PhD project

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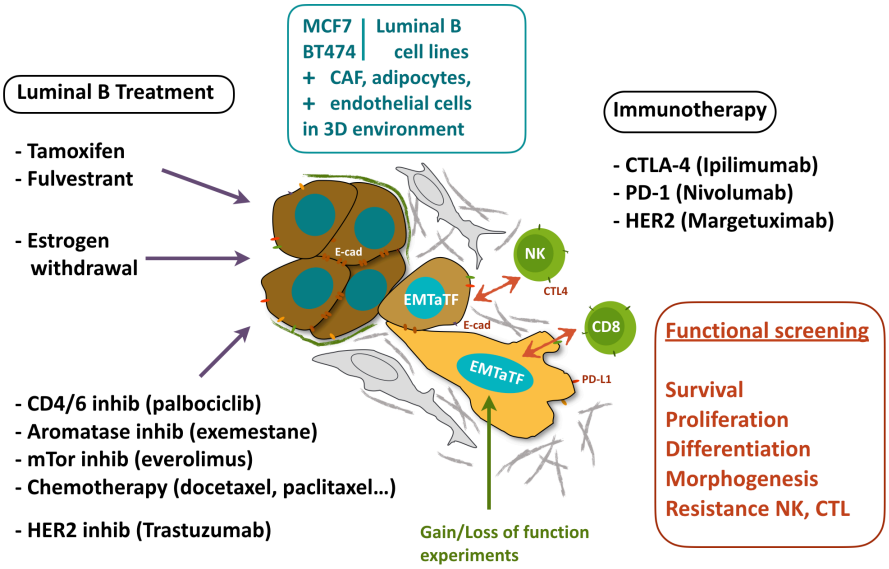
Title: Impact of EMT pathways on Luminal B breast cancer heterogeneity, progression, resistance and immune response in long-term composite microfluidic 3D organoid models.

