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617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Dissecting Subtype-Specific Tumor-Time Interactions and Underlying Hidden Drivers in Pediatric Acute Myeloid Leukemia Via Single-Cell Multi-Omics

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Introduction: Pediatric acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by various chromosomal abnormalities and somatic mutations. Investigating the origin of tumorigenesis and deciphering the complex interplay between AML cells and their surrounding tumor immune microenvironment (TIME) is crucial to understand the underlying mechanisms driving disease progression and response to therapy. Recent advancements in single-cell multi-omics technologies enabled us to characterize the transcriptional (GEX) and epigenetic (ATAC) landscapes of both tumors and TIMEs. Leveraging our in-house algorithm, scMINER (single-cell Mutual Information-based Network Engineering Ranger, Ding et al., 2023), this study aims to build a comprehensive cell type atlas in diverse pediatric AML bone marrow samples and construct intra- and inter-cellular networks, enabling the identification of hidden drivers that shape subtype-specific AML-TIME interactions.

Methods: Using CD45 and CD33 as selective markers by flow cytometry, we have successfully isolated blasts and enriched for T cell and B cell populations for 10X single-cell multiome profiling across AML molecular subtypes, including *UBTF*-TD (n=1), *NPM1* (n=1), and *KMT2A* rearrangement (n=1). We interrogated the GEX data using scMINER, including two key steps: (i) mutual information-based clustering analysis (MICA) and (ii) mutual information-based network inference engine (MINIE). MICA characterizes the intrinsic nonlinear similarity of gene expression distributions among cells, enabling a high resolution of clusters. MINIE reverse-engineers cluster-specific intracellular gene networks and infers protein activity of transcription factors (TF) or signaling factors (SIG) drivers based on the expression of its predicted regulon targets in the corresponding cluster. The activity profiles overcome the dropout effects of single-cell RNA-seq data and reflect protein activities across all cells. ArchR is used to analyze ATAC data. CellChat is used to construct and evaluate intercellular networks.

Results: AML cells from three samples were divided into four major clusters, and we labeled them as NPM1, KMT2A, UBTF_one, and UBTF_two based on sample subtypes (Fig.1A). MICA managed to obtain distinct AML cell sub-clusters. Comparison with single-cell profiles of normal hematopoietic cells and annotation with marker genes revealed their correspondence with different myeloid developmental stages. Notably, the KMT2A cluster shared a high similarity in cell type proportion with the UBTF_two cluster, and the NPM1 cluster is closer to the UBTF_one cluster. This result demonstrated significant tumor heterogeneity within and across AML molecular subtypes. With the help of intracellular gene networks generated by MINIE, we identified multiple subtype-specific hidden drivers by differential activity analysis. For example, KMT2A and UBTF_two clusters exhibited elevated *SAMHD1* levels, consistent with previous GSE data (Zhang et al., 2022). Chromatin accessibility analysis of ATAC data showed increased peaks near *SAMHD1* in KMT2A and UBTF_two clusters, further supporting this observation. *IRF8* showed a similar expression pattern to *SAMHD1*, suggesting its context-dependent role across AML subtypes. Accurate annotation of myeloid and immune cells greatly enriches the preciseness of the AML and TIME interaction patterns, which vary across AML molecular subtypes (Fig.1B). Interestingly, cells originated from *KMT2A* cluster exhibited similar interaction patterns to UBTF_two, while NPM1 and UBTF_one were grouped. This result indicated that different AML molecular subtypes may partially utilize the same mechanisms of tumor-TIME interaction, resulting in similar tumor phenotypes.

Conclusions: Accurate clustering and well-defined annotation of myeloid and immune cells enabled us to build a comprehensive cell type atlas in AML samples. The subtype-specific network and the following analysis identified candidate hidden drivers that possibly contribute to the heterogeneity of tumor populations. AML subtypes sharing the same hidden drivers have similar cell type proportions and interaction patterns with TIMEs. This observation implies that AML subtypes share cell type-specific dependencies, which can be therapeutic targets to overcome the refractoriness posed by tumor heterogeneity.

Disclosures No relevant conflicts of interest to declare.

