Novel computational approaches to study CRC tumour microenvironment organoids using scRNA-seq

Ferran Cardoso Rodriguez[1,2*], Xiao Qin[1], Petra Vlckova[1], Jahangir Sufi[1], Maria Ramos Zapatero[1], James Opzoomer[1], Javier Herrero[2] , Christopher J. Tape[1*]

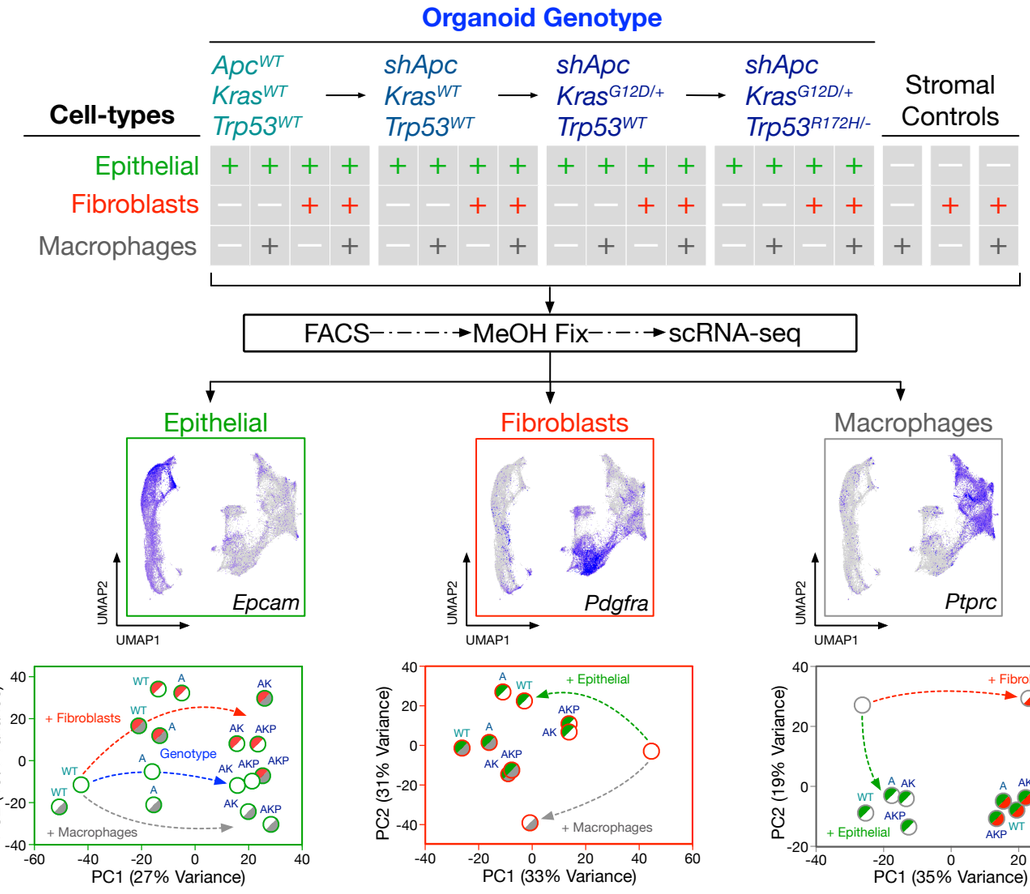
[1] Cell Communication Lab, Department of Oncology, University College London Cancer Institute, 72 Huntley Street, London, WC1E 6DD, UK.
[2] Bill Lyons Informatics Centre, University College London Cancer Institute, 72 Huntley Street, London, WC1E 6DD, UK.

* Correspondence: ferran.cardoso.19@ucl.ac.uk | c.tape@ucl.ac.uk

INTRODUCTION

Colorectal cancer (CRC) tumours present as a heterocellular setting where the colon epithelia harbouring oncogenic mutations interacts with the surrounding stromal and immune compartments. Despite epithelial organoids being used to model CRC there is a lack of complex coculture systems that also model the tumour microenvironment (TME). Here we present such a system, able to model both the oncogenic and microenvironmental axes, and report how scRNA-seq analysis reveals that stromal cells modulate differentiation in normal colonic epithelia but fail to do so in the altered CRC organoids.

