ImmunoTools *special* Award 2014

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Immunotherapy against Breast Cancer Metastasis based on Targeting Signal Transducer and Activator of Transcription 3 (Stat3)

Metastasis is responsible for as much as 90% of cancer-related deaths, yet it remains the hardest challenge to obtain a successful treatment. Conventional therapeutics, which efficiently target actively proliferating cells within the primary tumor, have little effect on quiescent or slowly proliferating cancer cells that comprise micrometastatic colonies. Early host defense mechanisms play a critical role in the outcome of metastatic disease. In this critical therapeutic condition, immunotherapy can help to prevent metastasis establishment and can contribute to micrometastasis clearance. However, the use of appropriate adjuvants able to trigger an antitumor immune response and time-course for immunotherapy (IT) administration remains poorly characterized. Breast cancer is the most frequent type of this disease in women and the second most deadly one. Recurrence at clinical stages I-II is 30%, but at stage III it soars to 70%. Given the fact that metastatic disease does not respond similarly to chemotherapies against primary tumor, we designed a project aimed at triggering an antitumor immune response to curb metastatic disease. IT is a safe and simple therapy with low/none secondary effects aimed to specifically search and destroy tumor cells avoiding the onset of metastasis. However, because of limited knowledge of the optimal adjuvant combination, tumor antigen heterogeneity and the schedule for rational application, active IT has been only very mildly successful.

Our laboratory has recently described an effective IT that prevents breast cancer metastasis, based on tumor cells with blocked signal transducer and activator of transcription 3 (Stat3). Activated Stat3 has long been recognized to act as an oncogene. It also mediates immune evasion by inhibiting anti-tumor innate and adaptive immune responses. We proposed to apply Stat3-inhibited breast cancer cells as a source of immunogens to induce an antitumor immune response. Thus, we designed a cancer vaccine in a preclinical setting based on the serial inoculation of Stat3-inhibited murine breast cancer cells as a source of immunogen to induce an anti-tumor immune response. Prophylactic or therapeutic administration of Stat3-inhibited breast cancer cells, decreased primary tumor growth compared with administration of control cells using 4T1 murine breast cancer model. We observed that 50% of the challenged mice were tumor-free, and the incidence of metastasis decreased by 90%. These results encouraged us to study the tumor phenotype responsible for this immunogenic effect.

We found that Stat3 inhibition increases the secretion of pro-inflammatory cytokines. We postulate that dissecting the specific cytokine/ chemokine profile obtained by Stat3 blockage will allow us to polarize an immune response triggered by an IT, to induce tumor clearance.

To achieve our aim we have already performed particle assays and cytokine antibody arrays to characterize the cytokine/ chemokine composition of the supernatant of Stat3-inhibited human and murine breast cancer cells. As a first step, we plan to elaborate both mix of human and of murine cytokines and chemokines based on the above mention results and culture with human and murine $CD8^+$, $CD4^+$ T lymphocytes and NK cells. Murine cells will be obtained from mice immunized with irradiated wild-type 4T1 cells. Then, we will evaluate on human and murine $CD8^+$, $CD4^+$ T lymphocytes and NK cells : i) the activation markers expression, ii) the migration towards tumor cells iii) the cytotoxic effect of $CD8^+$ T cells and NK cells against tumor cells. As positive control we will use supernatant of 4T1 cells or human breast cancer cells transfected with siRNA to Stat3. These important *in vitro* results will lead us to the formulation of an adjuvant to be administrated together with irradiated 4T1 cells in BALB/c mice and to evaluate its effectiveness in metastasis prevention.

The ImmunoTools special AWARD would be of tremendous value for our project because will allow formulating the mix of cytokines and chemokines necessary for our *in vitro* research. Also we will study activation markers such as CD69 by flow cytometry, we will investigate migration, using SDF1α as a positive control and will evaluate the apoptosis of tumor cells using Annexin V staining. The ELISA kits for TNFα and IL-8 will be essential for monitoring the Stat3 knockdown effect on human breast cancer cells. Obtaining the ImmunoTools special AWARD will contribute to the understanding of the complexity of the cytokines/chemokine network useful for triggering an effective antitumor immune response.

ImmunoTools *special* AWARD for **Roxana Schillaci** includes 23 reagents

PE - conjugated anti-human CD69, Annexin V,

APC - conjugated Annexin V,

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

recombinant human cytokines: rh GM-CSF, rh IL-6, rh IL-8, rh IL-12, rh IL-15, rh IL-17, IFN-gamma, rh SDF1alpha

PE - conjugated anti-mouse CD8, Gr1,

APC - conjugated anti-mouse CD11b, CD19,

recombinant mouse cytokines rm IL-2, rm IL-15 DETAILS more AWARDS