Super-enhancer landscapes reveal novel epigenomic patient subtypes and druggable dependencies in human AML

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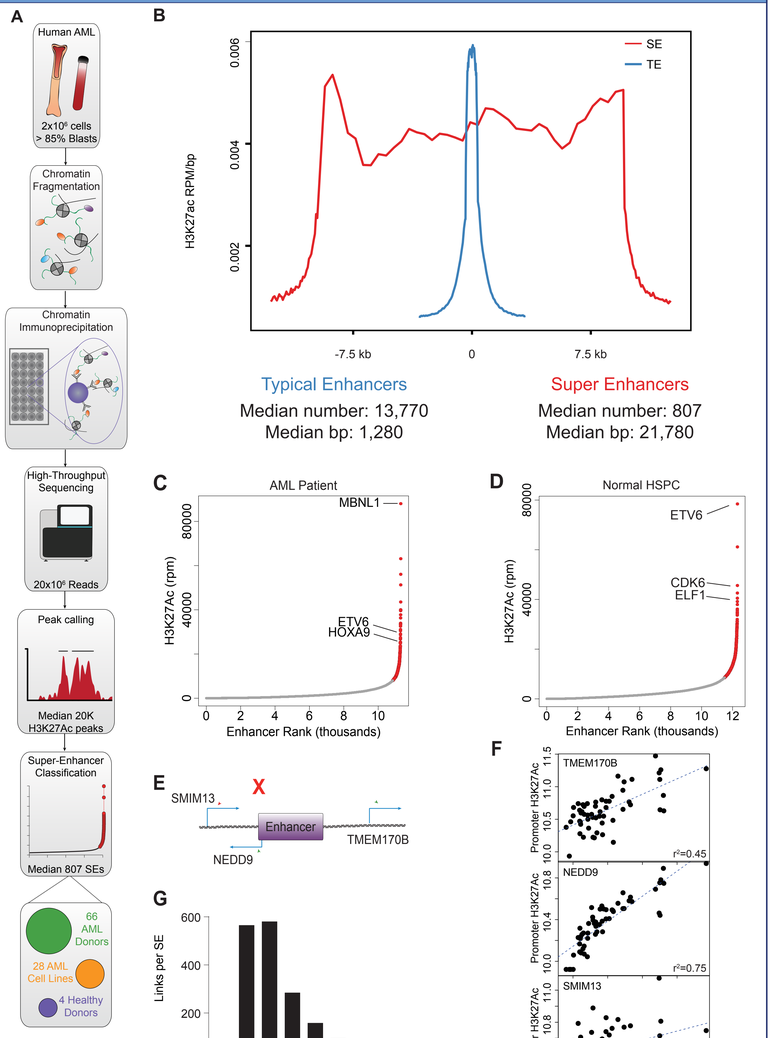
Abstract