① Multiplexed scRNA-seq Reveals Differential Regulation of CRC-TME Organoids by Oncogenes and Stromal Cells

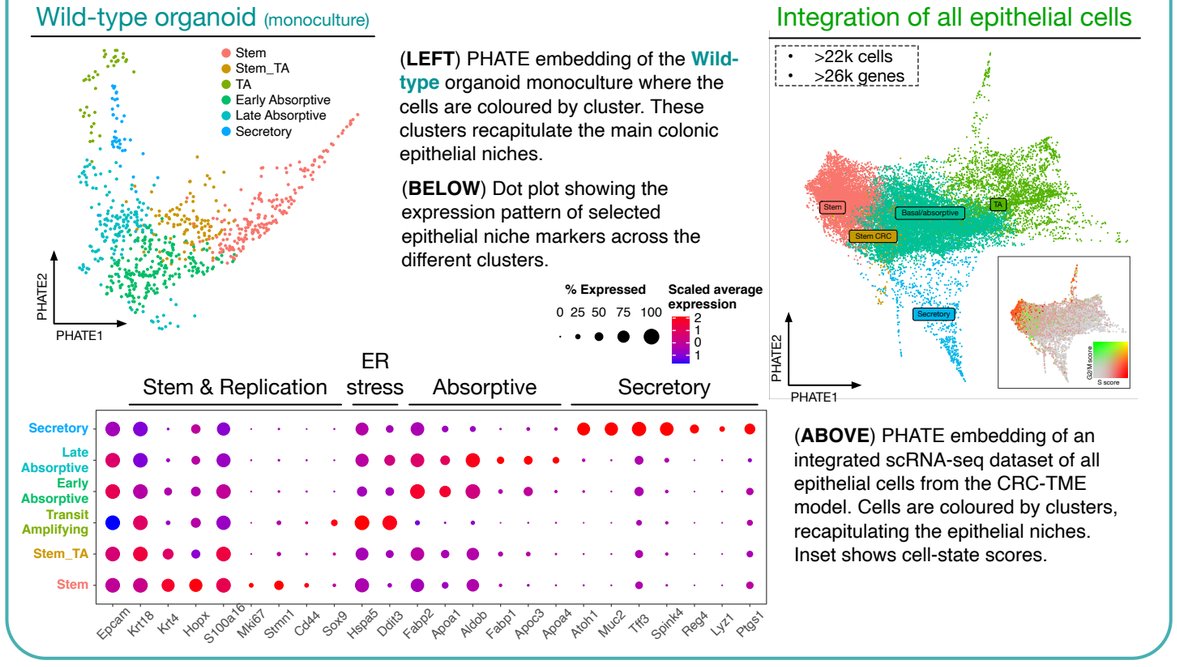


(**TOP**) CRC-TME model. Murine colonic organoids with a range of **genotypes** mimicking CRC progression were cultured in the presence or absence of **fibroblasts** and/or **macrophages** and analysed via droplet-based scRNA-seq.

(**MIDDLE**) UMAP distributions of an integrated scRNA-seq dataset containing all cells from whole CRC-TME model. Using the expression of canonical cell type markers we can resolve **epithelial** cells, **fibroblasts**, and **macrophages**.

(**BOTTOM**) EMD-derived PCA spaces of all cells in each of the three cell-types. In the **epithelial organoids** the genotype and microenvironment axes are recovered by PC1 and PC2 respectively.

