In vivo Intratumoral Electroporation of Gp96-Ig/Fc-OX40L Stimulates CD8+ T cell Cross Priming to Tumor-Specific Neoantigens

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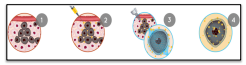
Abstract

- Cancer immunotherapy relies on presentation of shared- and neo- antigens from a patient's tumor cells for recognition and clearance by the immune system. However, the tumor microenvironment deploys multiple strategies to evade immune recognition and often remains non-immunogenic, which is one of the challenges that need to be addressed when designing new therapies.
- We set out to test whether intratumoral electroporation of Gp96-Ig/Fc-OX40L, a re-engineered molecular chaperone, designed to export and deliver MHC I-associated antigens to APCs in context of OX40L expression, would generate a robust anti-neoantigen CD8+ T cell response. To assess antigen-specific CD8+ expansion, mice were adoptively transferred with OT-I cells after B16.F10-ovalbumin cells were injected to generate primary and contralateral melanotic tumors. Contralateral tumors were monitored to assess whether a systemic CD8+ T cell response could be elicited following primary tumor electroporation. IT electroporation of DNA expressing Gp96-Ig/Fc-OX40L in the primary tumor triggered a significant expansion of antigen-specific OT-I cells, which was absent in control mice. Remarkably, increases in antigen-specific OT-I cells correlated with regression of both the treated primary and untreated contralateral tumors.
- We further validated our findings in a CT26 mouse colorectal cancer tumor model, in which the expression of Gp96-Ig/Fc-OX40L from electroporated DNA stimulated an expansion of antigen-specific CD8+ T cells and again led to regression of both the treated primary and untreated contralateral tumor.
- Our findings demonstrate that *in situ* manipulation of intratumoral cells to express Gp96-Ig/Fc-OX40L stimulates potent antigen-specific cross priming to tumor specific neoantigens that culminates in robust systemic anti-tumor response. These findings provide exciting proof-of-principal and warrant further investigation into the direct delivery of molecular chaperones such as Gp96-Ig/Fc-OX40L and/or pro-inflammatory molecules for elevating the immunogenicity of tumors for a potent anti-tumor CD8+ T cell response.

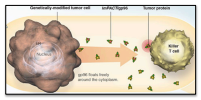
Introduction

Intratumoral DNA delivery by electroporation



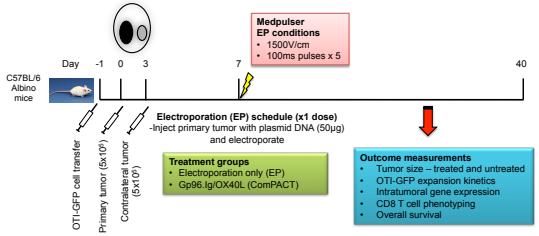
Step 1 – tumor cells are identified
Step 2 – DNA-based molecules are injected in the tumor
Step 3 – Electrical pulses are delivered via electrodes, which increases the permeability of the cell membrane
Step 4 – DNA-based molecules enter the cell nucleus and express the immuno-modulatory molecules

Chaperone and adjuvant functions of Gp96.Ig



- Gp96.Ig serves as a molecular chaperone and adjuvant that presents tumor-associated neoantigens to APCs to trigger an anti-tumor CTL response
- Fc-OX40L secreted from the tumor cell serves to enhance co-stimulation of CTLs

Experimental Design – B16.F10-ova melanoma tumor model



Results

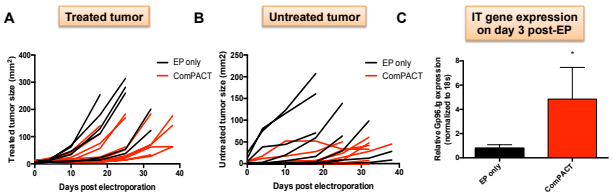


Figure 1. Intratumoral delivery of Gp96.Ig/Fc-OX40L (ComPACT) expressing DNA via electroporation (EP) leads to delayed tumor progression of both treated (primary) and untreated (contralateral) B16.F10 melanoma tumors. A. C57BL/6 albino mice (n=9 mice per group) bearing melanotic B16.F10-ova tumors were electroporated with saline (EP only) or ComPACT DNA expressing Gp96.Ig/Fc-OX40L. Tumor sizes were measured using a digital caliper and monitored over a 40 day time period post-EP. B. The size of the contralateral untreated tumor following electroporation was monitored over the same time period. C. Intratumoral mRNA expression of Gp96.Ig in ComPACT EP mice was confirmed by qPCR on day 3 following EP (n=3 mice per group). mRNA levels were normalized to 18s and * denotes p<0.05. The EP only control was set to 1.

