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Acute Leukemias

SINGLE-CELL SEQUENCING REVEALS PATHWAYS AND PERTURBATIONS PREDICTIVE OF CENTRAL NERVOUS SYSTEM (CNS) DISSEMINATION IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)

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Introduction: Prognosis of B-ALL has improved particularly through targeted treatment and immunotherapy. However, a proportion of patients still experience disease dissemination to sanctuaries including the CNS which is not easily targetable by novel agents, thus representing a primary unmet clinical need. Here we aim to dissect mechanisms underlying CNS dissemination at single-cell resolution.

Methods: We performed single-cell RNA sequencing (scRNA-seq) on 15 B-ALL diagnostic bone marrow (BM) samples - 2 with hematologic and CNS relapse, 4 with isolated CNS relapse, 3 with hematologic relapse and 6 in continuous complete remission (n=15-20.000 cells/sample) - to characterize involved cell types, and explore differential clusters and their related transcriptomic fingerprints.

Results: We identified ~200 differentially expressed genes (DEGs) between CNS+ vs CNS- samples defining a "CNS signature" enriched in genes crucial for signaling pathways including E2F transcription factors, MTORC1, TNFa and IL2-STAT5, along with metabolic pathways including oxidative phosphorylation and adipogenesis (Figure 1A). Crucial genes for leukemic blasts survival/signaling/migration were upregulated, including tyrosine-kinase ZAP70, cytokine receptor IL15RA, integrin subunit ITGA5, as well as genes described in cancer metastasis programs (adhesion molecules: CD99, SERPINE1, MUC4; fucosylation: POFUT1; sialylation: ST3GAL3 and ST8SIA4). This list also included genes previously described as predictive for CNS dissemination (Sapienza MR et al, Hematol Oncol. 2023). We then applied this "CNS signature" at the single-cell level. Pseudobulk GSVA analysis showed significantly higher scores between CNS+ vs CNS- samples (Figure 1B). By applying single-cell level GSVA analysis, we then identified a group of clusters with significant higher scores in CNS+ vs CNS- samples (Figure 1C-D). Pseudotime analysis revealed a convergent differentiation route from highly immature progenitor stem-like blasts to more mature B naïve cells (Figure 1E). By leveraging reference immune transcriptomic datasets using SingleR to infer each single cell type we found that predicted B-cell maturation stages matched pseudotime analysis. Overall, clusters and cell types with the highest enrichment of the "CNS signature" and a higher contribution of CNS+ samples were those corresponding to more immature progenitor states (Figure 1E-F-G). These data demonstrate that patients who experience CNS dissemination have - since from diagnosis - a proportion of immature stem-like blasts upregulating key migration molecules and functional hubs responsible for later extravasation and dissemination to the CNS.

Conclusions: Our scRNA-seq data on 15 B-ALL diagnostic samples show that patients with CNS+ ALL expose already at diagnosis, an expanded population of immature stem cell blasts that upregulate key migration molecules and functional hubs responsible for subsequent extravasation and dissemination into the CNS.

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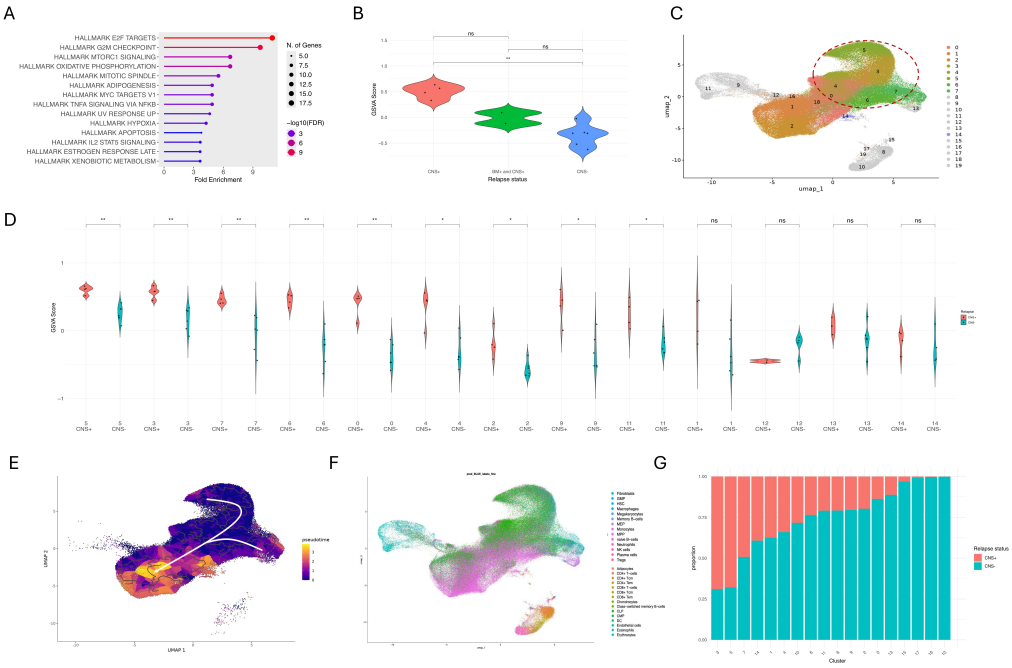

Figure 1. (A) Enrichment scores of genes found to be upregulated in CNS+ ALL samples *vs* CNS- ones ("CNS signature"). (B) GSVA scores obtained by pseudobulk analysis confirming the enrichment of the CNS signature in CNS+ ALL samples at single-cell resolution; DIFF_R: control group of patients with both BM relapse and CNS+, shows intermediate upregulation, confirming the specificity on the signature for the CNS+ cases. (C) Uniform Manifold Approximation and Projection (UMAP) reduction of CNS+ and CNS- samples, with the more immature clusters highlighted in red. (D) Violin plot illustrating the differential expression of the CNS signature (GSVA score) in the more immature clusters. (E-F) Pseudotime analysis demonstrating convergent evolution trajectories from stem-like progenitor cells to more mature B cells. The same analysis is matched using SingleR to infer each single cell type. Clusters corresponding to immature blasts are enriched with the CNS signature (see panel C). (G) Barplot showing the relative proportions of CNS+ *vs* CNS- samples in the various clusters depicted in the UMAPs (clusters 12 and from 15 to 19 are not shown because they are composed by only one sample and by few cells).