Efficacy and limitations of cytokine complex therapy on pancreatic ductal adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is the most common form of pancreatic cancer and the 3rd leading cause of cancer mortality1. The disease is often relentless and metastatic at diagnosis. PDA is resistant to chemotherapy and the immunosuppressive microenvironment suppresses antitumor lymphocyte infiltration and activity2. Our lab is developing immunotherapies for PDA and we have identified mechanisms of resistance3-4 indicating that combinatorial therapies may be more effective.

Cytokine complexes are cytokines in combination with their receptor or specific antibodies. They can enhance in vivo cytokine potency via increased half-life and signaling5. Treatment with IL-2/IL-2R (IL-2C) or IL-15/IL-15R (IL-15C) complexes can enhance both T cell and NK cell antitumor activity in a melanoma model6. We examined the impact of IL-2 or IL-15 complexes in a preclinical PDA model. We show that IL-15/IL-15R complex has the greatest antitumor activity yet is not curative.

We identify that both intratumoral NK cells and tumor-specific T cells upregulate activating/inhibitory receptors PD-1 and NKG2A and a large fraction of these cells also secrete IL-10. NKG2A is an inhibitory receptor upregulated with chronic antigen7. NKG2A dimerizes with CD94 and binds to HLA-E/QRa-1b, a non-classical MHC-I, and inhibits NK and CD8 effector function through ITIM signaling8. As a strategy to enhance cytokine complex therapy, I designed chimeric NKG2A receptors which can bind to the extracellular domain of NKG2A with stimulating intracellular signaling domains. These will be validated for cell therapy in combination with IL-15/IL-15R complex in PDA mouse models.

Specific Aims

Specific Aim 1: Determine the effect of IL-2 and IL-15 Complex treatment on survival and tumor size in a murine model of pancreatic cancer.

Hypothesis: Treatment with cytokine complexes will expand effector lymphocytes and decrease tumor radiation and prolong survival in treated mice.

Specific Aim 2: Develop and test chimeric NKG2A receptors to enhance cell therapies for pancreatic cancer.

Hypothesis: Co-opting NKG2A inhibitory signaling in engineered lymphocytes will render cell products with superior antitumor activity and synergize with cytokine complexes in PDA.

Figure 1. Schematic for testing cytokine complexes in vivo

Figure 2. Impact of cytokine complexes on tumor growth and survival in a PDA animal model.

Figure 3. Tumor-specific CD8+ T cells and NK cells express PD-1, NKG2A and IL-10 in PDA.

Specific Aim 2: Generate chimeric NKG2A receptor constructs for as a potential strategy for enhancing lymphocyte function versus PDA.

Conclusions:

• IL-15 has the most pronounced antitumor activity for PDA.
• Tumor recurrence despite therapy supports the need for a combinatorial treatment strategy.
• A higher proportion of intratumoral lymphocytes expressed PD-1, LAG3 and PD-10 compared to spleen suggesting that the tumor microenvironment is inducing a suppressive phenotype in infiltrating effector lymphocytes as part of its immune evasion.

Future:

• Examine the phenotype and function of host immune cells immediately following cytokine complex treatment
• Test safety and efficacy of cytokine complex therapy in combination with engineered T Cell therapy and other immunotherapies
• Test the impact of prolonged cytokine complex treatment

Specific Aim 2: Generate chimeric NKG2A receptor constructs for as a potential strategy for enhancing lymphocyte function versus PDA:

• Four murine constructs were generated and three have been cloned into a PDA genome

Figure 4. Murine chimeric NKG2A receptor constructs. A) NKG2A inhibitory receptor (Red), NKG2C activating receptor (Green), and Chimeric NKG2A receptor (chNKG2A) dimerized with CD94 (Blue) interacting with HLA-E (Purple). Constructs were ordered as gBlocks (IDT) and cloned into the entry vector pENTRY (Invitrogen). B) Successful cloning (arrows) was confirmed by restriction digest. Constructs 2-4 were then cloned into the MIGR1 retrovector. Final construct cloning will be confirmed by sequencing.

Figure 4. NKG2A chimeric receptors for engineering lymphocytes

References