Multi-omic single-cell analysis uncovers a blast crisis-like subpopulation in the paired chronic phase cells from CML patients at diagnosis

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Background

In the tyrosine kinase inhibitor (TKI) era, overcoming drug resistance and improving prognosis for late-stage CML patients remain challenges. TKIs show limited effectiveness against CML stem/progenitor cells, crucial for relapse and disease progression. Further, this compartment exhibits great heterogeneity in cell type composition, self-renewal, differentiation, leukemia-propagating capacities, and therapeutic responses at different disease stages, warranting studies at the single-cell level.

Objective

We aim to profile gene expression and chromatin accessibility changes in $CD34^+$ stem/progenitor cells from paired chronic phase (CP) to blast crisis (BC) patients at single-cell resolution, to uncover cellular heterogeneity and identify molecular drivers of disease progression and TKI resistance.

Methods

We performed simultaneous 3' single-cell RNA and ATAC sequencing on $CD34^+AnnexinV^-PI^-$ cells from paired CP and myeloid BC samples (BC blasts up to 63%), cultured *in vitro* with or without nilotinib (5μM) for 16 hours, from three CML patients (total 12 samples). 49,698 cells (~4,000 per sample) were retained after stringent quality control with a median of 2,619 genes and 5,936 peaks detected per cell. R packages including Seurat, Signac, chromVAR, and clusterProfiler were used for bioinformatic analyses.

Results

UMAP analysis revealed a highly distinct separation of BC cells from paired CP cells in all patients. Nilotinib treatment induced notable shifts in cells relative to untreated counterparts, with a more

pronounced effect on chromatin states than transcriptomes. We next investigated cell type composition changes from CP to BC and identified diverse cell populations in CP, including hematopoietic stem cells (HSCs) and progenitors of various lineages. BC samples predominantly contained cells resembling lymphoid-primed multipotent progenitors (LMPPs, 50-93% vs. 0-20% in CP). Conversely, HSCs and granulocyte-monocyte progenitors decreased to 0-2% and 0-35% in BC compared to 7-19% and 19-66% in CP.

Interestingly, we identified a subpopulation of CP cells presenting near BC-specific cluster in two out of three patients, comprising 20% and 3.3% of CP cells. Differential expression analysis revealed 96 genes commonly dysregulated ($|\log2FC|>0.5$, $p<0.05$) in this subpopulation compared to matched bulk of CP cells. Of these, 84 genes were also consistently dysregulated in BC cells ($|\log2FC|>0.5$, $p<0.05$), suggesting transcriptomic similarities of CP subpopulation with BC cells. Gene set enrichment analysis demonstrated that this BC-like subpopulation exhibited upregulated genes related to cellular quiescence, WNT signaling, interleukin 12/18 and STAT5 signaling, and downregulated genes of cellular proliferation, DNA replication, E2F targets, and hematopoietic lineage, compared to the bulk of CP cells ($|NES|>1$, $p<0.05$). Moreover, transcription factor (TF) motif enrichment analysis indicated enriched activity of STAT, SNAI, TCF, and depleted activity of GATA in BC-like subpopulation and BC cells, relative to the bulk of CP cells ($p<0.01$). We further identified BC-specific molecular changes across patients, including significant overexpression of oxidative phosphorylation, apoptotic signaling, and protein processing genes ($NES>1$, $p<0.01$), alongside reduced activities of CEBP TFs crucial for myeloid differentiation ($p<0.01$).

Conclusion

Our study identified key molecular changes and epigenetic rewiring during disease progression. Importantly, identification of the BC-like subpopulation in CP cells that already acquire BC characteristics offers an opportunity to discover biomarkers predictive of BC transformation in CML.