A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and Therapeutic Targets in Acute Myeloid Leukemia

Highlights

- Optimized CRISPR platform for identification of genome-wide genetic vulnerabilities
- Catalog of genetic vulnerabilities in acute myeloid leukemia cell lines
- KAT2A inhibition induces myeloid differentiation and apoptosis
- KAT2A inhibition arrests the growth of primary AML cells, but not of normal progenitors

In Brief

Tzelepis et al. optimize a CRISPR-Cas9-based platform for the performance of genome-wide recessive screens and apply it to identify genetic vulnerabilities of human AML cells. They identify several known therapeutic targets including BRD4, DOT1L, and MEN1, and numerous additional candidates. They provide data proposing KAT2A as a potential therapeutic target.
A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and Therapeutic Targets in Acute Myeloid Leukemia

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SUMMARY

Acute myeloid leukemia (AML) is an aggressive cancer with a poor prognosis, for which mainstream treatments have not changed for decades. To identify additional therapeutic targets in AML, we optimize a genome-wide clustered regularly interspaced short palindromic repeats (CRISPR) screening platform and use it to identify genetic vulnerabilities in AML cells. We identify 492 AML-specific cell-essential genes, including several established therapeutic targets such as DOT1L, BCL2, and MEN1, and many other genes including clinically actionable candidates. We validate selected genes using genetic and pharmacological inhibition, and chose KAT2A as a candidate for downstream study. KAT2A inhibition demonstrated anti-AML activity by inducing myeloid differentiation and apoptosis, and suppressed the growth of primary human AMLs of diverse genotypes while sparing normal hemopoietic stem-progenitor cells. Our results propose that KAT2A inhibition should be investigated as a therapeutic strategy in AML and provide a large number of genetic vulnerabilities of this leukemia that can be pursued in downstream studies.

INTRODUCTION

The successful adaptation of the Streptococcus pyogenes-derived type II clustered regularly interspaced short palindromic repeats (CRISPR)-Cas system for genome editing is transforming the landscape of genetic research in many organisms (Cho et al., 2013; Cong et al., 2013; Jinek et al., 2012; Mali et al., 2013). Furthermore, the system’s high efficiency and flexibility make it ideal for use in genome-wide recessive genetic screens. In fact, recent proof-of-principle studies have demonstrated the potential of this technology to identify cell-essential genes in mammalian cells (Koike-Yusa et al., 2014; Shalem et al., 2014; Shi et al., 2015; Wang et al., 2014). Previously, this was typically conducted using RNA interference (RNAi) in the form of short interfering RNA (siRNA) or short hairpin RNA (shRNA) libraries (Boutros and Ahringer, 2008; Luo et al., 2009; Schlabach et al., 2008; Silva et al., 2008; Zuber et al., 2011). Such screens have made important contributions to biology, but their success has been moderated by the varying efficiencies of siRNAs/shRNAs for the stringent and specific suppression of target genes required for genome-wide studies (Boutros and Ahringer, 2008; CRISPR-Cas9-based functional genomics may be able to overcome such limitations and, therefore, hold great promise in re-shaping cell-essentiality screens. In cancer research, such screens can be applied to identify genetic vulnerabilities of cancer cells that can be used to develop new anti-cancer treatments. Recent reports on CRISPR screens on several cancer cell lines have demonstrated their power (Hart et al., 2015; Wang et al., 2015). A human malignancy in urgent need of additional therapies is acute myeloid leukemia (AML), a devastating disorder with a long-term survival rate of less than 30% (Ferrara and Schiffer, 2013). Steady progress in deciphering its molecular pathogenesis has been made over the last few decades with a dramatic acceleration in recent years, particularly as a consequence of advances in cancer genomics (Cancer Genome Atlas Research Network, 2013; Welch et al., 2012). Despite such progress, the therapeutic landscape of AML has changed little.
for 40 years, with cytarabine still representing the last significant advance (Evans et al., 1961). Although the improved molecular understanding of AML permits some optimism that progress may be forthcoming, an alternative approach for the identification of therapeutic targets is the agnostic interrogation of AML genomes for genetic vulnerabilities using the CRISPR-Cas9 technology. Here, we make significant improvements in this technology and apply these to perform such a screen in AML.

