ImmunoTools *special* Award 2014

Ana Patrícia Pereira Cardoso, PhD student

Supervisor: Doctor Maria José Oliveira

NEWTherapies Group, INEB
Rua Campo Alegre, 823, 4150-180 Porto, Portugal

The role of macrophages on gastric and colorectal cancer cell invasion-related activities

Invasion is the hallmark of malignancy and therefore one of the most appealing targets for anti-cancer therapy. To design efficient therapeutic tools it is vital to dissect the molecular crosstalk established between cancer cells and other elements of the tumour ecosystem. Macrophages, in particular, are widely present within the tumour microenvironment and recent evidences suggest critical roles in breast cancer cell migration, invasion and metastasis. In gastric and colorectal cancer, however, their role is still poorly documented and the existing data is essentially contradictory. We recently reported that macrophages stimulate gastric and colorectal cancer invasion and motility through EGFR Y^{1086}, c-Src, Erk1/2 and Akt phosphorylation and smallGTPase activity. Moreover, we identified EGF as an important pro-invasive and pro-motile factor released by macrophages (AP Cardoso *et al*, Oncogene, 2013). We now propose to study the contribution of activated pro- and anti-inflammatory macrophages in these invasion-related activities and angiogenesis. Due to their functional plasticity, these cells are susceptible to molecular cues present at the tumor microenvironment, which polarize them towards an activated phenotype.

Pro-inflammatory or classically activated macrophages are induced by IFN-γ, microbial products such as LPS, and cytokines like tumor TNF-α.

They are generally characterized by inflammatory, microbicidal and tumoricidal activities with high antigen presenting capacity, high IL-12, IL-23, IL-6 and nitric oxide (NO) and reactive oxygen intermediates (ROI) and low IL-10 production. On the other hand, anti-inflammatory or alternatively activated macrophages are polarized in response to IL-4, IL-13, IL-10 or glucocorticoid hormones, and are described to have low IL-12 and IL-6 and high IL-10 production and a superior ability to scavenge, repair and remodel tissue and to promote angiogenesis.

Our final goal is to develop a therapeutic strategy able to modulate macrophages, *in loco*, to suppress their cooperation with cancer cells and to enhance their immune response against the tumour. Pro-inflammatory cytokines are potential candidates to incorporate in chitosan-based delivery systems, since they are potent activators of immunostimulatory monocytes and macrophages, up-regulate antigen presenting components in tumour cells increasing tumour immunogenicity, and affect cancer cell proliferation and survival. The incorporation of these cytokines in a delivery system able to target macrophages at the tumour site would enhance therapeutic efficacy, reduce systemic side effects and could even provide a vehicle to other chemotherapeutic agents.

ImmunoTools provides valuable reagents to the accomplishment of this project, which would be of great advantage in practically every task. Namely, human recombinant cytokines will be used in the polarization of human macrophages towards a pro- and an anti-inflammatory phenotype. Both macrophage populations will be characterized in terms of cytokine profile (by ELISA) and cell surface receptors expression (by flow cytometry). Differences in the angiogenic potential of distinct macrophage populations will be also evaluated by cytokine analysis in the conditioned medium. The evaluation of candidate factors responsible for different macrophage-derived stimulus of invasion, motility and proteolysis will be investigated by incorporation in assays involving cancer cells alone. Finally,

the ultimate goal of this work will involve the incorporation of human recombinant pro-inflammatory cytokines in therapeutics driven delivery-systems, requiring reliable and high quality products.