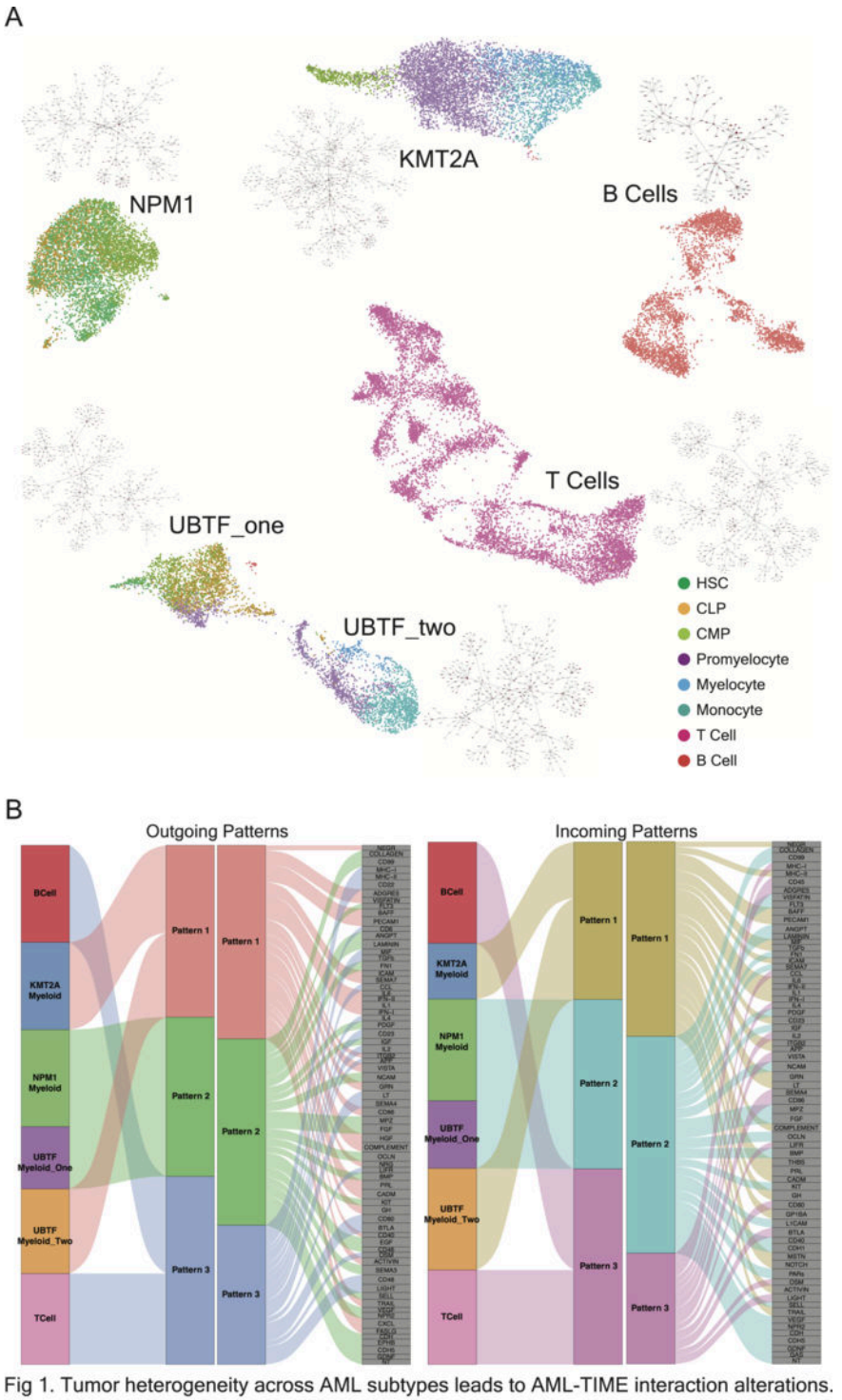

Fig 1. Tumor heterogeneity across AML subtypes leads to AML-TIME interaction alterations.
A, UMAP representation of major cell types and subtype-specific intracellular gene networks generated by scMINER.
B, River plots of AML-TIME intercellular network patterns.

Figure 1

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