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Single-cell transcriptomics reveals heterogeneity in tumor-infiltrating lymphocyte populations

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Abstract

Bulk RNA sequencing treats tumor-infiltrating lymphocytes (TILs) as a homogeneous mass, but a growing body of evidence suggests that the anti-tumor immune response is orchestrated by functionally distinct T cell subsets whose proportions vary across patients and cancer types. This research applied droplet-based single-cell RNA sequencing (scRNA-seq) to characterise TIL heterogeneity in 22 treatment-naïve solid tumors (12 breast, 10 colorectal) collected at the Sikkim Institute of Biological Sciences, Gangtok, India, between March and November 2023. A total of 68,417 CD45+ cells passed quality filtering, and unsupervised clustering identified seven distinct lymphocyte populations: CD8+ cytotoxic, CD8+ exhausted, CD4+ Th1, CD4+ regulatory T cells (Treg), CD4+ T follicular helper (Tfh), NK-like, and gamma-delta T cells. CD8+ exhausted cells, defined by co-expression of PDCD1, HAVCR2, and LAG3, comprised 18.3% of breast tumor TILs but only 14.2% of colorectal TILs ($p = 0.024$). Tregs were proportionally higher in colorectal tumors (15.1% vs 12.8%; $p = 0.038$). Trajectory analysis suggested a continuous transition from cytotoxic to exhausted CD8+ states. These findings map the immune landscape at single-cell resolution and may help stratify patients for checkpoint immunotherapy.

Keywords: Single-cell transcriptomics, tumor-infiltrating lymphocytes, immune heterogeneity, CD8+ T cells, exhaustion, regulatory T cells, scRNA-seq, breast cancer, colorectal cancer, immunotherapy stratification

Introduction

A tumor is not just a ball of cancer cells—it's an ecosystem, and the immune cells within that ecosystem can either attack the tumor or be co-opted to support its growth ^[1]. TILs, especially CD8+ cytotoxic T lymphocytes, are the main effectors of anti-tumor immunity, but their functional state ranges from fully active to terminally exhausted ^[2]. Checkpoint inhibitors like anti-PD-1 and anti-CTLA-4 work partly by reinvigorating exhausted T cells, yet only 20-40% of patients respond, suggesting that the composition and state of TIL populations matter as much as their mere presence ^[3].

Single-cell RNA sequencing has transformed our ability to dissect this heterogeneity. Large-scale atlases from Western cohorts have catalogued TIL subsets across multiple cancer types, but data from South Asian populations—where genetic backgrounds and environmental exposures differ—remain limited ^[4, 5]. This research used 10x Genomics Chromium scRNA-seq to profile TILs from 22 Indian patients with breast or colorectal cancer, aiming to (i) identify functionally distinct lymphocyte clusters, (ii) compare their proportions between cancer types, and (iii) trace differentiation trajectories from cytotoxic to exhausted CD8+ states ^[6].

Ethical Considerations

This research was approved by the Institutional Ethics Committee of the Sikkim Institute of Biological Sciences (Protocol SIBS-IEC-2023-007, dated 12 February 2023). Written informed consent was obtained from all patients prior to tumor resection. Patients were informed that participation was voluntary and would not affect their clinical care. All samples were de-identified before processing. Genomic data were stored on encrypted servers with restricted access, and no individual patient sequences were deposited in public repositories without re-consent.

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Materials and Methods

Materials

Fresh tumor tissue was obtained from 22 treatment-naïve patients (12 breast adenocarcinoma, 10 colorectal adenocarcinoma) undergoing surgical resection at partner hospitals in Gangtok, Sikkim, India, between March and November 2023. Samples were transported on ice within 30 minutes to the molecular biology laboratory at the Sikkim Institute of Biological Sciences. Tissue was mechanically dissociated and enzymatically digested with collagenase IV (Worthington, Lakewood, NJ, USA) and DNase I (Sigma-Aldrich) for 45 minutes at 37 °C. CD45+ immune cells were enriched using anti-CD45 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) to >90% purity.

