Proposal of Master Research Project / Proyecto fin de Master for the academic year 2025-26

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Title of the MRP/TFM:
Characterization of fibroblastic populations in breast tumors: Impact on cancer progression

Summary of the Project:
Intratumoral cell heterogeneity is a hallmark of cancer evolution, influencing metastatic potential and patient outcomes. This diversity arises from the accumulation of genetic mutations and genome-independent mechanisms, such as cancer cell phenotypic plasticity, mainly through epithelial-to-mesenchymal transitions (EMT; Cell 2016). Recently, we have demonstrated that cancer cells can adopt one of two opposing EMT trajectories: an embryonic-like path that promotes metastasis or an adult-like path that fosters anti-tumour inflammation (Nature Cancer , 2024). We have also found that the activation of the EMT factor Prrx1 drives the invasive trajectory and therefore, the acquisition of metastatic properties. After this analysis of cancer cells, we now propose to study their interaction with the tumor microenvironment (TME), as it is well known that the interaction of tumour cells with the TME has a major influence on cancer progression towards metastasis. Multiple cell populations have been characterised within the tumour stroma, including cancer-associated fibroblasts (CAFs), immune cells including myeloid (such as macrophages, called TAMs in cancer) and lymphoid, and vascular cells. For this TFG we will focus on CAFs.

Recent studies indicate that in addition to cancer cell heterogeneity there is also a high variety and plasticity in CAFs, with different subclasses such as myofibroblastic and inflammatory types. These two subtypes are reminiscent of the two types of EMT trajectories (invasive and inflammatory) that we have found in cancer cells. The classification of multiple CAF types comes among others from the pioneering work of Fatima Mechta-Grigoriou's laboratory (Institut Curie, Paris), with whom we are collaborating. For this TFM, we propose to start characterizing CAFs subpopulations in tumours (control or deficient for Prrx1) in breast cancer models using a pluridisciplinary approach as described below.

Methods and technology involved in the MRP/TFM Project:

1. **Characterization of Cancer Cell-Stromal Interactions.** With the help of our computational scientist, we will perform *in silico* analysis of our single cell RNAseq data to infer putative interactions between cancer cells and CAFs (in control and Pmutant tumours). Integration of these data with spatial transcriptomics from the lab will confirm the likelihood of interactions and the putative molecular mechanisms (cell-cell adhesion complexes and signalling pathways mediated by identified ligand-receptor pairs), all analysed in relation to the described EMT trajectories.

2. **Experimental Validation in *In Vitro* Models.** We will initiate the validation *in vitro* of the most promising interactions identified after data integration that are altered in the presence/absence of Prrx1, including the generation of tumouroids in collaboration with Kristina Haase (EMBL Barcelona).

The most promising candidates will be later subjected to i*n vivo* functional analysis in mouse models to assess their potential as therapeutic targets, all of which will be the subject of a PhD project. Preclinical validation will be also performed during the Thesis in patient samples from the MD Anderson Cancer Foundation tumour bank.

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