**RESULTS**

**Optimization of Genome-wide CRISPR-Cas9 Dropout Screens**

We and others have demonstrated that the CRISPR-Cas9 system can be adapted for use in functional screens in the form of pooled guide RNA (gRNA) libraries, and that enrichment screens for genes whose inactivation confers resistance to toxins, chemotherapeutics, and targeted cancer treatments can be successfully conducted (Koike-Yusa et al., 2014; Shalem et al., 2014; Wang et al., 2014; Zhou et al., 2014). However, when we applied statistical analyses (Li et al., 2014) to our own genome-wide screen data in mouse embryonic stem cells (ESCs), we were able to identify only a small number of genes depleted to significant levels (Figure 1A). We reasoned that this may be secondary to non-uniform CRISPR-Cas9 efficiency across the large numbers of gRNAs in the library, leading to reduced technical and statistical robustness. To identify factors that affect gRNA efficiency, we first compared nucleotide composition between efficient and inefficient gRNAs in the mouse ESC screen. This analysis revealed strong nucleotide biases between positions 16 and 20 (Figure 1B). These biases also have been observed in human cells (Wang et al., 2014) as well as Caenorhabditis elegans (Farboud and Meyer, 2015), suggesting that they may be an intrinsic feature of the current S. pyogenes CRISPR-Cas9 platform.

To increase CRISPR-Cas9 efficiency, we first tested a gRNA scaffold optimized for CRISPR imaging (Chen et al., 2013) and found that, consistent with the results shown in a recent report (Dang et al., 2015), gRNAs with the improved scaffold exhibited significantly higher knockout efficiency than those with the conventional scaffold (Figures S1A and S1B). In addition, to generate an optimal gRNA library, we re-designed gRNAs for the mouse genome using a new design pipeline (see Supplemental Experimental Procedures) and generated a murine lentiviral gRNA library (version 2 [v2]) composed of 90,230 gRNAs targeting a total of 18,424 genes (Table S1). We then tested the performance of the v2 library, with regard to depletion (dropout) of genes, with the same experimental setting as with our first version (v1). With the optimized platform, many more genes were depleted at statistically significant levels (360 and 1,680 genes depleted at a false discovery rate [FDR] of 0.1 with the v1 and v2 library, respectively; Figure 1C; Data S1). Furthermore, the nucleotide biases observed in v1 were not observed in the v2 library (Figure 1D), indicating that on-target efficiency prediction (Doench et al., 2016; Wang et al., 2015) may not be necessary with the improved gRNA scaffold. The abundances of gRNAs targeting non-expressed genes (fragments per kilobase transcript per million mapped reads [FPKM] ≤ 0.5) remained the same as the initial pool (plasmid), whereas large numbers of gRNAs with increased or decreased abundance in surviving ESCs were readily observed for expressed genes (FPKM > 0.5) (Figure 1E). At the gene level, the vast majority of depleted genes were expressed at FPKM > 0.5 in mouse ESCs (Figures 1F and 1G). Taken together, these data show that the sensitivity of our optimized CRISPR dropout screens for detecting cell-essential genes is markedly increased, whereas the off-target effects are negligible.
The Kolmogorov-Smirnov test was used in (A). See also Figures S1–S3, Tables S1, S2, and Data S2.

We proceeded to perform dropout screens in clonal Cas9-expressing HT-29 cells. Cells were harvested every 3 days from days 7 to 25 after transduction, and gRNA sequencing was performed (Data S2). As with the mouse ESC screen, a comparison between the screening results and RNA sequencing (RNA-seq) data revealed that the vast majority of depleted genes were expressed in HT-29 cells (Figures S3A and S3B), indicating that off-target effects were also negligible in our human CRISPR library. We identified approximately 2,000 depleted genes at a cutoff of FDR 20% and found that essential biological processes were enriched among them (Figures S3C–S3E).

