Project 7: Characterization of colorectal cancer cell states as a function of their microbial microenvironment

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MOTIVATION

Colorectal cancer (CRC) cells display molecular heterogeneity that cannot be described solely by their genetic alterations. Single cell studies have identified recurrent transcriptional states of malignant epithelial cells across patients, including drug tolerant persister and residual disease states that survive therapy and seed relapse (Joanito et al, 2022; Cañellas-Socias et al, 2022; Pelka et al, 2021). Patient derived organoids reproduce several of these states and provide an experimentally tractable system in which the transitions between them, cancer cell plasticity can be dissected at single cell resolution.

The tumour microenvironment is a major source of signals that can influence cancer cell plasticity and therapy response. Cancer associated fibroblasts (CAFs), a key stromal component of CRC, have been shown in published organoid studies and ongoing work in the Garnett lab to reshape cancer cell phenotypes under treatment pressure. Within the PERSIST-SEQ consortium, our analysis of CRC organoid lines exposed to targeted and cytotoxic therapies, with or without fibroblast conditioned medium (FCM), identified recurrent regenerative plastic programmes marked by reduced lineage commitment, facilitating trans_differentiation towards non canonical, therapy tolerant fates. Together, these findings provide a quantitative single cell framework for comparing how different microenvironmental signals shape lineage plasticity and regenerative potential in organoid models.

The microbiome is a second, largely unexplored source of microenvironmental signals, and one particularly pertinent to CRC. Fusobacterium nucleatum is among the most reproducibly CRC enriched taxa, a recent multi-cohort meta analysis (6,779 samples across 27 studies) confirmed a robust, generalisable CRC microbiome signature detectable in both stool and tumour tissue (Pekel et al, 2026) and has been linked experimentally to tumour progression, immune modulation and chemoresistance (Bullman et al, 2017; Kostic et al, 2013; Yu et al, 2017). Crucially, microbial products act directly on tumour biology mechanistically: F. nucleatum derived formate acts as an oncometabolite that increases cancer stemness and invasion through AhR signalling (Ternes et al, 2022), F. nucleatum activates Wnt/β catenin signalling, and colibactin from pks+ Escherichia coli imprints a mutational signature detectable in human CRC genomes (Pleguezuelos-Manzano et al, 2020) and is linked with the increasing incidence of early onset CRC. Whether microbial signals drive cancer cells towards the same states as stromal signals — for instance, converging on the stromal regenerative plastic programme through a distinct upstream axis or towards distinct states, is unknown.

Integrating microbial signals into a broader microenvironmental framework will allow us to determine how they influence CRC lineage plasticity and phenotypic transitions, and whether their effects converge with or differ from those induced by stromal signals.

Here we propose to go beyond extending the fibroblast or stroma paradigm: we will generate new single cell data on microbiome conditioned CRC organoids to resolve how the microbiome and F. nucleatum in particular reshapes cancer cell plasticity. Applying a similar defined conditioned medium logic, selected microbiome derived metabolites will be applied to patient derived CRC organoids and profiled by single cell transcriptomics to characterise how they influence cancer cell states. By combining these controlled perturbation experiments with pathway informed computational modelling and comparison against spatial and in vitro datasets, we will test whether distinct microenvironmental perturbations drive CRC cells towards shared or distinct molecular states.

SPECIFIC OBJECTIVES

Our proposed work aims to answer the following questions:

- Which cancer cell states are induced in CRC organoids by selected microbiome derived signals?
- Do microbial and CAF derived signals drive cancer cells towards shared or distinct states and by modulation of state identity or of transition timing?
- Which signalling and metabolic pathways are consistently associated with these transitions across experimental systems and mutational contexts (APC, KRAS, TP53)? How do genotypes affect the response to the external response? Genotype -> phenotype or MSI vs MSS
- Are the molecular responses identified in vitro detectable in spatial regions of human CRC associated with bacterial presence?
- We will address these questions using patient derived CRC organoids as a controlled perturbation system, building directly on the analytical framework established for FCM. Specifically, we will address the following aims.
- Do microbiome derived signals reprogram healthy and malignant colonic epithelium differently, i.e. does oncogenic genotype (notably APC loss) license a plastic response that normal crypt cells resist?

