(PB3933) GENETICALLY FAITHFUL, TRANSCRIPTIONALLY TRANSFORMED: MICROENVIRONMENT-DRIVEN DIVERGENCE IN MATCHED LBCL PRECLINICAL MODELS

Topic: 21. Lymphoma biology & translational research

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Background

Patient-derived xenografts (PDXs) and cell lines (PDCLs) of large B-cell lymphoma (LBCL) broadly retain primary tumor genetics, yet bulk transcriptomic profiling cannot resolve whether they preserve the intratumoral transcriptional heterogeneity that can drive clonal evolution and determine drug response.

Aims

To resolve, at single-cell resolution, transcriptional landscapes of matched primary tumor, PDX, PDCL, and PDCL-based xenografts (CDX) from LBCL patients, and to determine whether model-specific transcriptional programs arise from clonal selection or epigenetic adaptation to distinct tumor microenvironments (TME).

Methods

PDXs from pathology-confirmed LBCL were propagated in NSG mice; PDCLs were established from the same tumours in vitro. CDXs were established by xenotransplantation of PDCLs into mice. Viable cells were CD45+ sorted and profiled by 5′ scRNAseq with paired BCR/TCR V(D)J sequencing. scRNAseq QC included ambient RNA correction (decontX) and scDblFinder + V(D)J-based doublet removal. Samples were analyzed in Seurat. Cell types were assigned using canonical marker genes; BCR V(D)J, combined with light-chain restriction and clonotype analysis, identified malignant B cells. Samples were additionally profiled using whole exome sequencing (WES).

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Results

We profiled 46,609 cells from two primary LBCL samples (P062, ascites; P144, peripheral blood) and PDX, PDCL, and CDX samples derived from the primary samples (8 samples in total, Fig. 1A). B-cell receptor (BCR) light-chain restriction confirmed 85–100% malignant purity across all samples (Fig. 1B); TME cells of human origin were present in PT and PDX only. On joint UMAP, malignant cells clustered strictly by sample of origin. In P062 (Fig. 1C), the dominant “proliferative” PT state (blue, 56% of PT) propagated to all models (61–96%), while an “immunologically active” state with MHC I/II antigen presentation and IgM expression (green, 41% of PT) was entirely lost upon derivation of both PDX and PDCL. Besides the dominant “proliferative” state, the PDCL acquired a new “hypermetabolic” state (orange, 36%), subsequently inherited also by the CDX (32%). In P144 (Fig. 1D), 98% of PT cells occupied a single “G1 quiescent” state characterized by expression of KLF2 (green). PDX partially preserved the "G1 quiescent" state (9%) and acquired 3 new transcriptional states: a "tumor-proximal" state (violet, 29%), a "proliferative" state (brown, 15%), and a "xenograft-adapted" state (orange, 47%). In contrast, PDCL occupied 3 new transcriptional states: "hypermetabolic" (blue, 65%), "quiescent" (red, 23%), and "hyperproliferative" (purple, 11%). CDX converged on the "xenograft-adapted" state observed in PDX (98%). BTG1, ZFP36L2, and FCMR defined a conserved PT program lost in all models. Integration of matched WES confirmed a shared copy-number landscape across all models in both patients, with HVCN1 p.W45R showing no cluster-specific enrichment in P144, establishing transcriptional divergence as epigenetically rather than genetically driven.

Summary/Conclusion

We demonstrated that PDX and PDCL cells, despite sharing the same genomic landscape with the donor primary lymphoma cells, may assume different to highly different transcriptional states, which must be considered in the context of specific research objectives. Moreover, our data suggest that CDX represent the least relevant models compared to PDX and PDCL, and should be avoided in preclinical research, and replaced by PDX.

