ImmunoTools *special* Award 2018

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Exploring the role of Activin-A in the regulation of anti-tumor immunity in lung cancer

Background: Lung cancer, which represents the most common type of cancer in the world, is characterized by high mortality rates and is responsible for nearly 20% of cancer-related deaths. While the immune system plays a crucial role in eradicating tumors, in the setting of lung malignancy, transformed cells often exhibit low immunogenicity and manage to escape immune surveillance. Considering the crucial role T effector cells play in the induction of protective anti-tumor immunity, the identification of factors that can enhance T cell-driven responses *in vivo* represents a key therapeutic approach for lung cancer eradication.
Activin-A is a pleiotropic cytokine that exerts essential roles in fundamental biological processes. Our group has extensively studied the role of Activin-A in the regulation of T cell-mediated immune responses generated in the lung airways, during inflammatory conditions. Pertinent to cancer, a growing body of evidence suggests that Activin-A is highly involved in cancer pathophysiology; still, its precise role in shaping anti-tumor immunity remains unexplored.

Preliminary findings/Hypothesis: Our preliminary studies reveal that, *in vivo* administration of Activin-A in mouse models of lung metastasis, results in regression of lung tumor foci number and growth, and enhances the overall survival of tumor-bearing hosts. The inhibitory effects of Activin-A on lung tumor progression are associated with enhanced anti-tumor immunity, as evidenced by an increase in effector T cell populations and a decrease in the frequencies of immune-suppressive cell types, such as Tregs and myeloid derived suppressor cells, in the lungs of tumor-bearing mice. Based on our preliminary findings, we have formulated the hypothesis that Activin-A constrains the development of lung cancer through the enhancement of T cell-mediated anti-tumor responses.

Project description: We have designed two objectives to test the aforementioned hypothesis. Our first objective is to decipher the *in vivo* effects of Activin-A on T cell-mediated immune responses in lung cancer. To address this, we will disrupt

Activin-A's signalling specifically on $CD4^+$ T cells using an inducible model of $CD4^+$ T cell-specific knockout of the ALK4 receptor (CD4ERt2/Cre/Acvr1bfl/fl). This set of experiments will determine whether blocking Activin-A's signaling on $CD4^+$ T cells *in vivo* will inhibit the development of potent anti-tumor immunity and exacerbate lung tumor development/progression.
Our second objective is to explore whether Activin-A enhances anti-tumor human T cell immune responses in lung cancer patients. To address this, we will obtain lung cancer specimens from patients and isolate tumor infiltrating $CD4^+$ and $CD8^+$ T cells. In this objective, we will elucidate whether *ex vivo* administration of Activin-A skews lung tumor infiltrating $CD4^+$ and $CD8^+$ T cells towards an activated phenotype and augments their anti-tumor effector functions.

Methodology: For our first objective, the phenotype (CD3, CD4, CD8a, CD19, CD11b, CD45, CD49b) and cell activation status (CD25, CD44, CD62L, CD80) of lung tumor-infiltrating leukocytes will be analyzed by flow cytometry. For our second objective, single-cell suspensions from lung cancer tissues will be prepared and $CD3^+CD4^+$ and $CD8^+$ T cells will be selected by FACS sorting. Tumor infiltrating T cells will be treated *ex vivo* with recombinant Activin-A or negative controls and stimulated with mitomycin-treated, CD3-depleted antigen presenting cells. Cell activation status (CD45, CD45RA, CD45RO, CD127, CD25, CD44, CD62L, CD69 etc) on Activin-A- or PBS-treated $CD4^+$ T cells will be measured by flow cytometry.

Significance: There is an imperative need to identify novel and more effective cancer immunotherapies that can boost specific aspects of the T cell response, without the adverse risk of inducing overt immune cell activation and autoimmunity. We anticipate that results from the proposed studies may uncover Activin-A as a novel critical controller that can enhance effector responses of tumor-reactive T cells, facilitate the regression of lung tumors and increase the overall survival. Receiving the ImmunoTools *special* Award will accommodate the accomplishment of our research goals, as it will provide us with the necessary research tools, including flow cytometry antibodies and recombinant proteins.

ImmunoTools *special* AWARD for **Ioannis Morianos** includes 25 reagents

FITC - conjugated anti-human CD3, CD11b, CD25, CD44, CD45RA, CD69, CD127
PE - conjugated anti-human CD4, CD45RO, CD62L, IFN-gamma, TNFa
PerCP - conjugated anti-human CD8, CD45

FITC - conjugated anti-mouse CD3e, CD4, CD11b, CD25, CD44, CD80
PE- conjugated anti-mouse CD8a, CD19, CD45, CD62L

recombinant human cytokine: rh Activin A active

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