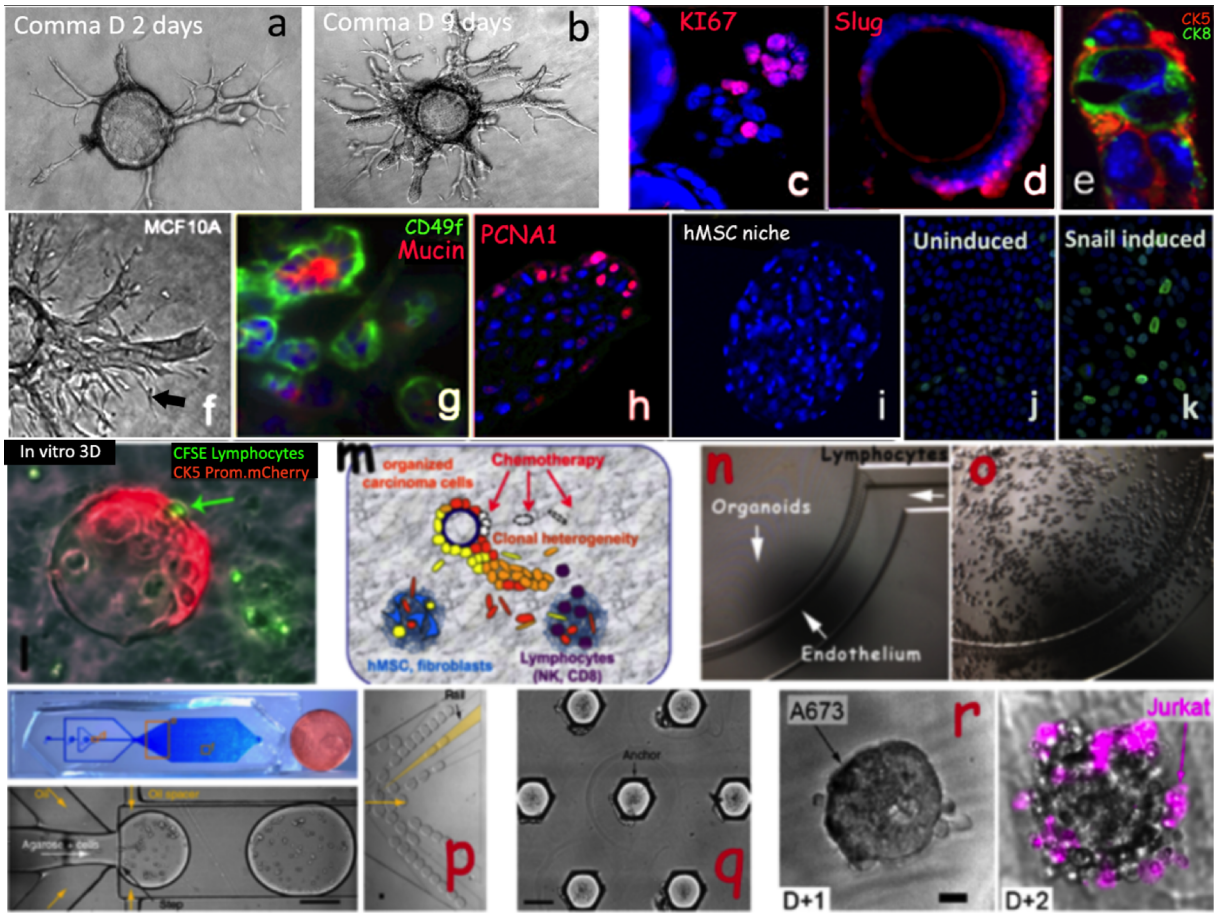
Introduction

Emergence and expansion of resistant tumor cell clones during cancer treatment is an essential issue for cancer therapy. It reflects a clonal evolution resulting from genomic instability, stemness pathways, immune escape and micro-environmental signaling. Designing appropriate long-term *in vitro* models to follow these events appears essential for functional analysis. Tumor heterogeneity emerges as a consequence of several pathways. Among them, epithelial-mesenchymal transition (EMT) pathways have recently emerged as particularly relevant. EMT-linked transcription factors belonging to the Snail, Twist and Zeb families (EMT-TF) have been found to induce resistance to chemotherapy and radiotherapy in various cancer types, *in vitro* and *in vivo*. EMT-TF are typically associated with inducing cell-cell dissociation, motility, invasiveness and stem cell-like properties. EMT has also been shown recently to modulate immune response. Our lab demonstrated that transfection of tumor cells with snail alters their susceptibility to immune induced lysis. Recent publications have shown how EMT process can interfere with immune response by modifying expression of membrane-bound molecules such as E-cadherin and PDL-1, involved in T cell recognition. In the tumor context, EMT genes are generally expressed de novo. They are activated through multiple pathways characterizing early tumor growth conditions (hypoxia, glycolytic metabolism, oncogenic activation, immune response, stromal reaction…). In the highly proliferative and high-risk breast carcinoma of Luminal B subtype, they are only expressed by a limited subpopulation of carcinoma cells, but significantly correlated with basal/progenitor markers and poor clinical outcome, as we published recently. Therefore, it appears urgent to better characterize EMT genes functional impact during luminal B breast carcinoma progression.

Thesis objectives

a- Characterize functionally EMT-TF roles during Luminal B breast carcinoma cell line progression in long term 3D culture models using reporters-GFP, gain or loss of function experiments and monitoring clonal heterogeneity, proliferation, dormancy and fate in response to chemotherapy.
b- Characterize carcinoma-immune cell interactions in these long term composite 3D culture models following chemotherapy. Design relevant microfluidic 3D models, including lymphocyte and NK cells.
c- Validate these results in clinical samples provided by Gustave Roussy.





In our model, mammary epithelial cells (CommaD: a-e or MCF10A: f-h) are grown on microsupport beads, allowing them to form tubule-like structures. In this configuration, proliferation is restricted to specific areas such as growing buds (c-h). EMTaTF such as Slug also express a specific localization pattern (d). Tubule-like processes show a differentiation pattern into basal and luminal layers, similar to *in vivo* organization in normal mammary tubules (e, g). Mesenchymal cells such as hMSC can be introduced within porous microsupports (i). Snail can be induced in engineered MCF10A cells (j-k). Reporter cell lines show live Slug promoter expression (l). Lymphocytes can be introduced directly in the culture medium (l) in our model (m). We now develop microfluidic approaches (m-o) with coculture and droplet array methodology (Tomasi et al. (2018) and Sart et al. (2018) (p), allowing array analysis (q) and exposure to lymphoid cells (r) in organoids growing in a 3D matrix.