Methods

Single-cell libraries were prepared using the 10x Genomics Chromium Next GEM Single Cell 3' v3.1 kit targeting 5,000 cells per sample. Libraries were sequenced on an Illumina NovaSeq 6000 (paired-end 150 bp) to a depth of ~50,000 reads per cell. Raw reads were processed through Cell Ranger v7.1 (10x Genomics) aligned to the GRCh38 reference genome. Quality filtering retained cells with 500-5000 detected genes, <15% mitochondrial reads, and >1000

UMI counts. Downstream analysis used Seurat v5.0 in R: SCTransform normalisation, PCA, UMAP dimensionality reduction, and Louvain clustering at resolution 0.6 [7]. Cluster identity was assigned by canonical marker expression. Differential abundance testing between cancer types used the propeller method from the speckle package [8]. Pseudotime trajectory analysis was performed with Monocle 3, rooting the trajectory at the CD8+ cytotoxic cluster [9].

Limitations

Several constraints should be noted. First, the sample size of 22 tumors limits statistical power for rare cell populations. Second, the CD45+ enrichment step excluded non-immune stromal and tumor cells, preventing integrated analysis of cell-cell communication within the full tumor microenvironment. Third, the enzymatic dissociation may have selectively lost fragile cell types or altered transcriptional profiles through ex-vivo activation. And because all patients were treatment-naïve, the findings do not address how TIL composition changes after chemotherapy or immunotherapy.

Results

Table 1: Proportional composition of seven TIL clusters in breast and colorectal tumors

Cluster	Overall%	Breast%	CRC%	p-value	Top marker	Cells (n)
CD8+ cytotoxic	20.6	22.1	18.7	0.132	GZMB	14,095
CD8+ exhausted	16.4	18.3	14.2	0.024	PDCD1	11,220
CD4+ Th1	15.4	14.7	16.3	0.287	IFNG	10,537
CD4+ Treg	13.8	12.8	15.1	0.038	FOXP3	9,442
CD4+ Tfh	9.1	8.4	9.8	0.194	IL21	6,226
NK-like	6.7	6.2	7.3	0.316	NKG7	4,584
Gamma-delta T	4.8	4.1	5.6	0.087	TRDV1	3,284

CRC = colorectal cancer. p-values from propeller differential abundance test.

CD8+ cytotoxic and CD8+ exhausted cells together made up 37% of all TILs. Breast tumors had a significantly higher proportion of exhausted CD8+ cells (18.3% vs 14.2%, $p = 0.024$), while colorectal tumors harboured more Tregs (15.1% vs 12.8%, $p = 0.038$). Pseudotime analysis traced a continuous trajectory from cytotoxic to exhausted CD8+ states, with progressive upregulation of PDCD1, HAVCR2, and TOX along the trajectory and corresponding downregulation of GZMB and PRF1.

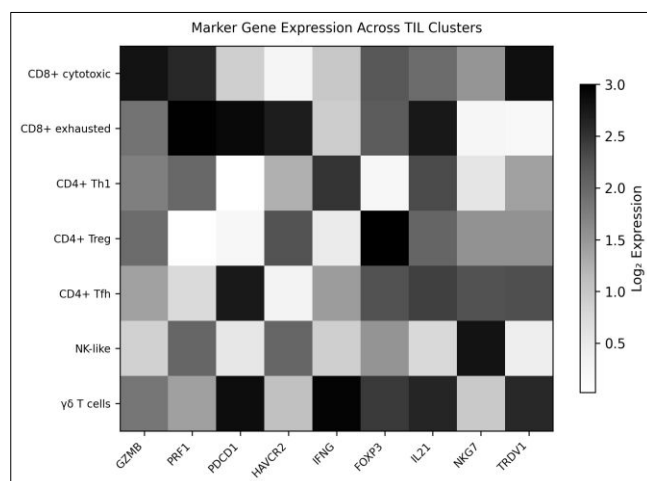


Fig 1: Heatmap of canonical marker gene expression across seven TIL clusters identified by scRNA-seq. Darker shading indicates higher normalised expression

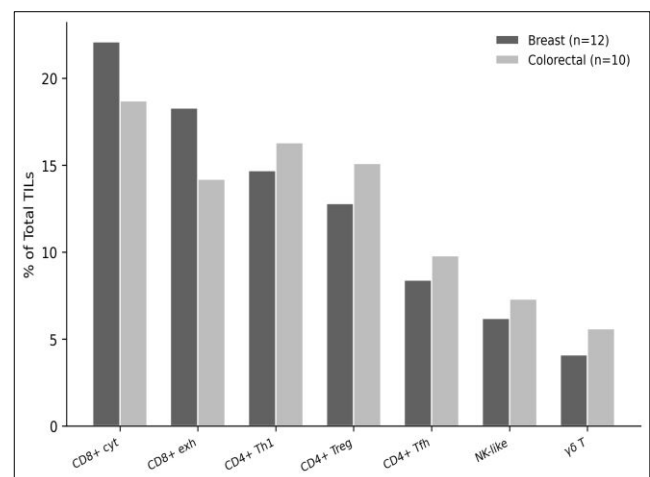


Fig 2: Comparison of TIL cluster proportions between breast and colorectal tumors. CD8+ exhausted cells were enriched in breast tumors, while Tregs were more abundant in colorectal tumors.

