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Seed or soil: Tracing the immune subsets in metastatic tumors

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Mapping the tumor-infiltrating immune subsets to an origin of malignancy or tissue niche is important for designing effective immunotherapies, yet it remains a challenging task. In this issue of *Cancer Cell*, Liu et al. pieced together this puzzle through a comprehensive single-cell analysis of colorectal cancer patients with liver metastases.

During tumor progression, the composition and phenotype of the immune cell types in the tumor microenvironment (TME) are jointly shaped by the malignant cells and the niche of the adjacent tissue (Binnewies et al., 2018). Although malignancy had been presumed to play a dominant role in this process, a growing body of literature has revealed that the niche of the adjacent tissue could also influence the induction and maintenance of certain immune populations in the TME. This influence is further broken down into tissue-specific immunity (Weisberg et al., 2021), which partially contributes to the TME heterogeneity across different cancer types, and other non-malignancy factors that co-exist in the TME, such as germline mutations and microbiome (Helmink et al., 2019; Sayaman et al., 2021). A precise mapping of the immune subsets in the TME to the malignancy, the tissue niche, or other contributing factors is highly desirable for the development of effective and low-toxicity immunotherapies. However, the co-existence of cancer cells and the tissue microenvironment makes it difficult to disentangle the sources of induction of the diverse immune cell subsets. Metastatic tumors (seed), on the other hand, maintain the genetic signatures from the primary tumor while migrating to a different organ-specific tissue environment (soil) (Fidler, 2003). Hence, an accurate linkage of the cellular components in the metastatic TME to their contributing tissue(s) of origin becomes available with multi-sectional autologous sampling and the cutting-edge single-cell transcriptomics profiling.

In this issue of *Cancer Cell,* Liu et al. analyzed the single-cell RNA-sequencing (scRNA-seq) samples from patients with colorectal cancer liver metastasis (CRLM), and they discovered several immune cell populations that are closely linked to either malignant cells or the tissue niche (Liu et al., 2022). First, the authors collected 101 matched samples from patients with CRLM; these samples covered the primary tumor, adjacent colon, mesenteric lymph node, liver metastases, adjacent liver, and peripheral blood. CD45^{+} immune cells were flow

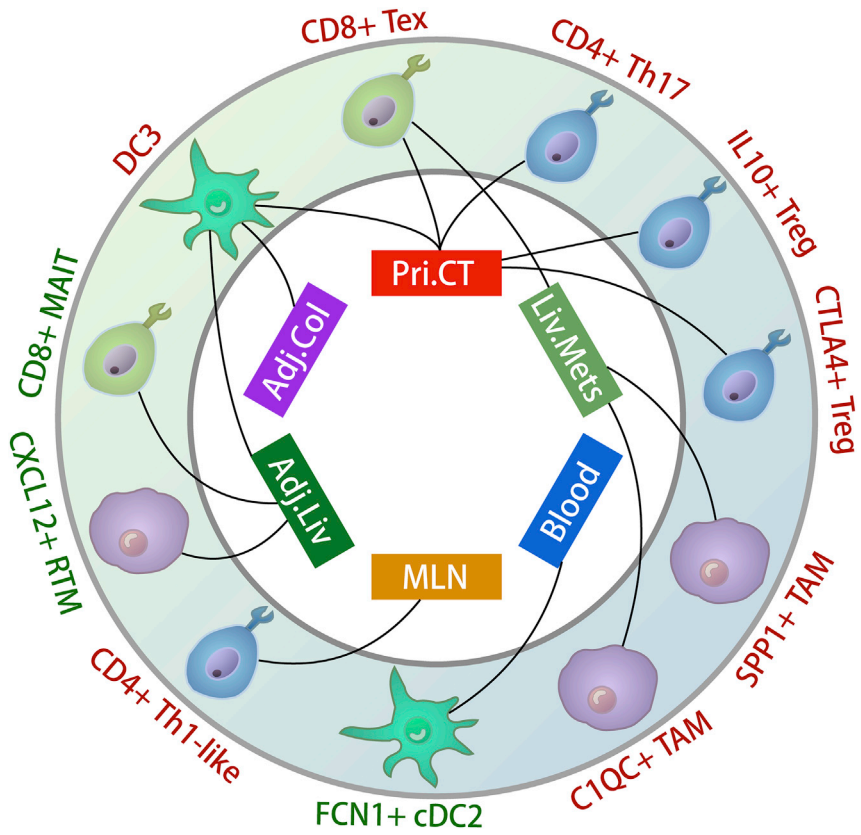

Figure 1. Enrichment of the major immune populations in CRLM patients
This figure summarizes the data presented in Figure 7 of Liu et al. (2022). The line connection indicates statistical enrichment (Ro/e > 3) of the related tissue except for DC3, with 1£Ro/e < 3.
Abbreviations: Pri.CT, primary tumor; Liv.Mets, liver metastasis; MLN, mesenteric lymph node; Adj.Liv, adjacent liver tissue; Adj.Col, adjacent colon tissue.
Color coding of the names of the immune cell subsets: red is for Malignancy-associated (M-type) and green is for Niche-associated (N-type).

sorted from these samples for scRNA-seq data generation, and a total of ~330K high-quality transcriptomes were acquired. Further, another 100 single-cell samples from non-metastatic colorectal cancer (CRC) or liver cancer patients were obtained from previous studies (Zhang et al., 2020; Zhang et al., 2019) and combined with the CRLM samples. By using the non-metastatic sample, Liu et al. identified tissue-specific immune populations, such as the liver-enriched KLRB1$^+$ resident memory CD8$^+$ T cells and the colon-enriched intraepithelial lymphocytes (IELs), etc. However, over half of the immune subsets cannot be unambiguously linked to malignancy or tissue origins because they could arise from more complicated biological processes.

