Genome editing-induced t(4;11) chromosomal translocations model B cell precursor acute lymphoblastic leukemias with KMT2A-AFF1 fusion

Feng Pan, … , Kara L. Davis, Michael L. Cleary


Find the latest version:

https://jci.me/171030/pdf
Genome editing-induced t(4;11) chromosomal translocations model B cell precursor acute lymphoblastic leukemias with KMT2A-AFF1 fusion

Feng Pan\textsuperscript{1,2}, Jolanda Sarno\textsuperscript{3,4}, Johan Jeong\textsuperscript{1}, Xin Yang\textsuperscript{1}, Astraea Jager\textsuperscript{3,4}, Tanja A. Gruber\textsuperscript{3}, Kara L. Davis\textsuperscript{3,4}, and Michael L. Cleary\textsuperscript{1}

\textsuperscript{1}Department of Pathology, Stanford University, Stanford, CA
\textsuperscript{2}Department of Molecular Medicine, the University of Texas Health Science Center at San Antonio, San Antonio, TX
\textsuperscript{3}Department of Pediatrics, Hematology, Oncology, Stem Cell Transplant and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA
\textsuperscript{4}Stanford Center for Cancer Cell Therapy, Stanford Cancer Institute, Stanford University, Stanford, CA

Corresponding Author:

Michael L. Cleary, MD
Lokey Stem Cell Research Building
Stanford, CA 94305
Phone: 650-723-5471
Email: mcleary@stanford.edu
To the Editor: The t(4;11)(q21;q23) chromosomal translocation fuses KMT2A to AFF1, the most common KMT2A-fusion partner, and is prevalent in BCP-ALL in both adults and children (1). Recently, CRISPR-mediated KMT2A rearrangement (KMT2Ar) in human umbilical cord blood (UCB) hematopoietic stem and progenitor cells (HSPCs) was used to model aspects of leukemia biology (2-4). Here, we induced chromosomal translocations between the KMT2A and AFF1 genes in primary human UCB HSPCs to model t(4;11) leukemia and performed multiomics analyses on the resultant gene-edited BCP-ALLs in comparison to patient ALLs and normal bone marrow from healthy donors (Fig. 1A).

CRISPR/Cas9 ribonucleoprotein (RNP) complexes targeting intronic breakpoint cluster regions in the KMT2A and AFF1 genes, respectively, were introduced into primary human UCB HSPCs to generate KMT2A-AFF1 gene fusions that mimic those in human ALLs (Fig. S1A-C and Fig. 1B). At various times of culture, gene-edited cells were transplanted into sub-lethally irradiated NSG mice, which succumbed to lethal hematological malignancies with latencies of 6 to 9 months, versus no abnormalities in Cas9 control mice (Fig. 1C and Fig. S1D-H). Flow cytometry showed leukemia cell phenotypes characteristic of BCP-ALL (CD19+CD33-) (Fig. S1F). The observed B lineage skewing occurred despite culture in myeloid-conditioned medium prior to transplantation, suggesting an instructive role of KMT2A-AFF1 and the reciprocal fusion in B lineage commitment in this model. Leukemia lineage and disease features were conserved in secondary transplant recipients, but with accelerated onset (Fig. 1C and Fig. S1I). Contrary to the oligoclonal composition of in vitro cultured cells, monoclonal KMT2A-AFF1 breakpoint sequences observed in primary BCP-ALLs indicated that gene-edited cells underwent selection for clonal leukemias in vivo (Fig. S1J).
To investigate the ontology of the gene-edited t(4;11) BCP-ALL cells, we performed single-cell mass cytometry and applied a B-cell developmental classifier, previously developed (5). This revealed that KMT2A-AFF1 gene-edited cells are arrested in an early stage of B-cell development, specifically at the pre-pro-B cell population when compared to engrafted Cas9 control cells (Fig. S1K). Cells harboring KMT2A-AFF1 translocation are classified in a less differentiated B-cell stage compared to primary samples with other prognostic translocations, corroborating prior findings (Fig. 1D) (5). Comparing classified leukemia cell subsets to their normal B-cell counterparts, high-dimensional phenotypes showed that gene-edited t(4;11) BCP-ALL shared tSNE space with corresponding patient BCP-ALL and PDX samples (Fig. 1E) but not human leukemia cell lines (Fig. S2A), suggesting that the KMT2A-AFF1 gene-edited cells overall recapitulate the same phenotype and intracellular state of primary B-ALL cells harboring t(4;11) translocation. Despite high expression of common BCP-ALL associated proteins such as CD19, CD34, and CD38, expression of others including CD133, PAX5, and BCL2 was more heterogeneous (Fig. S2B-D), indicating the phenotypic heterogeneity of t(4;11) BCP-ALL. Consistent with previous studies (6, 7), KMT2A-AFF1 gene-edited cells are largely restricted to the immature negative CD10 expression (Fig. S2C).