Cancer cells often exhibit genomic instability associated with multiple copy number alterations (Beroukhim et al., 2010; Bignell et al., 2010; Zack et al., 2013). To investigate whether copy number affects CRISPR efficiency, we analyzed the distributions of dropout p values for individual genes according to their copy numbers and found no noticeable differences in dropout efficiency for genes with up to five copies (Figure 2A), although genes with three copies showed a modest but statistically significant reduction (adjusted \( p = 0.0217 \)). By contrast, genes with eight copies, located on the Myc-centered distal region on chromosome 8, displayed a depletion pattern, which was very distinct to that of the surrounding region (Figures 2B and S2A).

A similar depletion pattern in a continuous chromosome segment was previously observed in a highly amplified region in K562 cells.
These results indicate that most genomic locations are amenable to dropout even when amplified and that knowledge of genome-wide copy number can help interpretation of genome-wide screens.

To investigate the timing of cell-essential gene depletion, we performed a longitudinal dropout analysis using the HT-29 dataset. A quarter of genes that were depleted at day 25 were already depleted by day 7, but the remaining cell-essential genes were depleted during the next 18 days (Figure S3D). An unsupervised cluster analysis of the depletion patterns identified seven clusters (Wang et al., 2015). These results indicate that most genomic disruptions are almost exclusively derived from those expressed at FPKM > 1000 (Figure 3A). We first determined that significantly depleted genes were likely to have phenotypic consequences on cellular growth and/or survival. We also compared dropout efficiency of known cell-essential genes according to the number of copies of the chromosomes on which they are located and we found no significant difference (Figures S5G–S5K), indicating that Cas9 disrupted genes equally effectively irrespective of copy number in our AML cell lines.

To identify AML-specific vulnerabilities, we focused on genes depleted in one or more AML, but not in either of the non-AML cell lines. Libraries identified essential genes with high precision and performance in human cancer cells. Our findings establish a technical framework for the performance and interpretation of genome-wide dropout screens using the CRISPR-Cas9 technology.

Identification of Genetic Vulnerabilities in AML

Having optimized our platform, we proceeded to perform genome-wide dropout screens in five AML cell lines (MOLM-13, MV4-11, HL-60, OCI-AML2, and OCI-AML3) and the fibrosarcoma line HT-1080 as a second non-AML reference. Similar to HT-29, bulk Cas9-expressing cells included a fraction of cells without Cas9 activity, but single-cell cloning effectively eliminated this population and showed uniform Cas9 activity (Figures S4A and S4B). The karyotypes of the selected Cas9-expressing clones were analyzed for all AML lines and found to agree closely with the published karyotypes of the parental lines (Figures S4C and S4D). The selected clones were transduced with the human CRISPR library, cultured for 30 days, and harvested to determine their gRNA content (Table S3; Data S2). The genome-wide screens, performed using two biological replicates per line, identified circa 1,000–1,500 depleted genes in each AML cell line (Figure 3A). We first determined that significantly depleted genes were almost exclusively derived from those expressed at FPKM > 0.5 in the corresponding cell line (Figures S5A–S5F), showing that off-target effects were very limited and that gene dropout libraries identified essential genes with high precision and performance in human cancer cells. Our findings establish a technical framework for the performance and interpretation of genome-wide dropout screens using the CRISPR-Cas9 technology.
66 genes essential to three or more and 5 genes essential to all five AML cell lines. Gene ontology analysis of these genes showed particular enrichment in processes pertaining to chromatin formation and modification and transcriptional regulation (Figure 3C), in keeping with the fact that AML is driven by corrupted epigenetic and transcriptional networks.

We also specifically checked for depletion of driver mutations present in the AML cell lines screened. First, we looked at MLL (also known as KMT2A) and found that gRNAs targeting the exons upstream of the MLL breakpoint region, and therefore predicted to disrupt the MLL-AF9 and MLL-AF4 oncogenes, were depleted in both MOLM-13 and MV4-11 (Figure 3D). In addition, gRNAs against FLT3 and NRAS showed specific depletion in cell lines carrying activating mutations in these genes, whereas NPM1 was depleted in four of the five AML lines including OCI-AML3 (Figure 3E). Interestingly, BCL2 was depleted in all AML cell lines except OCI-AML3, which carries a BAX pE41fs*33 mutation (Figure 3E), suggesting BAX mutations as candidate mediators of resistance to BCL2 inhibitors, a promising therapeutic strategy in AML (Chan et al., 2015; Pan et al., 2014).