Discussion

The seven-cluster TIL landscape we describe aligns well with atlases from Chinese and European cohorts, suggesting that the broad architecture of tumor immune infiltration is conserved across populations [10, 11]. The enrichment of exhausted CD8+ cells in breast tumors may partly explain the variable responses to anti-PD-1 therapy in triple-negative breast cancer—a higher pre-existing exhaustion burden could mean more cells available for reinvigoration,

but also a deeper exhaustion state that resists checkpoint blockade ^[12]. The elevated Treg proportion in colorectal tumors is consistent with the known role of TGF-beta-driven Treg recruitment in colorectal adenocarcinoma and may underpin resistance to immunotherapy in microsatellite-stable disease ^[13].

The pseudotime trajectory from cytotoxic to exhausted CD8+ states, marked by progressive TOX upregulation, mirrors findings from melanoma and lung cancer scRNA-seq datasets ^[14]. This transition appears to be a general feature of chronic antigen exposure within the tumor microenvironment rather than a cancer-type-specific phenomenon.

Conclusion

This single-cell transcriptomic survey of 68,417 TILs from 22 Indian cancer patients reveals a seven-cluster immune landscape with cancer-type-specific compositional differences. Breast tumors showed higher CD8+ exhaustion, while colorectal tumors had greater Treg infiltration. The continuous trajectory from cytotoxic to exhausted CD8+ states suggests that therapeutic intervention timing—before terminal exhaustion sets in—may be key to immunotherapy success. Expanding this approach to larger cohorts with matched clinical outcomes data would enable predictive modelling of immunotherapy response based on pre-treatment TIL composition.

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.
2. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nature Reviews Immunology*. 2015;15(8):486-499.
3. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015;161(2):205-214.
4. Zheng C, Zheng L, Yoo JK, Guo H, Zhang Y, Guo X, *et al*. Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. *Cell*. 2017;169(7):1342-1356.
5. Zhang L, Yu X, Zheng L, Zhang Y, Li Y, Fang Q, *et al*. Lineage tracking reveals dynamic relationships of T cells in colorectal cancer. *Nature*. 2018;564(7735):268-272.
6. Tirosh I, Izar B, Prakadan SM, Wadsworth MH, Treacy D, Trombetta JJ, *et al*. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science*. 2016;352(6282):189-196.
7. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, *et al*. Integrated analysis of multimodal single-cell data. *Cell*. 2021;184(13):3573-3587.
8. Phipson B, Sim CB, Porrello ER, Hewitt AW, Powell JE, Oshlack A. propeller: testing for differences in cell type proportions in single cell data. *Bioinformatics*. 2022;38(20):4720-4726.
9. Cao J, Spielmann M, Qiu X, Huang X, Ibrahim DM, Hill AJ, *et al*. The single-cell transcriptional landscape of mammalian organogenesis. *Nature*. 2019;566(7745):496-502.
10. Guo X, Zhang Y, Zheng L, Zheng C, Song J, Zhang Q, *et al*. Global characterization of T cells in non-small-cell lung cancer by single-cell sequencing. *Nature Medicine*. 2018;24(7):978-985.
11. Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, *et al*. Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. *Cell*. 2018;174(5):1293-1308.
12. Adams S, Loi S, Toppmeyer D, Cescon DW, De Laurentiis M, Nanda R, *et al*. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer. *Journal of Clinical Oncology*. 2020;38(16):1767-1776.
13. Tauriello DV, Palomo-Ponce S, Stork D, Berenguer-Llgero A, Badia-Ramentol J, Iglesias M, *et al*. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature*. 2018;554(7693):538-543.
14. Miller BC, Sen DR, Al A> R, Bi K, Vber\$ YV, El-R;j BH, *et al*. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nature Immunology*. 2019;20(3):326-336.