ImmunoTools *special* Award 2014

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TUMOR CELL-STROMA INTERACTION: HISTAMINE ACTION IN RADIATION-INDUCED BIOLOGICAL RESPONSES CONCERNING BREAST CANCER CELLS INVASIVENESS

Radiotherapy, an important therapeutic tool in oncology, may raise numerous side effects including an increase in the proliferative and invasive capacity of surviving tumor cells, with the consequent increase in the risk of metastasis.

Epithelial-mesenchymal transition (EMT) is a physiological process that if improperly activated in epithelial cancers may contribute to invasion and metastasis. Tumor cell-stroma interaction also plays an important role in such processes. Ionizing radiation (IR) in turn acts on both tumor cells and the microenvironment, by modulating signaling pathways involved in EMT.

We have previously investigated various aspects concerning histamine action in tumor biology, demonstrating its ability to inhibit cell proliferation through different mechanisms of death and the initiation of differentiation. We have also studied the ability of histamine to regulate EMT in tumor cells, demonstrating that it can block not only some events involved in the EMT process but also the increase in migration and invasiveness produced by IR. Moreover, we showed that soluble factors secreted by fibroblasts can induce EMT in tumor cells and that this effect can be blocked by pretreatment of the fibroblasts with histamine.

The objective of this project is to study the action of histamine in radiation-induced biological responses related to invasiveness of breast cancer cells and some aspects of tumor cell-microenvironment interaction. We will focus on three points:

1) *To assess the ability of histamine to blocking RI induced EMT process in breast cancer cell lines with different epithelial phenotype and invasive capacity.*
We will investigate EMT-functional markers such as migration, gelatinolytic activity, cell migration and invasion. We will also study the expression of recognized epithelial

and mesenchymal markers such as E-Cadherin, N-cadherin, Vimentin and beta-catenin related to EMT induction. Since recent studies have suggested a strong correlation between cancer stem cells and EMT, we will also evaluate the possible expression of stem cell markers and mesenchymal stem cell markers: CD24, CD44, CD50, CD105, CD106 by different methodologies (flow cytometry, indirect immunofluorescence, immunoblot) and the generation of mammospheres.

2) *To study the effect of histamine and IR on bidirectional communication between tumor cells and fibroblasts by soluble factors*.
We will investigate cell communication through soluble factors secreted in the conditioned media: IL-6, IL-8, and TGF- beta. We will evaluate the morphological and functional changes induced by histamine and ionizing radiation culturing breast cancer cells with the conditioned media from fibroblasts and *vice versa*. We will also investigate changes in cell proliferation and death.

3) *To determine the ability of histamine to modifying in vivo metastatic competence of irradiated tumors xenotransplanted in nude mice.*
The *in vivo* response to IR is very complex process and must be analyzed in context considering not only isolated cells but tissues and the whole body.
Nude mice will be sc xenotransplanted with tumors generated by the inoculation of a human breast cancer cell line. We will evaluate tumor growth, histological features, angiogenesis and lung metastasis. We will also determine the expression of epithelial, mesenchymal and stem cell markers in solid tumors and metastatic focii.

A better understanding of the IR effects in relation to the induction of EMT in tumor cells and metastasis development in a dynamic multicellular milieu might contribute to the design of new therapeutic strategies considering not only intrinsic tumor radio-resistance but invasiveness. The ImmunoTools reagents will be very useful for our research work:

ImmunoTools *special* AWARD for **Gabriela Martin** includes 23 reagents

FITC - conjugated anti-human CD24, CD29, CD44, CD105, mouse isotype control IgG1, mouse isotype control IgG2a, mouse isotype control IgG2b, Annexin V

PE - conjugated anti-human CD24, CD44, CD50, CD61, mouse isotype control IgG1, mouse isotype control IgG2b,

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

recombinant human cytokines: IL-6, IL-8, TGFbeta3 DETAILS more AWARDS