**Genetic and Pharmacological Validation of the Screening Results**

To validate the results of our screen, we first demonstrated genetically the cell-essential nature of the five dropout genes shared by all AML cell lines (Figure S5M). We then selected eight dropout genes and a control non-dropout gene (HDAC6) for targeted inhibition using genetic and pharmacological approaches. We first followed a gene-by-gene knockout approach using the CRISPR-Cas9 system. Two gRNAs (one from our library and one new) were designed per gene, and the relative growth of gRNA-transduced and non-transduced cells were compared in competitive co-culture assays. Results were in close agreement with the findings of our dropout screens (Figures 4A, 4B, and S6N). We then tested the ability of existing clinical compounds to inhibit the growth of the five AML cell lines and again found these to be in consonance with the findings of our genome-wide screens (Figure 4C). MAP2K1 (also known as MEK1) and MAP2K2 (also known as MEK2) are thought to have redundant functions, but OCI-AML2 was sensitive to depletion of either gene. To test MEK1/2 dependency in the other AML cell lines, we devised a lentiviral dual gRNA expression vector (Figures S1C and S1E) and found that HL-60 and OCI-AML3 were sensitive only to double MEK1/2 knockout. This differential sensitivity to MEK1/2 genetic perturbation was mirrored in responses to the dual MEK1/2 inhibitor trametinib.

Reassured by the concordance between the results of our screening and validation experiments, we searched the “druggability” of the 492 genes specifically depleted in our AML cell lines using the Drug Gene Interaction database (DGIdb) (Griffith et al., 2013) and found that 227 (46%) of the genes are in druggable categories (Figure 4D; Table S4). Among these were 33 genes, for which “clinically actionable” compounds are available, which overlap only partially with the “Kinase” and “histone modification” categories. However, the majority of genes in the druggable categories were not previously considered potential therapeutic targets (Figure 4D). Of note, at least 12 dropout genes, including BRD4, that were essential to at least three AML cell lines, as well as to HT-29 and HT-1080, are targets of clinical inhibitors (Table S4), indicating that “pan-essential” genes should not be dismissed as potential therapeutic targets.

**Selection of Rational Therapeutic Development Targets**

Our approach thus far has enabled us to define a set of genes that are essential to AML, but not to either of two solid cancer cell lines. However, it is probable that some of these AML-essential genes are also essential to normal blood cells including hemopoietic stem cells (HSCs), and such genes may not represent plausible therapeutic targets. Because currently no methods are available for systematic identification of essential genes in normal HSCs, we took an alternative strategy to identify therapeutic targets. In particular, we hypothesized that genes displaying cell line or oncogene specificity were less likely to cause toxicity to normal HSCs but could still be relevant to multiple AML genotypes. To do this, we compared the cell-essential genes of the MOLM-13 and MV4-11 cell lines. These both carry an internal tandem duplication in the FLT3 gene (FLT3-ITD) and multiple copies of chromosome 8, and exhibit comparable response to DOT1L and BRD4 inhibitors, but harbor the distinct, but related, fusion genes, MLL-AF9 (MOLM-13) and MLL-AF4 (MV4-11), known to directly establish leukemogenic transcriptional programs. Looking at the depleted genes (FDR < 0.2), we noted that MOLM-13 and MV4-11 showed significant overlap, but also many differences (Figure 5A; Table S3). Among these differentially essential genes, we selected two druggable genes for further study: the histone acetyltransferase gene KAT2A (also known as GCN5) and the spliceosome kinase gene SRPK1. We also chose CHEK1, a known therapeutic target (Daud et al., 2015; Zabludoff et al., 2008), as a control gene with a similar depletion pattern in both. In addition, we chose AURKB and HDAC6 as control essential genes to both and HDAC6 as essential to neither cell line (Figure 5A).