To facilitate the linkage tracing of immune cells in the patients with metastatic tumors, Liu et al., developed the PhenoAligner method, which implements a k-Nearest Neighbor search to map the cells from the query group (liver metastasis samples) to the reference groups (other samples). Specifically, it applies Pearson's correlation to compare the gene expression profile of each query cell to the reference tissues, and it labels the cell with the tissue type that has the highest similarity. For each query cluster for each reference tissue type, it then calculates a PhenoAligner index, which is the proportion of query cells that is labeled as the corresponding tissue type. According to PhenoAligner indices, the immune subsets in the liver metastasis group were divided into three categories: Malignancy-associated (M-type), Niche-associated (N-type), and Combo-associated (C-type). Under this framework, Liu et al. performed a detailed examination of the major immune populations in the liver metastasis (Figure 1). First, investigation of exhausted CD8$^+$ T cell (Tex) subpopulations revealed that the dysfunctional layilin$^+$ (*LAYN*) T cells displayed high gene expression similarity to the T cells in the primary tumor, and thus they were classified as M-type. By tracing the T cell receptors (TCRs) that were shared across different tissues, the authors confirmed that the TCRs in the *LAYN*$^+$ Tex cell cluster significantly co-occurred in both primary tumors and liver metastases. Due to the low cellular mobility of the exhausted T cells in the TME, it is likely that malignant cells in the liver metastases expressed the same cancer-associated antigens and induced the clonal expansion of the same groups of antigen-specific CD8$^+$ T cells. Based on the evidence of TCR sharing, recently activated effector memory T cells in the blood are a probable origin of the shared T cells in both tumor locations. The following analysis of CD4$^+$ T cells revealed four M-type clusters, including two helper (Th17 and Th1-like) and two regulatory (Treg-IL10 and Treg-CTLA4) subsets, all of which were significantly enriched in the primary tumors. To explain the recreation of these CD4$^+$ T cell clusters in distal tumors, Liu et al. came up with two possible mechanisms: (1) precursor T cells already existed in the tissue and clonally expanded upon activation with the same cancer antigens; and (2) these T cells were independently recruited by the cancer cells in the different tissues. By tracking TCRs in the CD4$^+$ T cells, the authors showed that Treg-CTLA4 and Th1-like subsets presented significant sharing between the primary and metastatic tumors, and this result implicates the first mechanism. In contrast, Th17 and Treg-IL10 T cells exhibited mutually exclusive TCR usage in the two tumor sites, and thus these cells might be induced by the commonalities from both tissue niches, such as the signatures related to gut milieu.

The myeloid cellular components in the liver metastasis also demonstrated interesting tissue-specific properties. Three tumor-associated macrophage (TAM) clusters were assigned as M-type, including *MKI67*$^+$, *SPP1*$^+$, and *C1QC*$^+$ TAMs. By comparing to the non-metastatic liver samples with CRC samples, Liu et al. made an interesting observation: *SPP1*$^+$

TAMs were common in non-metastatic CRCs but missing in non-metastatic liver cancers, and yet in CRLM patients, these cells were dominantly observed in the liver metastases. Further validation with a fluorescence-activated cell sorting (FACS) experiment confirmed the existence of *SPP1*$^{+}$ TAMs in the metastatic tumors. These results strongly support the possibility that the malignant cells are the primary driver for the *SPP1*$^{+}$ macrophages. The same analyses were performed for the dendritic cell (DC) populations. Liu et al. observed that the cDC2–C1QC cluster carried the same molecular signature as the recently reported DC3 population carried (Diao et al., 2018), and this cluster was also enriched in both tumor sites and was classified as the M-type. Subsequent survival analysis using TCGA data implicated the molecular signatures of both *SPP1*$^{+}$ TAM and DC3 subsets as being associated with poor clinical outcomes in multiple human cancers.

In this work, Liu et al. threaded the diverse immune components in the metastatic tumors to their probable origins. The computational analysis revealed that some of these immune subsets were clearly malignancy driven, and this finding may prompt future clinical investigations of these cells to uncover new therapeutic opportunities. In addition, the observation that the TCRs of *CTLA4*$^{+}$ Tregs were shared between primary and metastatic tumors suggests that the clonal activation of these Tregs is specific to cancer- associated antigens, not to tissue-associated antigens, and this may be worth experimental follow-up. Another interesting finding is the higher enrichment of *SPP1*$^{+}$ TAMs in metastases than in the primary tumors, which indicates a lineage-specific, malignancy-driven mechanism for macrophage polarization or recruitment. Overall, Liu et al. have advanced the knowledge of the tumor immune microenvironment through meticulous study design and comprehensive single-cell analysis. The high-quality scRNA-seq data generated in this study will also provide a valuable resource for future investigations of immune responses during tumor migration.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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