

Protective role of STING against gliomagenesis: Rational use of STING agonist in anti-glioma immunotherapy

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Abstract (48 words)

We recently reported that STING contributes to antiglioma immunity by triggering type I IFN induction in glioma microenvironment. Moreover, intratumoral administration of STING agonist improved the efficacy of peptide vaccination in a mouse glioma model, suggesting the rational use of STING agonists in the immunotherapy of brain tumor.

Text (up to approximately 900 words)

Glioma accounts for approximately 40% of all primary brain tumors and are responsible for approximately 13,000 cancer-related deaths in the US each year, representing a large unmet medical need. We have previously demonstrated the protective role of type I interferon (IFN) signaling against glioma progression using a clinically relevant *de novo* mouse glioma model, and identified single nucleotide polymorphisms (SNPs) in human *IFNA* genes associated with the prognosis of glioma patients¹. However it remained elusive how type I IFNs were induced in the glioma microenvironment. We have recently demonstrated that type I IFNs are induced in *de novo* glioma tissues and directly impact on functions of immune cells². Glioma-bearing brain tissues showed higher levels of type I IFNs than non-tumor-bearing brains. Using a type I IFN-reporter mice (tdTomato mice), type I IFN signaling was detected in a variety of glioma-infiltrating immune cell populations. Especially, type I IFN signaling down-regulated *Foxp3* and *Tgfb1* expression levels and immuno-suppressive activity in CD4⁺ T cells, while up-regulated *Tbx21* and *Ifn γ* expression levels and cytotoxic activity in CD8⁺ T cells, suggesting that type I IFN signaling directly enhances antitumor activity of T cells in the glioma-microenvironment.

We next focused on the signaling mechanism responsible for the type I IFN production in the glioma microenvironment. STING (stimulator of IFN genes) plays a critical role as one of the adaptors for cytosolic DNA sensing thereby triggering type I IFN production³. Due to the presence of apoptotic or necrotic cells in the tumor microenvironment, we hypothesized that double strand DNA (dsDNA) such as genomic DNA (gDNA) from the dead cells would induce type I IFN signaling through STING. Using wild type (WT) and STING-deficient (*Sting*^{Gt/Gt}) mouse-derived macrophages, we demonstrated *in vitro* that gDNA up-regulated type I IFN mRNA levels at least partially in a STING-dependent manner, implying that STING mediates type I IFN induction during glioma progression. When we induced *de novo* gliomas in WT

and *Sting*^{Gt/Gt} mice, glioma tissues of *Sting*^{Gt/Gt} mice showed lower levels of type I IFN mRNA and type I IFN-inducible ISG54 protein compared with those of WT mice. Furthermore, glioma-bearing *Sting*^{Gt/Gt} mice showed shorter survival, associated with increased immuno-suppressor cells, such as CD11b⁺ Gr-1⁺ immature myeloid cells and CD25⁺ Foxp3⁺ CD4⁺ regulatory T cells, and lesser IFN γ -producing CD8⁺ T cells in the glioma microenvironment compared with WT mice. These observations suggest that STING is at least partially responsible for spontaneous type I IFN production in glioma and positively affects the activity of immune cells in the glioma microenvironment (**Fig. 1**).

On the basis of these observations, we attempted to evaluate if administration of STING agonist would enhance the antiglioma immunity. Intratumoral administration of STING ligand, cyclic-di-GMP (c-di-GMP) significantly prolonged survival of glioma-bearing mice, increased *Ccl5* and *Cxcl10* mRNA levels, and enhanced infiltration of effector cells, such as CD4⁺ T cells and CD8⁺ T cells compared with control treatment in a STING-dependent manner. Also, in the *de novo* glioma model, c-di-GMP treatment significantly inhibited glioma growth. These suggest that direct intratumoral administration of c-di-GMP enhances antiglioma immunity by enhancing the recruitment of T cells into the brain tumor site. Moreover administration of c-di-GMP significantly enhanced the efficacy of peptide vaccinations targeting a tumor-specific antigen by recruiting peptide-specific CD8⁺ T cells in the tumor site. This strongly supports the development of combination strategy with vaccine and a STING agonist. Although we selected c-di-GMP as a STING ligand in this study due to its commercial availability, there are more agonists for STING such as c-di-AMP, cGMP-AMP (2'3'cGAMP or 3'3' cGAMP), or 10-carboxymethyl-9-acridanone (CMA)^{4, 5}. Moreover, while activities of bacterium-derived c-di-GMP can be dependent upon single nucleotide polymorphisms (SNPs) in human *STING*, metazoan-derived 2'3'cGAMP stimulate STING regardless of the SNP status⁶. These observations warrant inclusion of 2'3'cGAMP in our studies evaluating

STING agonists as adjuvants in cancer immunotherapy⁷. On the basis of our current study, early-phase clinical studies are warranted to evaluate the safety and efficacy for intratumoral administration of a STING agonist in patients with glioma as well as other tumors.

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Figure legend

Figure 1. Protective role of STING against glioma by initiating type I IFN signaling in the glioma microenvironment. CD11b⁺ macrophages produce type I IFNs, in a partially STING-dependent manner, in response to dsDNA. We hypothesize that dsDNA is released from dead cells, such as tumor, stroma and immune cells, in the glioma microenvironment. Type I IFNs induce chemokine production to recruit CD4⁺ T cells and CD8⁺ T cells that contribute to the anti-glioma immune response.

Figure 1