To test whether the observed essentialities for KAT2A and SRPK1 are indeed attributable to the different MLL oncogenic fusions rather than other differences between MOLM-13 and MV4-11, we developed a genetically defined experimental model (Figure 5B). First, we generated mice expressing Cas9 constitutively under the control of the ubiquitous EF1a promoter from the Rosa26 locus (Figure S6). Rosa26Cas9/− mice reproduced in the expected Mendelian ratios exhibited normal long-term survival and had normal hemopoietic stem and progenitor cell numbers, and normal proportions of blood cell subtypes (Figures 5C, 5D, and 5E). Cas9-expressing stem-progenitor cells exhibited comparable colony-forming and serial replating activity to wild-type (WT) cells and displayed highly efficient Cas9 function (Figures 5E and 5F). These results indicate that Cas9 expression has no detectable effect on the hemopoietic system and that any phenotype observed in gRNA-expressing cells is likely caused by genetic perturbation of a target gene. Rosa26Cas9/− mice were crossed to FIt3+/− mice (Lee et al., 2007), and lineage-negative hemopoietic progenitors from Rosa26Cas9/−;FIt3+/− double transgenic mice
were transduced with retroviral vectors expressing MLL-AF4 (Montes et al., 2011) or MLL-AF9 (Dawson et al., 2011) and cultured in vitro for 10–12 days, displaying similar exponential growth rates and a myeloid phenotype (Figure 5G). The cells were then independently transduced with individual lentiviruses carrying one of two gRNAs against the Kat2a, Srpk1, Aurkb, Chek1, Hdac3, and Hdac6 genes. In keeping with the results of our screen, this revealed significant differences in cell growth between MLL-AF4- and MLL-AF9-driven cells transduced with Chek1, Kat2a, and Srpk1 gRNAs, whereas Aurkb and Hdac3...
gRNAs were equally effective against both cell types (Figure 5H). In addition, we tested the essentiality of each gene to the non-leukemic mouse multipotent HPC-7 cells, which represent an early blood stem-progenitor cell and are capable of generating functional hematopoietic cells in vivo (Wilson et al., 2016). As shown in Figure 5H, all three of the MLL-AF9-specific essential genes had no effects on proliferation of HPC-7 cells. Taken together, these results support our strategy to use oncogene-specific essentialities for candidate prioritization and provide genetic evidence that KAT2A and SRPK1 are attractive drug...
targets. We chose to investigate KAT2A further because this gene is essential to three of the five AML cell lines studied (MOLM-13, OCI-AML2, and OCI-AML3), and as such may be relevant to a wider group of AML patients. Of note, the OCI-AML3 line carries a mutation in NPM1, which is also found in 25%–35% of primary AMLs.

Mechanistic Insights into the Effects of KAT2A Inhibition in AML

First, using two separate gRNAs, we confirmed that genetic disruption of KAT2A reduced the growth of MOLM-13, OCI-AML2, and OCI-AML3, but not MV4-11 and HL-60 (Figures 6A and 5N). We confirmed that targeting with KAT2A-specific
The Student's t test was performed in (B), (E), and (F). See also Table S5. N, normal karyotype; ND, not determined.

KAT2A inhibition showed suppression of leukemic cell growth in vivo and human primary AML cells. The HOXA9 protein was associated with the MLL-AF9 leukemogenic program such as changes in gene expression including downregulation of genes such as HOXA9, HOXA10, MEIS1, and MYC, and concomitant upregulation of genes associated with myeloid differentiation including ANPEP (CD13), ITGB2 (CD18), ITGAM (CD11b), and IL17RA (CD23) (Figures 6E and 6F). In keeping with these effects, we performed RNA-seq analysis on the sensitive MOLM-13 line after a 48-hr exposure to MB-3. We identified significant disruption was associated with a significant reduction in the expression of the leukemia-associated genes HOXA9, HOXA10, MEIS1, and MYC (Figure 6G). Microscopic and flow cytometry analyses of MOLM-13 cells after a 48 hr exposure to MB-3 confirmed monocytic-macrophage differentiation (Figure 6H) and increased CD13 surface expression (Figure 6I), whereas neither was observed in the MB-3-resistant MV4-11 cells. Furthermore, prolonged incubation with MB-3 caused a marked increase in apoptosis of MOLM-13 and OCI-AML3, but not MV4-11 (Figure 6J). Taken together, these results indicated that KAT2A inhibition suppresses AML cell proliferation through inhibition of leukemogenic transcriptional programs and induction of differentiation leading to cell death by apoptosis.

