STING restraints tumor-associated myeloid-derived suppressor cell (MDSC) 
induction by TBK1-SOCS1-STAT3 axis in nasopharyngeal carcinoma 

Chuanxia Zhang, Jun Cui and Jiang Li

Collaborative Innovation Center for Cancer Medicine, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou 510060, China.

Introduction
Myeloid-derived suppressor cells (MDSCs) contribute to immune suppression in many cancers. As a DNA virus-related tumor, nasopharyngeal carcinoma (NPC) promotes MDSC differentiation to create an immune-suppressive microenvironment. However, whether antiviral signaling is involved in the regulation of MDSCs in NPCs is not clear. Here, we determine that STING, a key adaptor in viral DNA sensing, plays a vital role in the regulation of MDSC differentiation and antitumor immunity in NPC. Decreased tumor STING level correlated with reduced survival in NPC patients. Mechanistic analyses revealed that STING repressed NPC-derived MDSC induction through enhancing the expression of SOCS1, which prevents STAT3 phosphorylation, thus inhibiting GM-CSF and IL-6 production to suppress MDSC induction. Additionally, we identified STING as a direct target gene of microRNA-24 (miR-24). Our findings reveal a novel mechanism where the exosomal miR-24-STING axis facilitates tumor escape by promoting MDSC induction through controlling the crosstalk between STAT3 and antiviral signaling.

Materials and Methods
- Immunohistochemistry (IHC) Assay
- MDSC Differentiation Assay
- Enzyme-linked Immunosorbent (ELISA) Assay
- Luciferase Reporter Assay
- Immunoblot Analysis
- Generation Knockout Cells by CRISPR/Cas9 Technology
- Statistical analysis (Data are represented as mean ± SEM when indicated, and Student’s t-test was used for all statistical analyses with the GraphPad Prism 5.0 software)

Results
1. Reduced STING expression correlates with poor prognosis in NPC patients
   - [Figure A: Reduced STING expression in NPC tissue compared to non-tumor tissue.]
   - [Figure B: Scatter plot showing the correlation between STING expression and DFS.]
   - [Figure C: Kaplan-Meier curve illustrating the survival difference between high and low STING expression.]

2. Knock down STING enhances tumor-associated MDSC differentiation by promoting IL-6 and GM-CSF secretion
   - [Figure A: CD11b expression in MDSCs with or without STING knockdown.]
   - [Figure B: IL-6 and GM-CSF secretion from MDSCs with or without STING knockdown.]

3. STING suppresses NPC-induced MDSC differentiation via inhibiting STAT3 signaling
   - [Figure A: Western blot showing STAT3 phosphorylation in NPC cells with or without STING knockdown.]
   - [Figure B: Flow cytometry showing IL-6 and GM-CSF secretion in NPC cells with or without STING knockdown.]

4. STING inhibits NPC-induced MDSC differentiation via TBK1 and SOCS1
   - [Figure A: Western blot showing SOCS1 and TBK1 expression in NPC cells with or without STING knockdown.]
   - [Figure B: Flow cytometry showing IL-6 and GM-CSF secretion in NPC cells with or without STING knockdown.]

5. MiR-24 directly suppresses STING expression in NPC
   - [Figure A: qRT-PCR showing miR-24 expression in NPC cells with or without STING knockdown.]
   - [Figure B: Western blot showing STING expression in NPC cells with or without STING knockdown.]
   - [Figure C: Flow cytometry showing IL-6 and GM-CSF secretion in NPC cells with or without STING knockdown.]

6. Working model of the regulation of MDSC differentiation by STING in NPC
   - [Diagram illustrates the regulatory axes between STING, SOCS1, and STAT3 in NPC cells.]

Conclusion
We propose a working model to illustrate how STING regulates MDSC differentiation in NPC (Figure 71). During EBV infection, STING works as an antitumor protein to activate TBK1-IRF3 signaling to up-regulate SOCS1. SOCS1 then interacts with STAT3 to inhibit its phosphorylation and dimerization. However, miR-24 carried by T-EXO in NPC can specifically target STING for down-regulation, which results in the persistent activation of STAT3 in tumor cells. Released IL-6 and GM-CSF from those cells eventually leads to the induction of MDSC differentiation to suppress T cell function. Furthermore, T-EXO-carried miR-24 can also enter into MDSCs to down-regulate STING and directly inhibit MDSC differentiation. Down-regulation of STING by T-EXO-carried miR-24 in different cell types finally facilitates MDSC differentiation to change the tumor microenvironment and leads to tumor progression.