Cryoadablation in combination with the checkpoint inhibitor anti-CTLA4 increased T cell activation in a murine breast cancer model

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Background/Hypothesis

A promising area of breast cancer cryoablation research is its combinational use with checkpoint inhibitors to enhance the antitumor response. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is an inhibitory receptor that acts as a negative regulator to T cells. [1]

Iplimumab, a monoclonal antibody against CTLA-4, has already been approved for melanoma and is currently being investigated in breast cancer. A pilot study of preoperative single-dose iplimumab and cryoadablation in women with early-stage breast cancer was safe and showed favorable intra-tumoral and systemic immunologic responses. [2] Using a murine cryoadablation model for high-risk metastatic breast cancer [3], we investigated cryoadablation in conjunction with a CTLA-4 inhibitor to enhance the anti-tumor T cell immune response.

Methods

• Balb/C mice were bilaterally transplanted with luciferase expressing metastatic breast cancer cells (4T1-12b-luc) into mammary fat pad.
• At 2 weeks, the mice were separated into two treatment groups. Group 1 (n=5) received cryoadablation alone. Group 2 (n=5) received 100 μg anti-CTLA4 intraperitoneal 24hrs pre-cryoablation as a T cell prime and post-cryoablation as an immune boost. For both groups, cryoadablation was only performed on the left tumor, and the right tumor served as proxy for metastatic tumor for absopal immune readout.
• One week post-cryoablation, both mouse groups were sacrificed and necropsied for tissue analysis.
• Isolated tissues were homogenized, and lysates were analyzed for systemic anti-CTLA4 antibody distribution by ELISA.
• Peripheral blood (PB) and spleen mononuclear cells were isolated and analyzed for T cell population CD4 and CD8 subsets and the activation marker ICOS by flow cytometry.
• Cryoadablated (left tumor) and absopal (right tumor) were analyzed for TILs of cryoablated tumor expressing T cells by hematoxylin and eosin (H&E) and activated cytotoxic T lymphocytes (CTLs) markers CD8/ICOS and Treg cell markers CD4/IFNγ by immunofluorescence.

Results

• Cryoadablation completely killed the left tumors for all mice as demonstrated by IVIS analysis.
• Mouse necropsies showed cryoadablated tumors undergoing coagulative necrosis.
• Tissue analysis for anti-CTLA4 antibody demonstrated systemic distribution with the highest levels detected in peripheral blood.
• Mice treated with both cryoadablation and anti-CTLA4 antibody had an increased percentage of CD4 and CD8 T cells expressing ICOS in peripheral blood and spleen compared to cryoadablation alone.
• Mice treated with combination of cryoadablation and anti-CTLA4 antibody had a significant increase in TILs in the cryoadablated tumors.
• Immunofluorescence analysis revealed significant increase of CD4 and CD8 T cells in the cryoadablated tumors compared to the absopal tumors.
• Mice treated with combinational therapy had increased T cells in absopal tumors with a significant increase in CD4+ T cells compared to cryoadablation alone.

Conclusions

Cryoadablation in combination with anti-CTLA4 antibodies increased T cell activation compared to cryoadablation treatment alone. The next step is to evaluate whether combinational therapy increases the absopal effect in controlling metastasis in long-term survival studies in our breast cancer murine model before proceeding to clinical trials.

References