Aim 1. Lineage and differentiation: Determine how microbiome derived signals (defined metabolites and, via FusoCRC, chronic F. nucleatum exposure) reshape the differentiation hierarchy of genotyped patient derived CRC organoids, using single cell transcriptomics to quantify the balance of $LGR5^{+}$ stem, secretory and absorptive lineages and to test whether microbial signals act through Wnt/β catenin to sustain a stem-like compartment. As a focused comparator, a small panel of wild type healthy colon organoids will be profiled under the same conditions; although more demanding to culture, and therefore restricted to the core exposures, this provides a normal epithelium baseline that separates microbiome-driven reprogramming from responses licensed by oncogenic genotype.

Aim 2. Cell state plasticity: Resolve whether microbial signals drive regenerative (revival), EMT or lineage plasticity, and whether microbial, CAF and therapy induced states converge on a shared attractor or remain distinct including whether combined CAF and microbial signals produce emergent states. Using pathway constrained comparison against our FCM reference and public CRC atlases, together with trajectory and optimal transport modelling, we will establish whether microbial signals alter state identity or, as FCM does, the timing of transitions.

Aim 3. Functional outcomes: Test the consequences of the induced states by (i) challenging conditioned organoids with chemotherapy and with KRAS inhibition to ask whether microbial signals

confer chemoresistance and increase tolerance to targeted therapy, as FCM does; (ii) assessing whether they shift the tumour towards an immune engaged ("hot") or immune excluded ("cold") phenotype, read out from antigen presentation, interferon and immune recruitment programmes and tested directly in co culture with iPSC derived immune cells, motivated by F. nucleatum being a known modulator of the CRC immune microenvironment (Kostic et al, 2013; Gur et al, 2015); and (iii) mapping the associated programmes onto spatially-resolved human CRC datasets with bacterial localisation to test whether they recur in native tumours and mark therapy relevant, immune excluded, relapse prone niches.

These aims yield concrete, falsifiable predictions grounded in our FCM results. First, because formate increases stemness and invasion and F. nucleatum activates Wnt/β catenin, we predict that microbial exposure will partially converge on the KRAS inhibitor induced revival/EMT programme through a distinct upstream route, quantifiable as overlap in programme or factor space. Second, we predict a microbiome timing interaction symmetric to the FCM effect, chronic bacterial exposure modulating the tempo of transitions, testable with the identical mixed effects framework. Third, because Wnt driven programmes are line selective in our data, we expect F. nucleatum to engage them preferentially in Wnt competent, APC mutant organoids, providing a testable interaction between mutational context and microbial signal. Fourth, if microbial signals expand the revival/persister state, conditioned organoids should show reduced killing by chemotherapy and by KRAS inhibition, a direct functional test linking microbiome driven plasticity to therapy tolerance. Fifth, we expect the microbial response itself to be conditioned by the inflammatory context: by priming organoids into immune "hot" or "cold" states with defined cytokine cocktails, we can test whether the same organoid responds differently to bacterial signals under distinct inflammatory settings, disentangling microbial from inflammation driven plasticity.

This multidisciplinary project combines the Garnett lab's expertise in patient derived organoid biobanks, functional perturbation and cancer pharmacogenomics including the world's largest CRC organoid drug response panel, and ongoing work priming organoids into immune "hot" or "cold" states with defined cytokine (e.g. interferon) cocktails with the Saez-Rodriguez lab's expertise in single cell integration, pathway informed multicellular modelling (e.g. MOFAcell, LIANA+) and mechanistic analysis of drug response. The cytokine priming capability is directly enabling: it allows the same organoid to be challenged with bacteria and bacterial metabolites across controlled inflammatory backgrounds, turning immune context from a confounder into an experimental variable. The project is deliberately complementary to two ongoing efforts: PERSIST-SEQ, which addresses the stromal (FCM) axis of CRC persister biology, and the CartoHostBug ERC Synergy project of the Saez-Rodriguez group that aims at spatially resolving host-microbiome interactions in CRC . By applying a shared framework across stromal, microbial and inflammatory signals and comparing them against a common cell state reference, the project connects distinct ecological drivers of CRC plasticity within a single, reusable analytical framework, and links controlled experimental perturbations to molecular patterns observed directly in human tumours.

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