Clinical Potential of KAT2A Inhibition in AML Therapy

We next investigated whether KAT2A inhibition reduces cell proliferation in vivo. We first introduced the luciferase gene into MOLM-13-Cas9 cells and then transduced the cells with either an empty gRNA scaffold or a gRNA targeting the KAT2A gene. After 3 days of puromycin selection, transduced cells were transplanted into immunocompromised Rag2<sup>−/−</sup>;Il2rg<sup>−/−</sup> mice, which were then imaged for bioluminescence until death. We found that KAT2A suppression was associated with a significant reduction in AML cell expansion (Figures 7A and 7B) and prolongation of mouse survival (Figure 7C), indicating that KAT2A inhibition suppresses AML cell proliferation in vivo. Encouraged by these results, we proceeded to test the effects of MB-3 on primary human AML cells. Treatment of 10 primary AMLs of diverse genotypes (Table S5) with MB-3 led to significant reduction of colony formation in methylcellulose media at both 100 and 200 μM MB-3. Detailed information can be found in Table S5. Mean values of 10 samples are shown in (E). Error bars represent SD. "p < 0.05.

(F) CFC efficiency of cord blood CD34+ cells (n = 4). The Student’s t test was performed in (B), (E), and (F). See also Table S5. N, normal karyotype; ND, not determined.
together, our results show that KAT2A inhibition does not exhibit adverse effects on hematopoietic stem-progenitor cells and offers itself as a potential anti-AML therapeutic strategy for future studies.

DISCUSSION

Despite important advances in understanding their genomic and molecular pathogenesis, many cancers including AML continue to represent unmet clinical challenges (Cancer Genome Atlas Research Network, 2013; Döhner et al., 2015). It is therefore crucial to develop additional therapeutic strategies by identifying vulnerabilities in cancer cells. This can be achieved by either hypothesis-driven mechanistic studies or hypothesis-free unbiased genetic screening. In AML, recent detailed mechanistic studies have identified DOT1L as a vulnerability of MLL-rearranged leukemia (Bernt et al., 2011), and both a mechanistic and an RNAi-based epigenetics-focused screen identified BRD4 as a therapeutic target against AMLs of different genotypes (Dawson et al., 2011; Zuber et al., 2011). Drug development against these targets has rapidly progressed and their therapeutic efficacy is now being tested in clinical trials. Nevertheless, despite these successes, AML remains a lethal disease for most patients, and a complete set of genetic vulnerabilities for this and other cancers remains unknown, leaving many candidates with a therapeutic potential undiscovered.

To this end, we optimized and validated a robust CRISPR-Cas9 platform for the performance of genome-wide essentiality screens and applied this to catalog genetic vulnerabilities in AML. Our results have not only confirmed known therapeutic targets but also revealed a large number of genetic vulnerabilities in the AML cell lines studied, many of which represent plausible direct or indirect targets for drug development. Importantly, the unbiased nature of genome-wide screens such as ours makes them a powerful instrument for the identification of such targets, which is both orthogonal and complementary to mechanistic studies of disease pathogenesis and also able to reveal both intuitive and non-intuitive vulnerabilities.

Nevertheless, not all genetic vulnerabilities represent viable therapeutic targets. An important hurdle in selecting these is the real possibility that any genes essential to AML cells may also be essential to normal hemopoietic and/or non-hemopoietic cells, making their pharmacological inhibition harmful. To select targets that are likely to exhibit minimal adverse effects and thus have a higher likelihood of success in drug development, we applied a differential essentiality filter to our screen dataset and identified and characterized a potential AML therapeutic target, namely KAT2A. Genetic or pharmacological suppression of KAT2A did not show detectable adverse effects in either mouse HPC-7 hematopoietic precursor cell line or human cord blood CD34⁺ cells, further supporting that our approach was valid. It would be important to identify any toxic effects on hemopoietic stem-progenitor cells using a potent and bioavailable KAT2A inhibitor. Of course, in the absence of comprehensive datasets from other normal cell types, we cannot rule out the possibility that KAT2A suppression can cause side effects, and the generation of such datasets would significantly enhance our ability to predict clinical toxicity and identify the most promising therapies. It is, however, noteworthy that at least a dozen targets that have been in clinical use already were essential to cell types other than AML, suggesting that valuable targets can be found even among genes within this category and may potentially have a broad spectrum of antitumor activity.