Correlation analysis of genome-wide chromatin accessibility and RNA-seq indicated distinct clustering of samples (Fig. 1F, G). Gene-edited t(4;11) cells, t(9;11) ALL cells (4), and patient KMT2A-AFF1 BCP-ALL grouped together more than other leukemia subsets (gene-edited KMT2A-MLLT3 AML, MPAL and KMT2A-AFF1 human cell lines), consistent with their phenotypic similarities. Notably, epigenomic and transcriptomic profiles of human cell lines
MV4;11, SEM, and RS4;11 differed from those of the gene-edited leukemias and primary patient samples (Fig. S3A-C), again suggesting that human KMT2A-AFF1 cell lines are poor surrogates for primary leukemia biology. The association between KMT2A-AFF1 BCP-ALL chromatin accessibility landscapes and gene expression programs was then assessed, which identified 17,756 accessible regions corresponding to 2,343 genes. These genes included established KMT2Ar and lineage-specific signatures, such as MEIS1, CDKN2A, and BCL11A. The group was enriched for specific gene ontology (GO) terms, including B cell receptor complex, Bcl-2 family protein complex, and acute lymphoblastic leukemia (Fig. S3D).

In summary, CRISPR editing in human HSPCs generates de novo t(4;11) leukemia that captures the phenotypic, transcriptional, and chromatin accessibility signatures of human KMT2A-AFF1 BCP-ALL, highlighting the advantages of gene-edited cells for modeling human disease. KMT2A-AFF1 drives distinct lymphoid gene expression programs leading to a developmental block at the earliest stages of B cell development. Although this model may not fully recapitulate leukemias that develop in patients particularly under immune surveillance, it can improve understanding of the pathogenesis of KMT2A-AFF1 BCP-ALL and facilitate the development of novel therapeutic and diagnostic approaches.
References

Figure 1: CRISPR-engineered KMT2A-AFF1 BCP-ALLs recapitulate proteomic, epigenomic, and transcriptomic features of primary patient leukemias.

(A) Schematic of the phenotypic, genetic, and epigenetic characterization of KMT2A-AFF1 cells generated through CRISPR/Cas9-mediated gene editing.

(B) FISH analysis of cells (day 26 in vitro culture) for KMT2A translocation using KMT2A break-apart probes.

(C) Survival curves for xeno-transplanted mice showing mean latencies for development of primary (n=18), and secondary (n=9) leukemias, respectively. P value was generated using Mantel-Cox log-rank test. **p < 0.01, ***p < 0.001.

(D) Percentage of cells from gene-edited KMT2A-AFF1 BCP-ALL and diagnostic KMT2A-AFF1 BCP-ALL patient bone marrow classified into developmental populations.

(E) tSNE projections of normal, gene-edited BCP-ALL, patient samples, PDX and human cell lines where each cell is represented by a dot with color-coded clusters.

(F) Unsupervised hierarchical clustering of the Spearman correlations from ATAC-seq data of gene-edited KMT2A-AFF1 BCP-ALLs, gene-edited KMT2A-MLLT3 leukemias (AML, ALL, MPAL), KMT2A-AFF1 patient samples, and KMT2A-AFF1 human cell lines.
(G) Principal component analysis of RNA-seq data from gene-edited BCP-ALLs, gene-edited leukemias (AML, ALL, MPAL), patient samples, and human cell lines.