② scRNA-seq Identifies Colonic Epithelial Differentiation

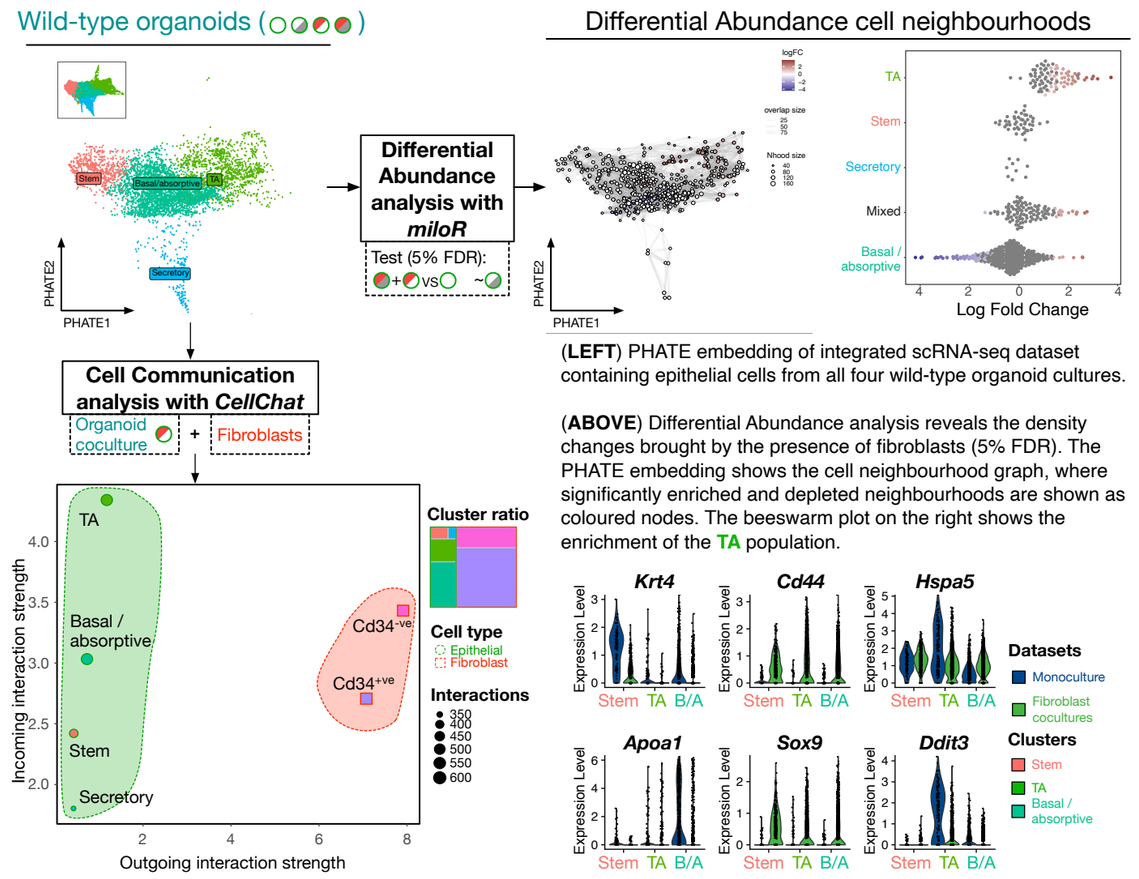


(**LEFT**) PHATE embedding of the **Wild-type** organoid monoculture where the cells are coloured by cluster. These clusters recapitulate the main colonic epithelial niches.

(**BELOW**) Dot plot showing the expression pattern of selected epithelial niche markers across the different clusters.

(**ABOVE**) PHATE embedding of an integrated scRNA-seq dataset of all epithelial cells from the CRC-TME model. Cells are coloured by clusters, recapitulating the epithelial niches. Inset shows cell-state scores.

③ Fibroblasts Promote the Epithelial TA Population, Perturbing their ER Stress Response



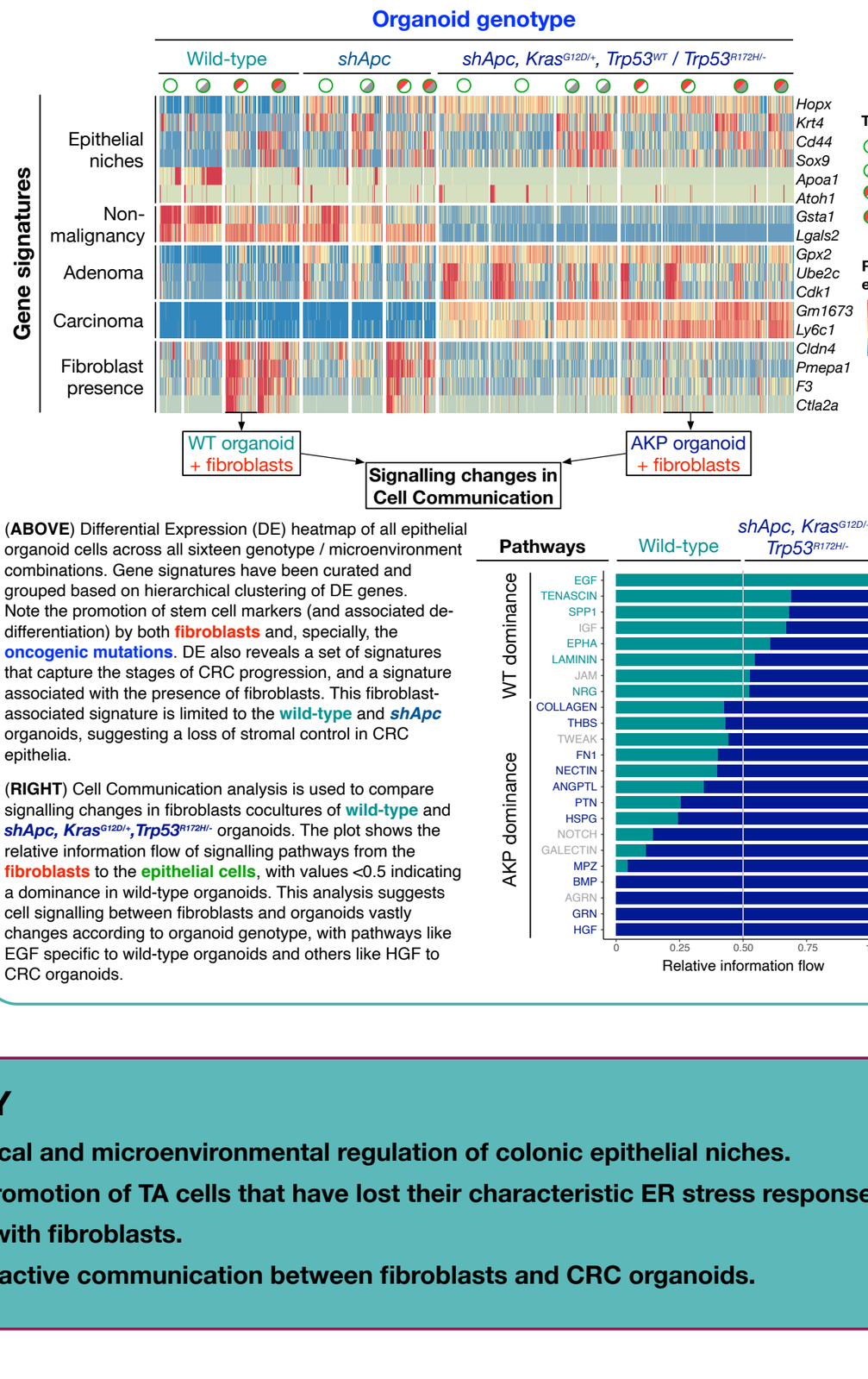
(**LEFT**) PHATE embedding of integrated scRNA-seq dataset containing epithelial cells from all four wild-type organoid cultures.