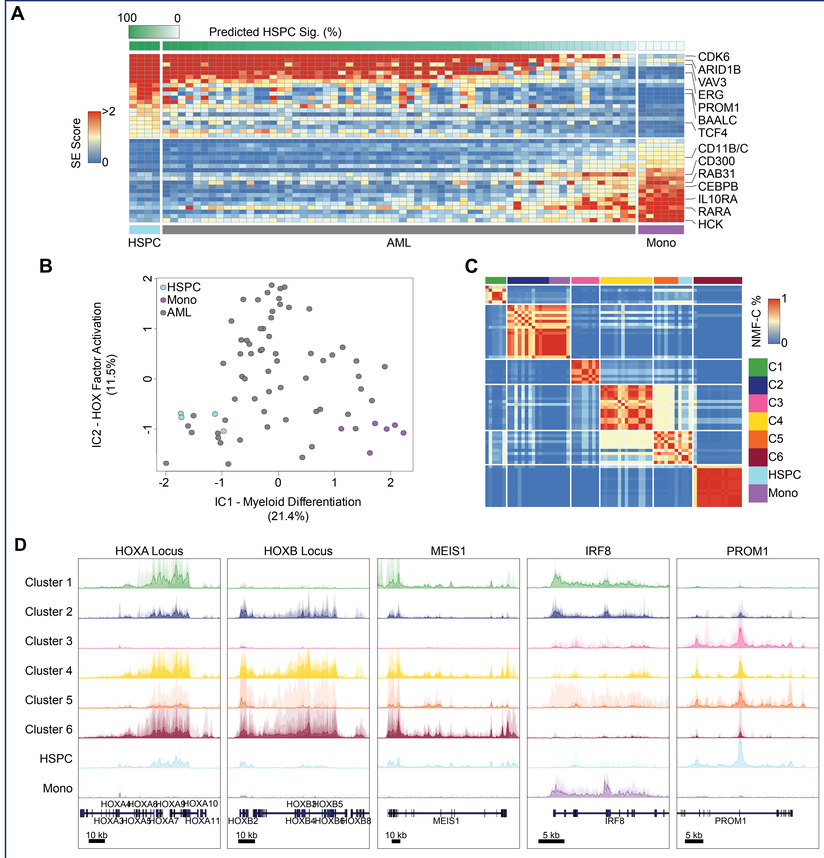
The bulk of translational cancer research to date has focused on somatic mutations in protein coding regions to identify putative oncogenic drivers. However, recent studies have shown that enhancer activity plays an important role in specifying and maintaining oncogenic cell state. Here, we present a mapping and analysis of the transcriptional cell state of acute myeloid leukemia (AML) via the H3K27Ac landscape, gene expression, and somatic mutations from 62 AML patients. The goal of this work is to identify the recurrent enhancer drivers of oncogenic cell states and translate that knowledge of the epigenome to discover novel therapeutic opportunities. Through a computational deconvolution of enhancer maps, we identify 6 epigenomically defined patient subtypes of AML. We demonstrate that while certain genetic lesions, such as MLL translocations and NPM1 mutations, do correlate with these subtypes, the epigenome provides a novel stratification of patients that is not fully specified by combinations of mutations. We develop a novel scoring of myeloid differentiation based on the enhancer landscape of healthy cells and use this score to show that enhancer subtypes are associated with the differentiation state of the underlying AML blasts. Enhancer subtypes are also clinically relevant as they are predictive of divergent overall survival, varying from a median overall survival of 9.2 months to a median overall survival that was not reached in our cohort. By using individual enhancer activity as a novel biomarker, we are able to predict the effect of existing therapies on cell line models. Finally, a network analysis of the super-enhancers underlying the patient subtypes suggests that one subtype of AML is specified in part by enhancer activation of the retinoic acid receptor alpha gene (RARA), and we demonstrate that RARA enhancer strength in cell-line and patient-derived xenograft models is predictive of response to a first-in-class selective RARα agonist, SY-1425 (tamibarotene). Taken together these findings highlight the importance and utility of understanding the enhancer landscape for patient stratification and the development of novel therapies.

Identification and linking of super-enhancers in human AML



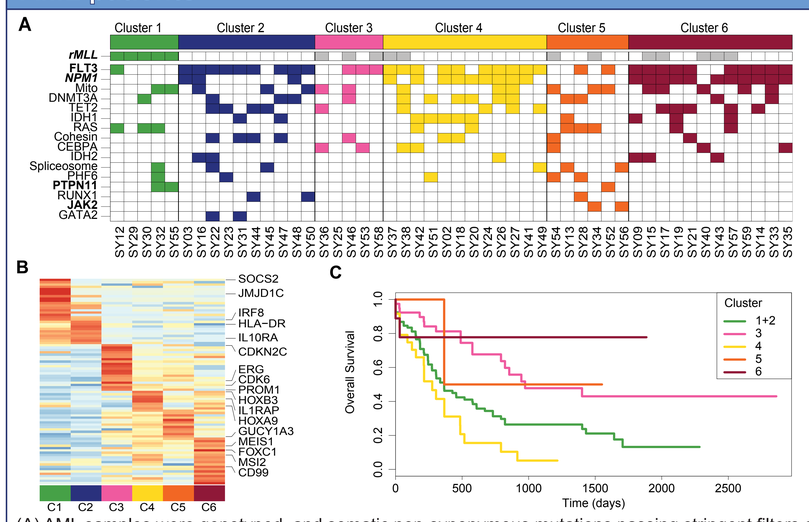
(A) Schematic of the SE calling pipeline used in this study.
(B) Metapeak representation of typical (blue) and super- (red) enhancers from an example AML map.
(C & D) Rank order representation of typical (black) and super- (red) enhancer strength genome-wide in an example AML blast sample (C) and an example healthy HSPC (D).
(E & F) Linking schematic for an example enhancer and three nearby genes (E), and correlation between H3K27Ac promoter signal and SE strength at the 3 genes (F).
(G) Histogram describing the number of called gene-SE links genome-wide.

