ImmunoTools *special* Award 2014

Ana Pinto, PhD student

Supervisor: Doctor Maria José Oliveira

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

The role of radiotherapy on macrophages and on macrophage-cancer cell communication

In colorectal cancer neoadjuvant radiotherapy improves locoregional control but has little or no effect on the development of distant metastases, which constitute the major cause of death. To design more efficient therapeutic tools, attention has to be paid to the other elements of the tumor ecosystem, also targets of those therapies, which may modulate and be modulated by treatment or by the response of the cancer cells themselves. After radiotherapy, enhanced macrophage influx is frequently observed at invasive fronts. Incoming monocytes may differentiate into M1 or M2 macrophages. M1 macrophages, also known as classically activated macrophages, are induced by INF-γ, LPS or TNF-α, produce pro-inflammatory cytokines, reactive oxygen species and nitric oxide (NO), reducing tumour growth and metastasis. In contrast, M2 macrophages, also designated by anti-inflammatory or alternatively activated macrophages, are polarized in response to IL-4, IL-13, IL-10 or glucocorticoid hormones. They are poor producers of nitric oxide, reactive oxygen intermediates and pro-inflammatory cytokines (e.g. IL-12, IL-1β, TNF-α, IL-6), but produce high levels of both scavenger receptor-A (CD204) and mannose receptor (CD206). Therefore, they are considered immunosuppressive and stimulators of tissue repair/remodelling, angiogenesis and tumour progression.

In macrophages, ionizing radiation is described to enhance the expression of several growth factors and cytokines such as IL1β, NO, IGF-1, PDGF, TNFα and VEGF. However, it remains to be known how radiotherapy influences macrophage polarization and behaviour, namely migration which is highly relevant for homing of macrophage to tumours, and the molecular mechanisms underlying macrophage-mediated cancer progression. Recurring to primary human monocyte-derived macrophages and human colorectal cancer resections, we aim to reveal, respectively, the main cytokine/chemokine/growth factor changes induced by radiation in macrophages and how this may affect macrophage-cancer cell communication in a tumour microenvironment context.

Our working hypothesis is that after radiotherapy multiple cytokines/chemokines are released by macrophages, affecting their polarization and migration, and influencing the communication with cancer cells and treatment outcome. To test this, we first isolated

monocytes from healthy blood donors, induced monocyte-macrophage differentiation with M-CSF and subjected these macrophages to conventional doses of ionizing radiation, as used for cancer patient's treatment. Upon different doses of irradiation, macrophage population will be analyzed by flow cytometry for the expression of lineage markers (CD45, CD14, CD80, CD86), M1/M2 phenotype markers (HLA-DR, CD163, CD206) and molecules involved in migration/adhesion (CD11a, CD11b, CD11c, CD9, CD18, CD29, CD33, CD43, CD44, CD50, CD53, CD54). We will also evaluate cytokines/chemokines/growth factors levels (IL-4, IL-6, IL-8, IL-10, IL-12, IL-23, TNF-α, VEGF, PDGF) by ELISA. As efficient controls of M1 and M2 macrophage polarization, we have stimulated non-irradiated human macrophages with LPS and IL-10 (see article from our team published in Oncogene, 2013).

Characterization of macrophage populations (number, localization, phenotype) and their relation with cancer cells (via adhesion receptors) upon irradiation will be also evaluated/validated by immunohistochemistry. We will have access to tumour specimens from colorectal cancer patients, which received or not radiotherapy before/after surgery and have not been submitted to other therapeutic regimens, available at the Hospital S. João Tumour Bank (Porto). This tumour bank, of national and international reference, is matched with a database of extensive patient clinic- pathological information, allowing the correlation of the molecular findings with the therapy protocol to which the patient has been submitted. The biological relevance of our *in vitro* findings will be then directly assessed by comparison with the molecular profile found in human specimens.

The ImmunoTools collection of reagents, recombinant cytokines and ELISA-sets selected will be crucial for an extensive evaluation of changes induced by radiotherapy on macrophage surface receptors and cytokines involved in macrophage polarization and migration. With the support of ImmunoTools our work will bring new insights on how radiotherapy modulates macrophage behaviour and how this affects macrophage/cancer cell communication, opening new perspectives for therapeutic intervention.

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

FITC - conjugated anti-human CD11b, CD18, CD29, CD33, CD43, CD45, CD53, CD54, CD86, HLA-DR,

PE - conjugated anti-human CD9, CD11a, CD14, CD44, CD45, CD50, CD80,

APC - conjugated anti-human CD11c, CD14,

human IL-4 ELISA-set for 96 wells, human IL-8 ELISA-set for 96 wells, human IL-12p40 ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

recombinant human cytokines: rh IL-10, rh M-CSF DETAILS more AWARDS