(**ABOVE**) Differential Abundance analysis reveals the density changes brought by the presence of fibroblasts (5% FDR). The PHATE embedding shows the cell neighbourhood graph, where significantly enriched and depleted neighbourhoods are shown as coloured nodes. The beeswarm plot on the right shows the enrichment of the **TA** population.

(**ABOVE**) Cell Communication analysis between **wild-type organoids** and **fibroblasts** in a coculture. The scatter plot shows the clusters according to the strength of their outgoing and incoming interactions. These results suggest a key signalling role of the fibroblasts, with the **TA** population monopolising the incoming signalling.

(**ABOVE**) Violin plots show the expression level of epithelial niche markers (**stem**: *Krt4, Cd44 and Sox9*, **absorptive**: *Apoa1*) and of ER stress response hallmarks (*Hspa5, Ddit3*) in wild-type organoids cultured with or without **fibroblasts**. Note the fibroblast-induced promotion of stem cell markers and break down of the ER stress response characteristic of the **TA** population.

④ Oncogenic Mutations Disrupt the Stromal Control of Epithelial Differentiation



(**ABOVE**) Differential Expression (DE) heatmap of all epithelial organoid cells across all sixteen genotype / microenvironment combinations. Gene signatures have been curated and grouped based on hierarchical clustering of DE genes. Note the promotion of stem cell markers (and associated de-differentiation) by both **fibroblasts** and, specially, the **oncogenic mutations**. DE also reveals a set of signatures that capture the stages of CRC progression, and a signature associated with the presence of fibroblasts. This fibroblast-associated signature is limited to the **wild-type** and ***shApc*** organoids, suggesting a loss of stromal control in CRC epithelia.

(**RIGHT**) Cell Communication analysis is used to compare signalling changes in fibroblasts cocultures of **wild-type** and ***shApc, Kras$^{G12D/+}$,Trp53$^{R172H/-}$*** organoids. The plot shows the relative information flow of signalling pathways from the **fibroblasts** to the **epithelial cells**, with values <0.5 indicating a dominance in wild-type organoids. This analysis suggests cell signalling between fibroblasts and organoids vastly changes according to organoid genotype, with pathways like EGF specific to wild-type organoids and others like HGF to CRC organoids.

SUMMARY

- **scRNA-seq of CRC-TME organoids enables the identification of differential genotypical and microenvironmental regulation of colonic epithelial niches.**
- **In wild-type organoids, Differential Abundance analysis reveals a fibroblast-driven promotion of TA cells that have lost their characteristic ER stress response.**
- **Oncogenic mutations de-differentiate CRC organoids and disrupt their interactions with fibroblasts.**
- **Despite the disruption of stromal control, Cell Communication analysis suggests an active communication between fibroblasts and CRC organoids.**

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References **1** Qin, X. *et al. Nat Methods* 1–8 (2020). **2** Dow, L. E. *et al. Cell* **161**, 1539–1552 (2015). **3** van Lidth de Jeude, J. F. *et al. Oncogene* **36**, 3397–3405 (2017). **4** Dann, E. *et al. bioRxiv* 2020.11.23.393769 (2020). **5** Jin, S. *et al. Nature Communications* **12**, 1088 (2021).