaros targets elucidates its contribution to mouse B-cell lineage specification and pre-B-cell differentiation. Blood 121, 1769–1782

51. Xiang, Y., Zhu, Z., Han, G., Ye, X., Xu, B., Peng, Z., Ma, Y., Yu, Y., Lin, H., Chen, A. P., and Chen, C. D. (2007) JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. Proc. Natl. Acad. Sci. U.S.A. 104, 19226–19231

52. Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, I., Bernstein, B. E., Emre, N. C., Schreiber, S. L., Mellor, I., and Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. Nature 419, 407–411

53. Bernstein, B. E., Humphrey, E. L., Erlich, R. L., Schneider, R., Bouman, P., Liu, J. S., Kouzarides, T., and Schreiber, S. L. (2002) Methylation of histone H3 Lys 4 in coding regions of active genes. Proc. Natl. Acad. Sci. U.S.A. 99, 8695–8700

54. Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wegschal, A., Feil, R., Schreiber, S. L., and Lander, E. S. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326

55. Barrett, A., Madsen, B., Copier, J., Lu, P. J., Cooper, L., Scibetta, A. G., Burchell, J., and Taylor-Papadimitriou, J. (2002) Characterisation and developmental expression of mouse Plu-1, a homologue of a human nuclear protein (PLU-1) which is specifically up-regulated in breast cancer. Mech. Dev. 119, S239–S246

56. Rhodes, D. R., Yu, J., Shanker, K., Deshpande, N., Varambally, R., Ghosh, D., B arte r e t t, T., Pandey, A., and Chinnaiyan, A. M. (2004) ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia 6, 1–6

57. Rhodes, D. R., Kalyana-Sundaram, S., Mahavisno, V., Varambally, R., Yu, J., Briggs, B. B., Barrette, T. R., Anstet, M. J., Kincead-Beal, C., Kulkarni, P., Varambally, S., Ghosh, D., and Chinnaiyan, A. M. (2007) Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. Neoplasia 9, 166–180

58. Song, C., Gowda, C., Pan, X., Ding, Y., Tong, Y., Tan, B. H., Wang, H., Muthusami, S., Ge, Z., Sachdev, M., Amin, S. G., Desai, D., Gowda, K., Robertson, G. P., Schijven, H., Muschen, M., Payne, K. I., and Dovat, S. (2015) Targeting casein kinase II restores Ikaros tumor suppressor activity and demonstrates therapeutic efficacy in high-risk leukemia. Blood 126, 1813–1822