Leveraging Single-Cell Analysis and Trajectory Inference to Dissect Phenotypic Heterogeneity in Colorectal Cancer Organoids

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In colorectal cancer (CRC), the intrinsic phenotypic heterogeneity characteristic of the normal intestinal epithelium is partially preserved, while stem cell dynamics, mirroring features of native hierarchical organization, actively contribute to its plasticity. This is manifested in the rerouting of normal differentiation paths into abnormal and unregulated developmental trajectories that actively drive tumor progression. The presence of transcriptionally and phenotypically distinct subpopulations within tumors is thought to play a fundamental role in sustaining tumor growth and influencing treatment response. In colorectal cancer (CRC), such phenotypic heterogeneity has increasingly been recognized as a major driver of therapy resistance and disease relapse. In addition to cancer stem cells, colorectal tumors also harbor subpopulations of differentiated cells — usually considered post-mitotic — distinguished by specific transcriptional signatures. However, the role and mutual interactions among these different subpopulations remain poorly understood.

In this study, we leveraged single-cell RNA sequencing to computationally infer differentiation trajectories, with the aim of exploring lineage hierarchies and dynamic relationships among transcriptionally distinct subpopulations in colorectal cancer. Specifically, we analyzed scRNA-seq datasets from patient-derived organoids (PDOs) established from liver metastases of CRC, both under normal growth conditions and following treatment with Cetuximab, an anti-EGFR monoclonal antibody. During normal tumor growth, we identified the presence of two well-established subpopulations: a stem-like cells subpopulation, and a differentiated one. Upon Cetuximab treatment, new subpopulations emerged, including cells characterized by high expression of WNT-related genes and a fraction of slow-cycling cells, which persisted even under therapeutic pressure. By applying trajectory inference tools, we reconstructed the graph structure of the single-cell data, allowing us to infer differentiation (or dedifferentiation) pathways and assess the global lineage architecture of each PDO. This strategy also enabled the integration of datasets across multiple time points, offering insights into the dynamic interplay between subpopulations under both steady-state and drug-exposed conditions.