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Immunochemotheraphy with 5-Fluorouracil and Interferon-alpha enhances the immunogenicity of murine pancreatic tumor via upregulation of a NKG2D ligands and MHC Class-I

Pancreatic cancer has the poorest prognosis of all gastrointestinal cancers driving the need for new therapeutic approaches. Adjuvant 5-Fluorouacil (5-FU) chemotherapy proved effective in increasing the survival of patients with resected tumors. Furthermore, the addition of Interferon-α (IFN-α) immunotherapy to 5-FU has shown encouraging clinical results. In previous *in vivo* studies we demonstrated that the immune modulation is indispensable in the anti-cancer effects mediated by this immunochemotherapy. Here we investigate the relevance of different immune cell populations namely, CD8+ T cells, CD4+ T cells, NK cells, Regulatory T cells (T regs) and dendritic cells, in the anti-cancer immune response mediated by IFN-α in combination with 5-FU using an orthotopic mouse model of pancreatic carcinoma.
Luciferase-transfected Panc02 cells were implanted in the pancreas of C57BL/6 mice. Five days later, mice were treated with 5-FU alone, 5-FU + IFN-α or with a vehicle control. In parallel, CD4+ T cells, CD8+ T cells, or NK cells were depleted by neutralizing antibodies. Depletion of CD11c+ dendritic cells (CD11c+ DCs) and T regs was performed using transgenic mice based on the diphtheria toxin receptor-mediated conditional and targeted cell ablation. Tumor volume was measured and splenic NK cells were isolated then used as effectors against Panc02 cells in a standard chromium release assay. In parallel, we performed flow cytometry analysis to evaluate the effects of different treatments on the expression of MHC class-I and NKG2D ligands (Mult-1 and Rae-1) in Panc02 cells *in vitro* and in the pancreatic tumors *in vivo*.

Additionally, we previously showed that IFN-α has anti-angiogenic properties in our mouse model. Furthermore, it has been proven that specific doses of some anti-angiogenic agents may normalize the tumor vasculature leading to improve availability of chemotherapeutic agents in the tumor tissue. Taken together, we wanted first to compare the anti-angiogenic properties of IFN-α with an anti-VEGF agent, second, to evaluate weather adding an anti-VEGF agent may enhance the outcome of the combination therapy.

While treatment with 5-FU+IFN-α significantly decreased tumor volume in comparison with control or 5-FU treatments, depleting CD8+ T cells, NK cells or dendritic cells (DCs) significantly reduced the anti-cancer effects mediated by the combination therapy. Interestingly, depleting T regs as well as adding an anti-VEGF therapy enhanced the anti-cancer effects of the combination therapy while depletion of CD4+ T cells did not significantly influence the immunochemotherapy-outcome. Tumors of 5-FU + IFN-α treated mice harbored higher numbers of infiltrating NK cells in comparison with control mice. Furthermore, NK cells isolated from spleens of 5-FU + IFN-α treated mice showed enhanced cytotoxicity against Panc02 cells. Moreover, 5-FU + IFN-α treatment increased the expression of MHC class-I and NKG2D-ligands: Mult-1 and Rae-1 on Panc02 cells both *in vitro* and *in vivo* that could be potential key for enhancing the immunogenicity of tumors.

It is essential to consider the clinical applications of this study. Based on our data, it is worthwhile to evaluate the expression of NKG2D ligands in human pancreatic tumors. A correlation between higher expression of NKG2D ligands and better treatment outcome (either with 5-FU or with the combination therapy) may provide a good predictive marker. Investigating predictive and prognostic markers may largely help in personalizing the therapy and providing the best efficacy with less toxicity. Taking all that into consideration will also help in designing the optimal clinical trial to address the efficacy of the 5-FU+INF-α immunochemotherapy for pancreatic carcinoma and to define the sub-population of patients who will benefit most from it.