Notwithstanding limitations in predicting clinical toxicity, our results demonstrate that KAT2A inhibition induces cellular differentiation and apoptosis of AML cells. Although the precise molecular basis of these effects will need to be investigated in future studies, the transcriptional changes associated with KAT2A inhibition suggest that the effects may be secondary to inhibition of leukemogenic transcriptional programs, in a manner reminiscent of BRD4 and DOT1L inhibition (Bernt et al., 2011; Dawson et al., 2011). KAT2A encodes a histone lysine acetyltransferase that functions within the multi-protein transcriptional co-activator complexes SAGA (Spt-Ada-Gcn5-acetyltransferase) or ATAC (Ada2a-containing); the former predominantly localizes at a subset of active promoters, whereas the latter localizes at distinct active promoters and enhancers (Krebs et al., 2011). As such, KAT2A influences diverse transcriptional programs and participates in multiple developmental and cellular processes (Wang and Dent, 2014). It has been shown that leukemia induction by MLL-AF9 requires the Myb-p300 interaction, which is thought to be responsible for the methylation-to-acetylation switch at the lysine-27 residue of histone H3 upon MLL-AF9 expression in HSCs (Pasini et al., 2010). One hypothesis is that a KAT2A-containing complex serves as a transcriptional coactivator that is also recruited to the target sites by MLL-AF9 and activates and/or maintains the leukemic transcriptional program. Alternatively, KAT2A might maintain the leukemic program through acetylation of non-histone proteins as exemplified by direct acetylation of the RUNX1/MDS1/EVI1 (Senyuk et al., 2003) and EZA-PBX1 (Holmlund et al., 2013) fusion oncoproteins by KAT2A and its homolog KAT2B (also known as PCAF). Further work is required to investigate the molecular function of KAT2A and determine the full therapeutic potential of this finding.

Our work demonstrates the power of unbiased genome-wide screens to catalog a comprehensive set of genetic vulnerabilities in cancer cells. Such catalogs enable not only the rapid identification of new targets and development of therapeutic strategies, but also generate hypotheses pertinent to the study of molecular mechanisms underlying tumorigenesis.

EXPERIMENTAL PROCEDURES

All reagents and detailed methods are described in the Supplemental Information.

Plasmids, Cell Lines, Mouse Lines, and Reagents

Guide RNA expression vectors with the improved scaffold, pKLV2-U6gRNAs (BssIII)-PKGpuro2A8FP-W and pKLV2.2-h7SkgRNAs(SapI)-U6gRNA5(BssIII)-PKGpuro2A8FP-W, for a single and dual gRNA expression, respectively, were generated in this study and have been deposited with Addgene. The optimized human and murine CRISPR libraries were also available through Addgene. Guide RNA sequences used in a gene-by-gene approach are listed in Table S6. All AML cell lines (MOLM-13, MV4-11, HL-60, OCI-AML2, and OCI-AML3), colon cancer cell line HT-29, and fibrosarcoma cell line HT-1080 were obtained from the Sanger Institute Cancer Cell Line Panel and were mycoplasma free. Cas9-expressing cell lines were generated by lentiviral transduction using pKLV2-EF1aBsd2ACas9-W, and Cas9 activity in individual
subclones was tested using a lentiviral reporter pKL2V2-U6gRNAiGFP-PKGBFP2AGFP-W. A Cas9-expressing mouse line was generated by inserting the human EFTa promoter-driven Cas9 expression cassette into the Rosa26 locus in mouse ESC line JMB (Pettitt et al., 2009) and is kept in the C57BL/6N background. See also Supplemental Information. All animal studies were carried out in accordance with the Animals (Scientific Procedures) Act 1986 (UK) and approved by the Ethics Committee at the Sanger Institute.