SE maps reveal key AML drivers, differentiation state, and distinct patient subgroups



(A) A predicted HSPC signature (green, top), derived from 40 HSPC- or monocyte-specific SEs.
(B) ICA decomposition of the SE matrix reveals the two most important sources of AML enhancer variance to be predicted HSPC signature and HOX factor activation.
(C) Non-negative matrix factorization consensus clustering distance matrix shows 6 distinct clusters.
(D) Clusters exhibit unique signatures at key regulatory factors, setting up cluster-specific transcriptional regulatory circuits (unique combinations of master TFs). Each cluster's individual H3K27Ac maps are shown as transparent area graphs with the median profile drawn over top with a solid line.

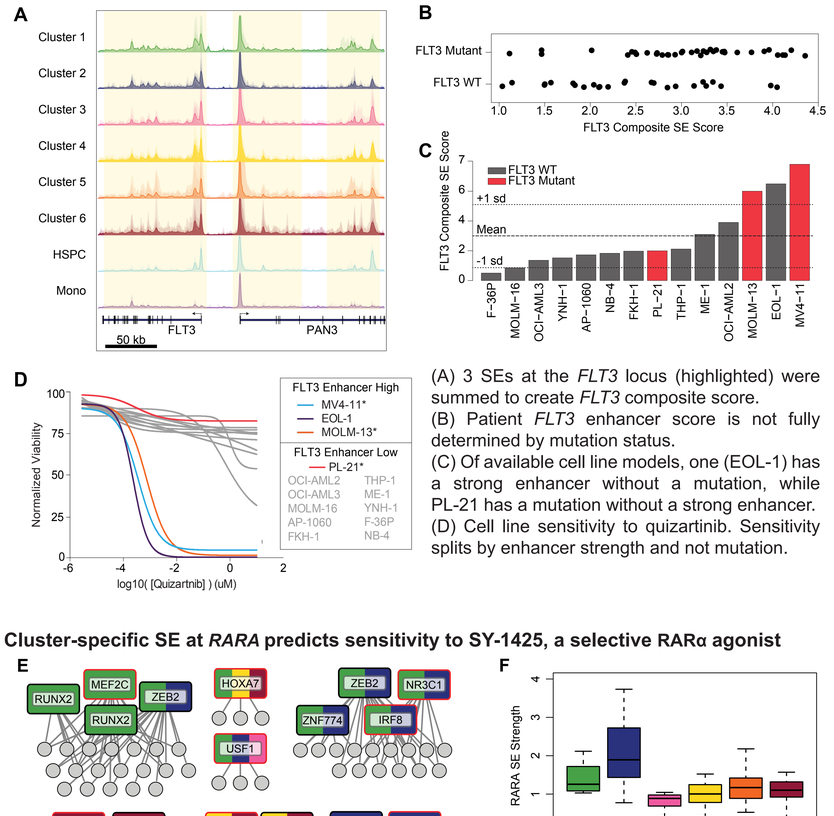
SE-derived novel subgroups are predictive of survival in AML patients



(A) AML samples were genotyped, and somatic non-synonymous mutations passing stringent filters are displayed as colored blocks. Gray - unknown; white - wildtype. Bold mutation names indicate a nominally significant association ($p < 0.05$) between mutation and subtype (Fisher's exact). Italic indicates significance after multiple hypothesis testing correction ($p < 0.001$). Only the most recurrent mutations ($n > 2$) are shown. rMLL = MLL rearrangement; Mito = Mitochondrial genes.
(B) Cluster-specific SEs were determined by identifying the SEs with the largest dynamic range in the NMF basis matrix. The heatmap visualizes these 88 SEs as their median SE score per cluster (row normalized) and key linked genes are highlighted.
(C) Using a shrunken centroids method on RNA-seq, cluster classifiers were derived for our cohort and used to classify the TCGA AML population. There is a strong ($p<.003$) association with survival, with cluster 4 having a 9 month median survival and cluster 6's median not yet met.