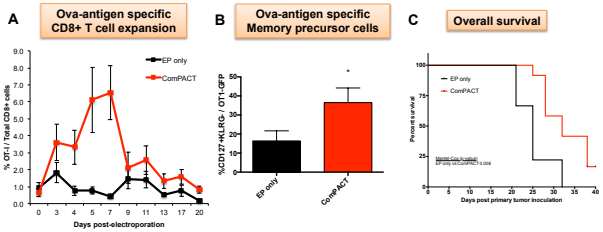
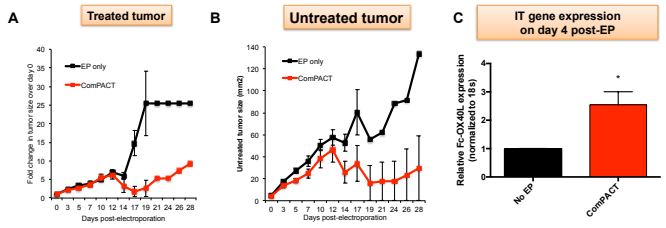


Figure 2. Intratumoral EP of ComPACT leads to ova-antigen specific CD8+ T cell expansion *in vivo*. A. C57BL/6 albino mice that were adoptively transferred with OT1-GFP cells and B16.F10-ova tumors were electroporated with either saline (EP only) or ComPACT DNA. The percentage of CD8+ OT1-GFP cells in peripheral blood was monitored over time by flow cytometry. B. Phenotypic analysis of ova-antigen specific CD8+ T cells on day 12 following EP by flow cytometry reveals increased numbers of CD127+/KLRG- memory precursor cells in mice EP'd with ComPACT. C. Overall survival of B16.F10 melanoma bearing mice EP'd with saline or ComPACT DNA . * indicates p<0.05. Statistical significance was determined by student t-test and Mantel-Cox test.

Results – CT26 colon cancer tumor model



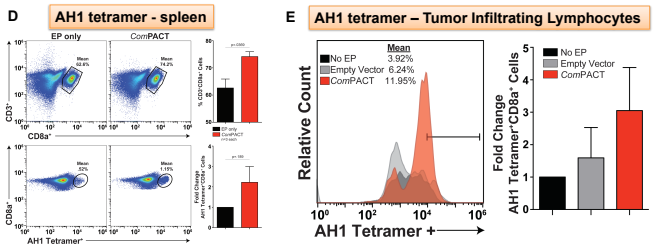


Figure 3. EP-based intratumoral delivery of ComPACT leads to regression of both treated (primary) and untreated (contralateral) CT26 colon cancer tumors. BALB/c mice (n=12 per group) bearing CT26 tumors were EP'd with saline or ComPACT DNA and tumor sizes, both treated (A) and untreated (B) were monitored over a 28 day period. C. Intratumoral mRNA expression of Fc-OX40L in ComPACT treated mice was confirmed by qPCR 4 days following EP (n=3 mice per group) *p<.05. D. Splenocytes from EP'd mice (n=3 mice per group) were dissected on day 6 post-EP and enriched for CD8+ cells using a commercial kit (Stemcell tech) and stained for AH1-tetramer+ (representing CT26 antigen-specific CD8+) cells. Cells were negatively gated to exclude cells positive for Nk1.1, Gr-1, CD11b and CD11c and subsequently pre-gated on CD3+. E. Tumors were isolated from mice on day 7 post-EP and enzymatically dissociated using a commercial kit (Miltenyi Biotec) and cells were stained for AH1-tetramer-positive cells as before. Representative histogram showing CD8+ AH1-tetramer+ double positive cells are shown. An empty vector control electroporated group was also included.

Key Points

- *In vivo* EP of DNA-based Gp96.Ig/Fc-OX40L into B16 melanoma and CT26 colon cancer tumors result in delayed tumor progression of treated and untreated tumors
- Intratumoral expression of Gp96.Ig/Fc-OX40L stimulates CD8+ T cell cross priming to tumor specific neoantigens and increases the frequency of circulating memory precursor T cells
- Electroporation-based delivery of Gp96.Ig/Fc-OX40L DNA in combination with other immuno-modulatory DNA could lead to synergistic anti-tumor activity

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