Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Visualisation of the Onco-immuno-microbial crosstalk in the human breast tumour microenvironment

Synopsis: Breast cancer is the most commonly diagnosed cancer type and the leading cause of cancer-related death in women worldwide. The tumour microenvironment (TME) is now recognized as an active player in cancer progression and response to treatments. A multi-dimensional network of non-malignant cells populate the TME, including fibroblasts, adipocytes and immune cells, namely T cells and macrophages. Evidence suggests that immune cells play an ambiguous role in regulating both protumour and antitumor immune responses in BC. Strikingly, a pan-cancer study found that BC has a particularly rich and diverse microbiome compared to other cancer types [1]. This suggests that intra-tumoural bacteria exist, are active, and more provocatively that they may play key roles in cancer pathogenesis and response to therapy. Indeed, some of these tumour-associated bacteria can activate immune responses against tumours, while others produce enzymes able to hamper chemotherapy, helping cancer cells escape from the immune system [2]. The complex interaction between microbiota, the immune system, and malignant cells is still poorly described. We hypothesize that the microbiome interacting with tumour cells, as well as with the host's immune system, may be a key determinant *in situ*, constituting an independent component of the TME.

We propose a MSc project to detect bacteria by microscopy and microbial DNA amplification, aiming at visualizing and quantifying the interaction between tumour cells and their TME components, namely immune cells and tumour-associated microbiota. The student will perform ***immunohistochemistry***, as well as ***fluorescent immunostaining*** assessed by ***microscopy***, to detect bacteria, tumour cells, and immune cells on tissue sections. To characterise the tumour architecture, we will use anti-pan cytokeratin (tumour cells), anti-α–smooth muscle actin (blood vessels), anti-CD3 and anti-CD68 (T cells and macrophages). We will use anti-lipopolysaccharide (LPS) and anti-lipoteichoic acid (LTA) to detect Gram-negative and Gram-positive-bacteria, respectively. This will provide information on selective niches and selective interaction of bacteria with tumour or immune cells in the TME. We will use ***QuPath*** and ***CytoMap*** softwares to determine location, density and phenotype and the nearest neighbour (tumour or immune cells) as a function of the presence of bacteria *in situ*. In addition, we will quantify the 16S ribosomal RNA (16S rRNA) gene, by ***real time RT-PCR***, to assess the bacterial load in tissue sections.

This project will be integrated in the iMM-Laço Hub initiatives and benefit from the collaborative and interdisciplinary environment of iMM and host teams. By dissecting the crosstalk between these different layers, this project will point towards the possible interactions between immune components and tumour cells supporting further research to unravel novel molecular signatures of cancer progression and provide novel insights with implications for breast cancer personalized therapy. Finally, this project will trigger hypotheses about the tripartite onco-immuno-microbial crosstalk in breast cancer that will be functionally tested *in vitro* and *in vivo*.

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Bibliography:

[1] Nejman, D. *et al.* The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* **368,** 973–980 (2020).
[2] Geller, L. T. *et al.* Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* **1160,** 1156–1160 (2017).