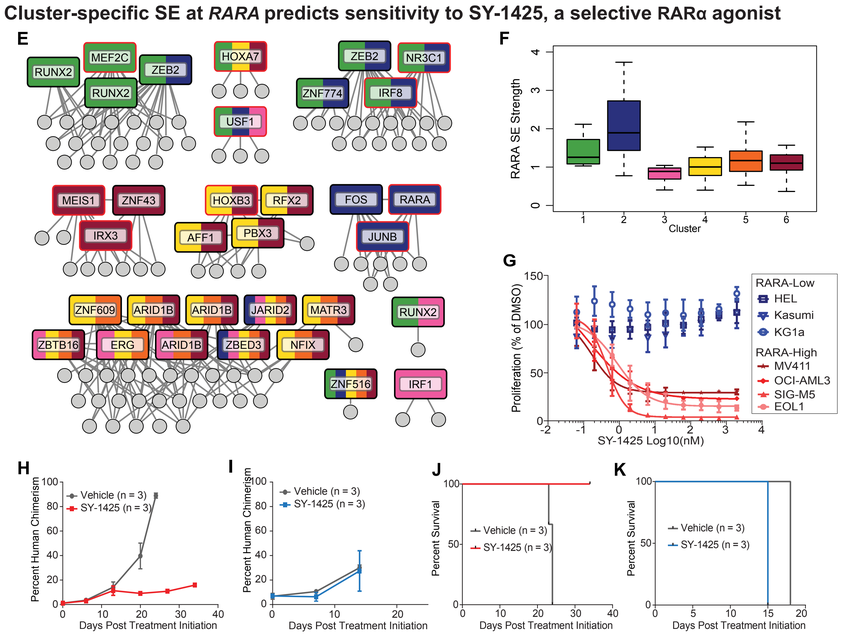
AML-specific SEs predict targets for therapeutic intervention

Cross-cluster SE at *FLT3* predicts sensitivity to quizartinib, a FLT3 inhibitor



(A) 3 SEs at the *FLT3* locus (highlighted) were summed to create *FLT3* composite score.
(B) Patient *FLT3* enhancer score is not fully determined by mutation status.
(C) Of available cell line models, one (EOL-1) has a strong enhancer without a mutation, while PL-21 has a mutation without a strong enhancer.
(D) Cell line sensitivity to quizartinib. Sensitivity splits by enhancer strength and not mutation.

Cluster-specific SE at *RARA* predicts sensitivity to SY-1425, a selective RARα agonist



(E) A mutual information network was constructed of cluster-specific SEs. TFs are labeled and colored with respect to cluster, while a red border indicates cluster-specific motif activity.
(F) Enhancer for *RARA*, a nuclear hormone receptor, shows strong cluster association.
(G) Anti-proliferative response of non-APL AML cell lines to SY-1425 as assessed by ATPlite for 3 RARA-low (blue) and 3 RARA-high (red).
(H-K) Efficacy and survival of SY-1425 in non-APL RARA-high PDX model (H & J, resp.) and AM7577 non-APL RARA-low PDX model (I & K, resp.). Tumor growth was monitored by measuring human CD45 positive cells in mouse circulation. Treatment was initiated 30 days after inoculation with patient derived human AML cells. Survival prolongation significant at p=0.03 (Mantel-Cox).

Conclusions

- Super-enhancer landscapes in human AML define the transcriptional regulatory circuits that govern this aggressive malignancy.
- The greatest sources of variance in AML enhancer landscapes are (1) the differentiation status of the AML and (2) the activity of a HOXA9/PBX3/MEIS1 transcription factor triad.
- NMF consensus clustering of patient SE maps reveals 6 novel subtypes of AML with strikingly distinct circuitry, mutational profiles, and clinical features such as overall survival.
- SEs can be used to identify biomarker-associated targets for therapeutic intervention.
- FLT3 enhancer activity spans multiple clusters and its strength is predictive for quizartinib sensitivity.
- The *RARA* enhancer is predicted to be a key subtype-specific driver, and both RARA-high cell line and patient-derived xenograft models show susceptibility to the RARα agonist SY-1425.
- Based on SY-1425's well-established safety and efficacy profile in R/R APL and our strong preclinical data, we have initiated a biomarker-directed Phase 2 clinical trial in genomically defined subsets of RARA-high AML and MDS patients (clinicaltrials.gov, NCT02807558).