ImmunoTools *special* Award 2014

Silvia Peppicelli, PhD student

Supervisor: Prof. Dr. Lido Calorini

Department of Experimental and Clinical Biomedical Sciences,
University of Florence, Viale G. Morgagni, 50,
50134 Florence, Italy

Acidic microenvironment and mesenchymal stem cells in melanoma progression

It is well known that tumors are composed of malignant tumor cells and nonmalignant benign cells. The “benign” tumor compartment includes blood vessels, infiltrating inflammatory cells, and stromal cells such as fibroblasts and mesenchymal stem cells (MSC). MSC are multipotent precursors endowed with the ability to differentiate into a variety of mesenchymal cells, including osteoblasts, chondrocytes, adipocytes, muscle cells, pericytes, reticular fibroblasts, and even neural cells. Large number of MSC is recruited into the stroma of developing tumors and recent evidences suggest that these cells play a role in facilitating cancer progression, influencing the behavior and aggressiveness of tumor cells. Indeed, the relationship between MSC and tumor cells appears dual: primary and metastatic tumors attract MSC in their microenvironment where they affect tumor cell survival, angiogenesis, motility and invasiveness; vice versa in the bone marrow MSC attracts tumor cells and contribute to a microenvironment that promotes osteolysis, tumor growth, survival, and drug resistance. Moreover, MSC are an important source of inflammatory cytokines that affect tumor and immune cells, thus have an immunomodulatory function.

The chaotic and incomplete vasculature of tumor is frequently responsible for a transient or persistent oxygen deficiency and tumor cells respond converting to an anaerobic respiration, driving the environment of most solid tumor to be acidic. Our past studies revealed that an acidic pH, that often characterizes the tumor, induces a more aggressive phenotype of melanoma cells.

We are now studying the role of acidity in the interaction between tumor cells and mesenchymal stem cells. We expose MSC for 24 hours to a pH 6.7 - 6.8 acidified medium or to a pH 7.4 standard medium and media conditioned by MSC grown in standard or acidic medium are used to grow human melanoma cells. We found that melanoma cells grown in medium conditioned by acidic MSC have a stimulated

invasiveness and motility compared to control cells. Moreover media conditioned by acidic MSC attract melanoma cells more than media conditioned by non acidic MSC. Moreover, we found that acidic MSC express high levels of cytokines including for example TGFβ, TNFα, IL-6, SDF-1, VEGF.

The ImmunoTools recombinant human cytokines (**IL-1β, IL-6, IL-8, IL-10, TGF-β, TNF-α, VEGF**) will be used to treat melanoma cells to study the involved factor released by acidic MSC which is able to stimulate the invasiveness and motility of melanoma cells. ImmunoTools human **SDF-1** and **CCL7**, chemotactic factors involved in tumor progression, will be used to test the chemotaxis of melanoma cells. Cell culture supernatant will be collected from mesenchymal stem cells and growth factor levels will be measured with ELISA (**human ELISA-set for human IL-6, human IL-8, human TNF-α,** and others**,** such as human TGF-β).

It is reported that CD63 antigen may play a role as a tumor suppressor gene, as its expression in human melanoma cells reduces tumor spread and metastasis. We will use the ImmunoTools anti-human **CD63** for flow cytometry to analyze the expression of CD63 in melanoma cells grown in medium conditioned by acidic MSC.

Finally we will investigate the capacity of media conditioned by acidic or non-acidic MSC to induce in melanoma cells apoptosis (using **Annexin-V)** or stemness (**CD20, CD29, CD38, CD44**).

ImmunoTools reagents will be essential for many analyses in this project. The possibilities provided by ImmunoTools reagents would be great to study the role of tumor acidic microenvironment in the crosstalk between melanoma cells and stromal cells.

ImmunoTools *special* AWARD for **Silvia Peppicelli** includes 25 reagents

FITC - conjugated anti-human CD20, CD29, CD38, CD45, CD47, CD63, CD105, IL-6, Annexin V,

PE - conjugated anti-human CD20, CD44, Annexin V,

human IL-6 ELISA-set for 96 wells, human IL-8 ELISA-set for 96 wells, human TNFa ELISA-set for 96 wells (each 3 reagents),

recombinant human cytokines: rh IL-1beta /IL-1F2, rh IL-6, rh IL-8, rh IL-10, rh MCP3 / CCL7, rh SDF-1α / CXCL12a, rh SDF-1β /CXCL12b, rh TGF-beta3, rh TNFα, rh VEGF-A/VEGF-165 DETAILS more AWARDS