ImmunoTools *special* Award 2013

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Role of tumour microenvironment on macrophage polarization: dissecting the associated mechanisms

Tumours are highly complex structures composed of cancer cells, extracellular matrix (ECM) components and distinct stromal cell types including fibroblasts, endothelial and immune cells. Growing evidence shows that the molecular crosstalk established between cancer cells and the surrounding environment has a crucial impact in tumour progression, by triggering and modulating invasion-associated activities such as cell-cell adhesion, cell-matrix interactions, growth, survival, angiogenesis, proteolysis and motility.

Macrophages are one of the best studied mediators of the tumour microenvironment and have been recently considered key elements, acting as modulators of breast cancer cell migration, invasion and metastasis. When bone marrow progenitor cells are mobilized into the periphery, they differentiate into monocytes, which in turn invade the tissues and differentiate into M1 or M2 macrophages, depending on the type of factors they are exposed to. Classical M1-macrophages produce pro-inflammatory cytokines, promoting tumour cytotoxicity and impairing tumour growth. In contrast, alternatively-activated M2-macrophages are stimulators of tissue repair and remodelling, angiogenesis and tumour growth and progression. In breast or ovarian cancer, it was shown that tumour-associated macrophages (TAMs) are usually accumulated in poorly vascularised regions of the tumour, sharing many characteristics of M2-macrophages, inducing low-inflammatory response, angiogenesis, tissue remodelling, tumour invasion and metastasis. However, in colorectal cancer, data regarding macrophage profile is rather contradictory. Most of the studies have been focusing on the effect of macrophages on tumour cells but little is known about the effect of tumour cells and ECM

components on macrophage polarization. Experimental observations evidence that tumour cells modulate their microenvironment but data on the role of the gastrointestinal microenvironment on macrophage polarization and behaviour is scarce. Bearing these results in mind, and knowing that macrophages are highly plastic cells, it is possible that tumours explore this characteristic in their benefit, at different stages of tumour progression.

The main goal of this PhD project is to elucidate how ECM and tumour cells modulate macrophage differentiation and polarization in colorectal cancer, contributing to disease progression. Therefore, our first step is to characterize the distribution of ECM components, such as laminin, fibronectin and collagens type I and IV, and to profile the distinct macrophage subpopulations at different stages of tumour progression, namely from non-tumour to primary or advanced tumours. Accordingly, we will perform immunohistochemistry analysis in paraffin-embedded tissues from colorectal cancer patients and available at the well-characterized Hospital de São João Tumour Bank, using specific antibodies directed to cell surface receptors (CD80, CD163, CD197, CD206) and cytokine/chemokines (VEGF, IL-6, IL-10, IL-12, IL-23, TNF-α). Additionally, and to complement this analysis, we will use fresh colorectal cancer surgical-resections from which we will isolate macrophages and perform flow cytometry for specific cell surface receptors, such as CD14, HLA-DR, CD80 and CD86.

At the end, molecular data will be crossed with patient clinicopathological information and correlated with disease progression, prognosis and patient overall survival. We hope that, by understanding the complexity of the tumour microenvironment, and the mechanisms of its regulation, we will be able to design more efficient tools for therapeutic intervention.

ImmunoTools has been instrumental in previous work developed by our team that was recently published in Oncogene (AP Cardoso, 2013), particularly by supplying antibodies for FACS analysis, ELISA kits or recombinant human cytokines for *in vitro* macrophage polarization. The results obtained were completely reliable and so we intend to perform the FACS analysis of macrophages isolated from fresh colorectal tissues using the ImmunoTools available antibodies.

ImmunoTools *special* AWARD for **Marta Pinto** includes 25 reagents

FITC - conjugated anti-human CD11b, CD45, CD80, CD86, HLA-DR, IL-6, Control-IgG1, Control-IgG2a and Control-IgG2b,

PE - conjugated anti-human CD3, CD8, CD11b, CD14, CD45, CD80, IL-6, Control-IgG1, Control-IgG2a and Control-IgG2b,

APC - conjugated anti-human CD8, CD14, IL-6, Control-IgG1, Control-IgG2a and Control-IgG2b

DETAILS