

POSTER PRESENTATION

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STING contributes to anti-glioma immunity via triggering type-I IFN signals in the tumor microenvironment

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While type-I interferons (IFNs) play critical roles in antiviral and antitumor activity, it remains to be elucidated how type-I IFNs are produced in sterile conditions of the tumor microenvironment and directly impacts

tumor-infiltrating immune cells. We report that both human and *de novo* mouse gliomas show increased expression of type-I IFN messages, and in mice, CD11b⁺ brain-infiltrating leukocytes (BILs) are the main source

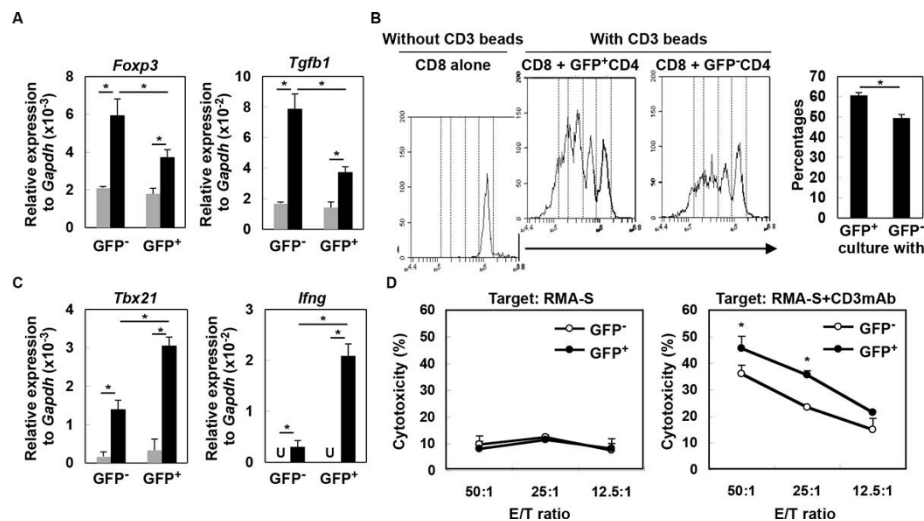


Figure 1 Type-I IFNs directly impact on T-cell functions in glioma-developing mice. (A) CD4⁺ cells from draining LN derived from glioma-developing tdTomato mice were sorted into GFP⁻ or GFP⁺ cells and incubated with (black bars) or without (grey bars) anti-CD3mAb. After 4 h, total RNA was extracted for evaluation of *Foxp3* and *Tgfb1* mRNA levels by qRT-PCR. (B) CFSE-labeled WT CD8⁺ T-cells were co-cultured with GFP⁻ or GFP⁺ CD4⁺ T-cells in the presence of CD3 beads. After 60 h, division of CFSE-labeled CD8⁺ T-cells gated by reactivity to PE-Cy7-conjugated anti-CD8mAb was evaluated by CFSE intensity. As a negative control, CFSE-labeled WT CD8⁺ T-cells were cultured without any stimulation (left panel). Histograms are representative of two independent experiments. The bar graph shows the percentage of CD8⁺ cells that have divided at least twice in each of two stimulation conditions (N = 4/group; *p < 0.05). (C) GFP⁻ or GFP⁺ CD8⁺ T-cells were incubated with (black bar) or without (grey bar) anti-CD3mAb. After 4 h, total RNA was extracted for evaluation of *Tbx21* and *Ifng* mRNA expression levels by qRT-PCR (U: undetected). (D) Cytotoxic activity of GFP⁻ and GFP⁺ CD8⁺ T-cells was evaluated by ⁵¹Cr-release assay. RMA-S cells untreated (left panel) or pretreated (right panel) with anti-CD3mAb (10 g/mL) were used as target cells. *p < 0.05 compared at the same E/T ratio.

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of type-I IFNs that is induced partially in a STING (stimulator of IFN genes)-dependent manner. Consequently, glioma-bearing *Sting*^{Gt/Gt} mice showed shorter survival, and lower expression levels of *Ifns* compared with wild-type mice. Furthermore, BILs of *Sting*^{Gt/Gt} mice show increased CD11b⁺ Gr-1⁺ immature myeloid suppressor and CD25⁺ Foxp3⁺ regulatory T (Treg) cells, while decreased IFN- γ -producing CD8⁺ T cells. To determine the effects of type-I IFN expression in the glioma microenvironment, we utilized a novel reporter mouse model, in which the type-I IFN signaling induces the *Mx1* (IFN-induced GTP-binding protein) promoter-driven Cre recombinase, which turns the expression of *loxP*-flanked *tdTomato* off, and turns green fluorescence protein (GFP) expression on, thereby enabling us to monitor the induction and effects of IFN signaling in the glioma microenvironment. CD4⁺ T cells that received direct type-I IFN signals (i.e., GFP⁺ cells) demonstrate lesser degrees of regulatory activity based on lower *Foxp3* and *Tgfb1* expression levels (Figure 1) as well as lesser suppression of CD8⁺ T cell proliferation (Figure B). IFN-sensed CD8⁺ T cells exhibit enhanced levels of Th1 markers, *Tbx21* and *Igfng* (Figure C), as well as cytotoxic T-cell activity based on reverse antibody-dependent T-cell-mediated cytotoxicity assay (Figure D). Finally, intratumoral administration of a STING agonist (cyclic diguanylate monophosphate; c-di-GMP) improves the survival of glioma-bearing mice associated with enhanced type-I IFN signaling, *Cxcl10* and *Ccl5* and T cell migration into the brain. In a combination with subcutaneous OVA peptide-vaccination, c-di-GMP increased OVA-specific cytotoxicity of BILs and prolonged the survival. These data demonstrate significant contributions of STING to antitumor immunity via enhancement of the type-I IFN signaling in the tumor microenvironment, and imply a potential use of STING agonists for development of effective immunotherapy, such as the combination with antigen-specific vaccinations.

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