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Chronic lymphocytic leukemia and Chronic Lymphoproliferative Disorders

SPATIAL TRANSCRIPTOMIC ANALYSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA LYMPH NODES REVEALS TUMOR MICROENVIRONMENT EVOLUTION DURING DISEASE PROGRESSION

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Introduction: In chronic lymphocytic leukemia (CLL), malignant cells bidirectionally interact with the tumor microenvironment (TME), creating pro-tumoral niches within lymph nodes (LNs) that sustain disease progression and therapy resistance. Understanding dynamic CLL–TME modifications is crucial to identify novel biomarkers and actionable cell-to--cell interactions. We applied Digital Spatial Profiling (DSP) to characterize tumor and immune populations—including T lymphocytes and macrophages—while distinguishing microanatomical areas within proliferation centers (PCs) from surrounding non-PC regions.

Methods: Seventeen FFPE CLL-infiltrated LNs were analyzed and grouped into three clinical subsets: A (n=6) indolent CLL; B (n=4) CLL requiring treatment within 6 months after LN biopsy; and C (n=7) relapsed/progressive CLL. Regions of interest (ROIs) were defined by multiplexed immunofluorescence using CD3, CD68, and MUM1 to identify T cells, macrophages, and neoplastic B cells, both inside and outside PCs. Normalized transcriptomic matrices were generated for each cell type and analyzed by Gene Ontology (GO) on differentially expressed genes ($p<0.05$, $|\log_2 FC|>0.2$), ranking pathways by Normalized Enrichment Score (NES) with FDR<0.05.

Results: A total of 295 ROIs were profiled (88 $CD3^+$, 106 $CD68^+$, 101 $MUM1^+$). CLL cells within PCs showed upregulation of MUM1, MYC, and PCNA, confirming active proliferation. PC-localized T cells were enriched in pathways related to proliferation and inflammation (STAT1, NF-κB1, CCL19), while macrophages displayed oxidative stress (SOD1, TRX2), chromatin remodeling, and inflammatory signatures, including LAG3 upregulation. Across clinical subgroups, group A T cell retained an immunologically active profile (TNF, IL17D, STAT5B), group B showed metabolic and chromatin-related activation (ENO1, AKT2, PTPN1), and group C exhibited a dysfunctional state with apoptosis and reduced motility (CR1, MYB, IL3RA). Group A macrophages displayed immune and motility enrichment, while in groups B and C activation, vesicle trafficking, and mitochondrial metabolism predominated. Tumor B cells in group A were enriched for activation and antigen-receptor signaling, whereas in advanced disease they showed cell-cycle, chromatin remodeling, and inflammatory pathways.

Conclusions: Within CLL LNs, both tumor and microenvironmental cells activate distinct transcriptional programs according to spatial localization. As the disease progresses, tumor and TME compartments co-evolve from an immunologically active state toward increasing dysfunction and immune alteration.

CHRONIC LYMPHOCYTIC LEUKEMIA AND CHRONIC LYMPHOPROLIFERATIVE DISORDERS

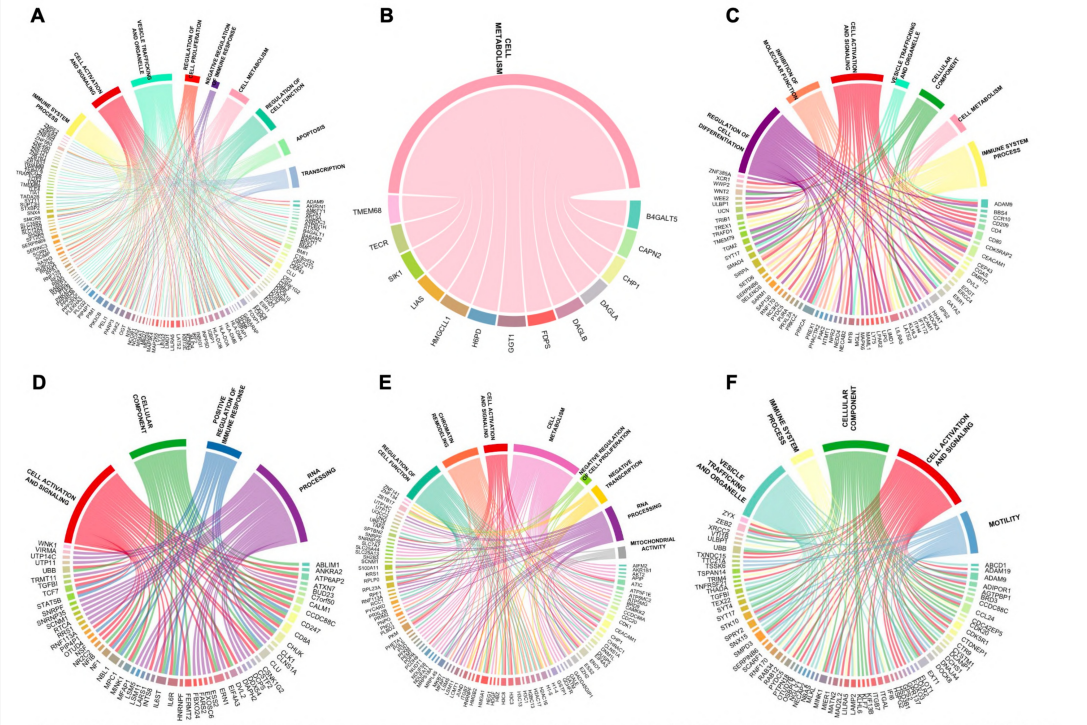

Figure 1. Chord diagram. A,B,C) Chord diagram representative of the GO pathways enriched in CLL-PC of Group A, Group B, Group C.; **D,E,F)** Chord diagram representative of the GO pathways enriched in CD3-PC of Group A, Group B, Group C.