The use of immunomodulatory cytokines has been shown effective in regressing a wide range of tumors. However, systemic delivery of recombinant cytokines can result in serious adverse effects, often life-threatening. DNA transfer via electroporation (EP) is a safe and effective method of delivering cytokines to target tissues. Intratumoral (IT) gene electrotransfer (GET) of Interleukin-12 (IL-12), a potent immunomodulatory cytokine, has demonstrated an acceptable safety profile and is well-tolerated and effective in Phase 2 clinical trials in metastatic melanoma and Merkel cell carcinoma. We sought to improve the systemic anti-tumor response of IT GET of IL-12 by improving IL-12p70 expression and electroporation conditions, which were evaluated in vitro and in vivo with a two-tumor syngeneic mouse model of melanoma. As functional IL-12p70 is a heterodimer, we compared different expression constructs to achieve high levels of IL-12p70 protein expression. IL-2/p70 expression from a plasmid that incorporated a picornavirus-derived co-translationally cleavable splice (P2A) was higher than constructs with an internal intronless entry sequence (RES) or a fusion of the p35 and p40 subunits. In functional in vitro assays, pIL12-P2A was superior to pIL12(RES) and the fusion protein. Using the murine B16.F10 tumor model, we show that IT EP of pIL12-P2A plasmid regresses the treated lesions in a dose-dependent manner compared to control treatments. Systemic effects of IT-expressed IL-12 were assessed by monitoring generation of an antigen-specific CD8 T cell response and regression of B16.F10 contralateral (untreated) tumors following primary tumor electrotransfer. IT-pIL12-P2A EP treatment with the P2A-linked construct resulted in a significant increase in antigen-specific CD8 T cells, as well as enhanced contralateral tumor growth inhibition suggesting the induction of a strong systemic anti-tumor immune response.

**Abstract**

**Low Voltage electrotransfer conditions improve intratumoral transfection efficiency and transgene expression.**

**Improvements to the IT-pIL12-EP platform (P2A linker + Low Voltage Electroporation) lead to suppression of tumor growth in a B16.F10 contralateral tumor model.**

**Our novel pIL12-P2A plasmid leads to increased expression of IL-12p70 and its downstream effector molecule IFN-γ in vivo. Applying Low Voltage electrotransfer conditions results in enhanced transgene expression in vivo by improving transfection efficiency.**

**Contralateral B16.F10 tumor regression model to assess the therapeutic effect of IT-pIL12-EP.**

**Gene expression changes in treated and untreated lesions were assessed by NanoString nCounter technology.**

**Summary and Conclusions**

- Our novel pIL12-P2A plasmid leads to increased expression of IL-12p70 and its downstream effector molecule IFN-γ both in vitro and in vivo.
- Applying Low Voltage electrotransfer parameters results in enhanced transgene expression in vivo by improving transfection efficiency.
- Modifications to plasmid design (P2A) or electrotransfer parameters (Low Voltage) can improve the contralateral systemic anti-tumor response of IT-pIL12-EP in a murine melanoma model.
- IT-pIL12(P2A)-EP with Low Voltage conditions results in the generation of circulating antigen-specific CD8 T cells (B16-OVA model).
- P2A-linked multigene constructs in combination with a Low Voltage generator will provide the platform for our future clinical studies.

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