(PF449) IMPROVED SINGLE-CELL LONG-READ GENOTYPING DEFINES CLONAL ARCHITECTURES, LINEAGE COMMITMENT AND TRANSCRIPTIONAL PROGRAMS IN SRSF2MUT AML

Topic: 03. Acute myeloid leukemia - Biology & translational research

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Background

SRSF2^{mut} AML carries poor prognosis and frequent relapse, yet its differentiation states and lineage-specific transcriptional programs remain incompletely defined. Linking genotype to phenotype at single-cell resolution is essential to dissect clonal architecture, malignant transformation, and therapy resistance. However, reliable identification of leukemic cells in scRNA-seq data remains challenging, even with targeted long-read genotyping. We hypothesized that systematic incorporation of patient-specific private mutations as leukemic barcodes would markedly improve malignant cell annotation and clonal resolution.

Aims

We streamlined and scaled our scRNA-seq genotyping workflow (*nanoranger v2*) to characterize the cellular and transcriptional landscape of *SRSF2*^{mut} AML.

Methods

Somatic mutations were selected by whole-exome sequencing based on expression at 5′ transcript ends in hematopoietic cells. Target transcripts were amplified in a 2-step PCR followed by Oxford Nanopore sequencing. Genotypes were integrated with scRNA-seq profiles to assign mutations to individual cells and transcriptional states.

Results

The *nanoranger v2* workflow replaces RNase H-dependent PCR with TaqMan-based preamplification for simultaneous interrogation of large mutation panels. Genotyping efficiency was on par with the original protocol (r=0.74, p<0.001), while substantially reducing cost and technical complexity.

We profiled 17 $SRSF2^{mut}$ AML cases at diagnosis, identifying a median of 9 (range 7-16) genotypable mutations per case and obtaining genotyping information in 71% (range 59-92%) of cells. To define clonal architectures, we analyzed single-cell co-mutational status, which revealed that 94.1% of private mutations were acquired after $SRSF2^{mut}$ and could therefore serve as robust leukemic markers. We found pre-leukemic clonal expansion in only 2 AML cases, where $STAG2^{S377fs}$ or $CBL^{END386del}$ frequently occurred in $SRSF2^{wt}$ cells. None of the AML-associated mutations were mutually exclusive, consistent with linear evolution. In sum, $SRSF2^{mut}$ is an early driver of secondary AML, which is dominated by highly selected leukemic clones.

We mapped differentiation hierarchies and demonstrated extensive AML involvement of progenitor compartments, with HSC, GMP and MEP populations being 81% (61-95%), 76% (65-96%) and 73% (66-93%) leukemic. In 75% of cases, full differentiation potential along myeloid and erythroid lineages was retained, which were 69% (50-96%) and 74% (6-93%) leukemic. Lymphoid progenitors were also frequently leukemia-derived (76%, 65-93%), yet differentiation into mature lymphocytes was heterogeneous across T (10%, 3-30%), NK (38%, 0-77%), or B cells (22%, 0-92%). Together, residual non-leukemic hematopoiesis is highly lineage-specific, despite near-total leukemic involvement of hematopoietic progenitors.

Finally, we defined a leukemic transcriptional signature based on 24,879 $SRSF2^{mut}$ vs. 11,321 $SRSF2^{wt}$ cells, revealing enrichment for core spliceosome components, DNA replication, cell cycle regulators, and chromatin-associated genes, consistent with enhanced RNA processing, proliferative capacity, and replicative stress. This signature was consistently higher in $SRSF2^{mut}$ cells across patients, indicating a conserved, transcriptional program.

Summary/Conclusion

We define single-cell differentiation hierarchies and conserved transcriptional programs of $SRSF2^{mut}$ AML, and establish a framework for interrogating leukemic evolution, relapse biology, and therapeutic resistance at scale.