Generation of Genome-wide Mutant Libraries and Screening
A total of 3 × 10^6 cells were transduced with a predetermined volume of the genome-wide gRNA lentiviral supernatant. Two days after transduction, the cells were selected with puromycin for 4 days and further cultured. For HT-29, approximately 1 × 10^6 cells were harvested every 3 days between day 7 and day 25 post-transduction. The AML cell lines and HT-1080 were harvested on day 25 post-transduction. See also Supplemental Information.

gRNA Competitive Proliferation Assay
Cas9-expressing cells were transduced with a lentivirus expressing a gene-specific gRNA, and the percentage of blue fluorescent protein (BFP)-positive cells was measured between days 4 and 12 post-transduction and normalized to the percentage of BFP-positive cells at day 4. See also Supplemental Information.

Drugs and Proliferation Assays
A total of 3 × 10^5 human or primary mouse cells were plated onto 96-well plates with vehicle or the indicated concentrations of compounds. Plates were measured 72 hr post-treatment using CellTiter 96 AQueous Non-Radioactive active Cell Proliferation Assay (Promega). See also Supplemental Information.

Adult Primary Leukemia and Cord Blood Sample Analysis
All human AML and cord blood samples were obtained with informed consent under local ethical approval (REC 07-MRE05-44). Primary AML cells or cord-blood-derived CD34+ cells were tested for colony-forming efficiency in H4435 semi-solid medium (Stem Cell Technologies) in the presence of the indicated concentration of MB3 or DMSO. Colonies were counted by microscopy 10–11 days (AML cells) or 12–14 days (CD34+ cells) after plating. See also Supplemental Information.

Statistical Analysis
Statistical analyses performed and the numbers of replicates were mentioned in the associated figure legends. Differences were considered significant for p < 0.05.

ACCESSION NUMBERS
The accession numbers for the CRISPR data reported in this paper are European Nucleotide Archive (http://www.ebi.ac.uk/ena): ERPP06734 (mouse ESCs), ERPP005600 (HT-29), and ERPP008475 (AML and HT-1080). The accession numbers for the RNA-seq data reported in this paper are European Nucleotide Archive: ERPP06662 and ERPP003933. The CRISPR toolkit and the CRISPR libraries are available from Addgene.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, six figures, six tables, and two datasets and can be found with this article online at http://dx.doi.org/10.1016/j.cellrep.2016.09.079.

AUTHOR CONTRIBUTIONS
G.S.V., K.T., and K.Y. conceived the study and designed the experiments. Y.L. and K.Y. designed the mouse and human gRNA libraries. H.K.-Y., S.O., and K.Y. generated the CRISPR toolkit and the Cas9 transgenic mice, and performed CRISPR screens in mouse ESCs and HT-29. K.T. performed CRISPR screens in the AML cell lines and conducted the validation and drug sensitivity assays with help from E.D.B., E.M., and K.Y. K.Y. performed the large-scale data analyses with help from K.T., F.I., E.D.B., H.P., and G.S.V. E.M., A.M., M.M., M.G., O.M.D., T.M., M.P., and J.C. performed cell culture and mouse experiments. O.M.D. and B.C. performed CRISPR screens for HT-1080, V.G. and U.M. generated and analyzed Cas9-expressing HT-29. M.L. performed analysis of RNA-seq data. J.S.-R. and B.J.P.H. contributed to study strategy, technical and analytical aspects. C.P. conceived and designed analysis of KAT2A inhibition in AML cell lines and in primary patient and cord blood samples, and performed the experiments with help from K.T. and A.F.D. S.I. supported RNA-seq analysis of MB3-treated MOLM-13 cells. P.G. and B.J.P.H. contributed with AML patient samples. K.T., E.D.B., G.S.V., and K.Y. wrote the paper with input from all authors.

CONFLICT OF INTEREST
G.S.V. is a consultant for Kymab and received an educational grant from Celgene.

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