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Melanoma secreted factors educate lymph node fibroblasts

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The lymph nodes are immune organs that lymphatic vessels directly link to the skin. Among skin cancers, melanoma is the most aggressive, spreading rapidly to lymph nodes. Lymph nodes are key organ for anti-tumor immune response, and yet a crossroad for metastasis in vital organs, where half of patients do not respond to actual treatments. To prevent the deadliest steps of melanoma, understanding how tumor cells make metastasis from the primary tumor in the lymph node is crucial. It is known that the primary tumor modifies the architecture of the targeted tissue by secreting soluble factors and extracellular vesicles, leading to the pre-metastatic niche. In the lymph node, Fibroblastic Reticular Cells (FRC) produce the network of extracellular matrix and secrete cytokines and chemokines that regulate T lymphocytes activation, their survival and migration. These functions are based on the precise compartmentalization of the lymph node between B zones and T zones. A key function of FRC is precisely to modulate intern architecture and elasticity of the lymph node thanks to their spontaneously contraction ability. FRC contraction is inhibited during infection and lymph node swelling by dendritic cells. This regulation of lymph node architecture seems to be fundamental for the modulation of the immune response. But in a tumoral context, the relation between FRC contraction ability and pre-metastatic education is not known. Using a syngeneic mice model of pre-metastatic education, we observed that the draining lymph node educated with melanoma secreted factors is bigger and has disorganized architecture. We observed also that it was more elastic using *Atomic Force Microscopy*.

My aim is to understand the effects of melanoma secreted factors on FRC contraction. Using an in vitro approach, I showed with *Matrigel and Collagen Gel Contraction Assay* that (1) secreted factors from melanoma cell lines inhibit the FRC contraction and (2) only factors secreted by invasive melanoma cell lines, and not proliferative cell lines, inhibit FRC contraction. I also showed (3) that this inhibition is associated to modifications in cell morphology and actin cytoskeleton; and (4) inhibition of transcription factors involved in mechanotransduction and contractility of cancer-associated fibroblasts (CAF), YAP and STAT3. Transcriptomic analysis (*DNA Chips*) of educated FRC gave us insights of specific signatures for contraction inhibition. Secretome analysis (*Mass Spectrometry*) of the invasive melanoma cell lines inhibiting FRC contraction is helping us to select candidates among tumoral factors that will be validated by a systemic siRNA-mediated depletion approach.

During this master class, I will discuss the biology of melanoma and the pre-metastatic education of the lymph node. I will present my main results and I focus on the adaptation of the Gel Contraction Assay to my primary cell model and the current strategy to identify the tumoral factors inhibiting FRC contraction.