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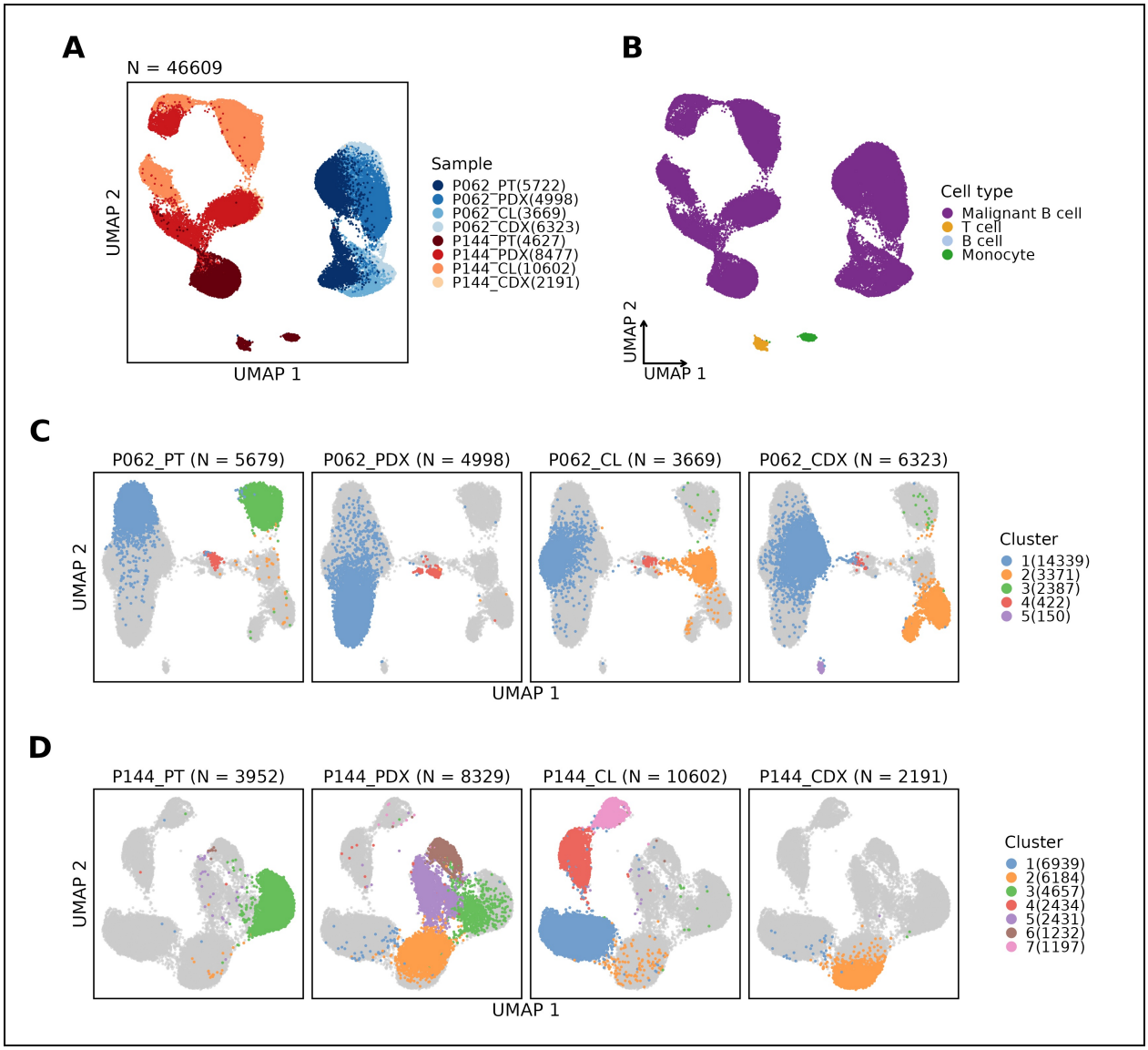


Figure 1. Single-cell transcriptional landscape of LBCL preclinical models. (A) Joint UMAP of 46,609 cells from two LBCL patients (P062, blues; P144, reds/oranges), coloured by sample of origin. Cell counts per sample in parentheses. (B) Same UMAP coloured by cell type; TME cells are restricted to PT and PDX. (C–D) UMAPs of malignant B cells from P062 (20,669 cells; C) and P144 (25,074 cells; D), each faceted by model (PT, PDX, PDCL, CDX). Cells are coloured by Leiden cluster (resolution 0.1 for P062, 5 clusters; resolution 0.3 for P144, 7 clusters); grey points represent cells from other facets shown for spatial reference.

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