Ohkuri et al. Journal for ImmunoTherapy of Cancer 2014, 2(Suppl 3):P228
http://www.immunotherapyofcancer.org/content/2/S3/P228

Journal for ImmunoTherapy of Cancer

POSTER PRESENTATION Open Access

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

Takayuki Ohkuri[1,6], Arundhati Ghosh[4,8], Akemi Kosaka[1,6], Jianzhong Zhu[4,8], Maki Ikeura[6], Michael David[9], Simon C Watkins[5], Saumendra N Sarkar[3,4,8], Hideho Okada[1,2,3,6,7*]

From Society for Immunotherapy of Cancer 29th Annual Meeting
National Harbor, MD, USA. 6-9 November 2014

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

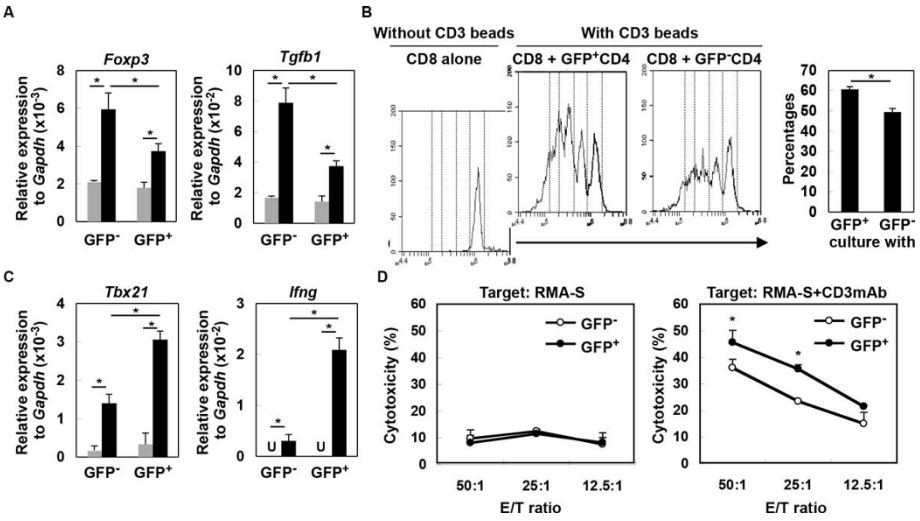

Figure 1 Type-I IFNs directly impact on T-cell functions in glioma-developing mice. (A) CD4$^+$ cells from draining LN derived from glioma-developing tdTomato mice were sorted into GFP$^-$ or GFP$^+$ cells and incubated with (black bars) or without (grey bars) anti-CD3mAb. After 4 h, total RNA was extracted 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-condjugated 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 ^{51}Cr-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.

[1]Department of Neurological Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA

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.

Authors' details
[1]Department of Neurological Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA. [2]Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA. [3]Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA. [4]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA. [5]Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA. [6]Department of Brain Tumor, University of Pittsburgh School of Medicine, Pittsburgh, PA. [7]Cancer Immunology, University of Pittsburgh Cancer Institute, Pittsburgh, PA. [8]Cancer Virology Programs, University of Pittsburgh Cancer Institute, Pittsburgh, PA. [9]Division of Biology, UCSD, La Jolla, CA.

Published: 6 November 2014

doi:10.1186/2051-1426-2-S3-P228
Cite this article as: Ohkuri *et al.*: STING contributes to anti-glioma immunity via triggering type-I IFN signals in the tumor microenvironment. *Journal for ImmunoTherapy of Cancer* 2014 **2**(Suppl 3): P228.