# The Effects of STING Downregulation in Human Osteosarcoma on Innate Inflammatory Signals

Shelby Baham[1], Raegan Abadie[2], Chin-Chi Liu[3], Sarah Keeton[3], Andrea Johnston[3], Sita Withers[3]

*Centenary College of Louisiana[1], University of Louisiana Monroe[2], Louisiana State University[3]*

## Abstract

Background: Osteosarcoma (OSA) is a highly aggressive cancer, for which additional treatments are urgently necessary. Modulation of the innate immune response is a promising strategy to encourage anti-tumor immunity. Stimulator of interferon genes (STING), is a key protein linking cytoplasmic DNA with innate inflammation.

Purpose: To quantify the expression of STING in human OSA cells. **Our hypothesis was that STING will be downregulated in OSA cells as a mechanism to hide from the body's immune system, and downregulation would result in diminished cytokine and chemokine production with STING agonism**.

Methods: We used real-time quantitative PCR (RT-qPCR) to measure the expression of STING in human OSA cell lines and the positive control cell line HeLa. We then activated STING with cGAMP, and measured the expression of RNA transcripts encoding IFNβ1, and the chemokine CXCL10.

Results: STING is variably downregulated in human OSA cell lines. STING-deficiency in these cell lines is associated with decreased pro-inflammatory cytokine and chemokine production.

Conclusions: STING downregulation may be a mechanism of immunoevasion by OSA cells.

## Background

**What is osteosarcoma (OSA)?**

- OSA is the most common primary bone tumor in humans.
- OSA is highly metastatic and predominantly affects adolescents.
- Unfortunately, survival rates have remained static since the 1980's(1).

**What Is the STING pathway?**

- cGAS detects cytoplasmic viral DNA, or self-DNA present in the cytoplasm due to genomic instability or DNA damage.
- cGAMP activates STING.
- STING induces transcription factors that stimulate production of type I interferons (IFN) and chemokines.

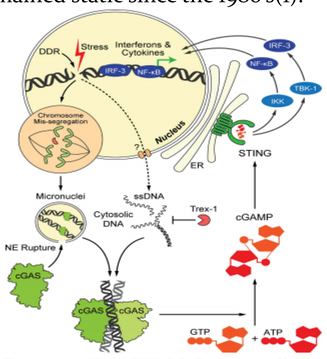



**Figure 1:** The STING Pathway (Li, T. and Chen, Z; Ref 2)

**What do we know?**

- Downregulation of the STING pathway in SAOS-2 and U2OS cell lines was found to be permissive to herpes simplex virus replication (3).
- INFβ, CCL5, and CXCL10 are necessary for proper recruitment of CD8+ cytotoxic T cells and natural killer cells (4).

**Why is this research important?**

- Downregulation of STING in OSA may make this cancer more susceptible to oncolytic viruses.
- Downregulation of STING in OSA may impede the ability of chemotherapy and radiation therapy to induce an anti-tumor immune response.

## Data & Results

### Verification of adequate RT-qPCR conditions:

**Table 1: Primer efficiencies.** Calculated from multiple 10x dilutions of RNA derived from HeLa treated with 10 µg/ml cGAMP.

| Primers | $R^2$ | % Efficiency |
|---|---|---|
| GAPDH | 0.992 | 126.4 |
| TBP | 0.997 | 122.9 |
| STING | 0.993 | 119.9 |
| INFβ1 | 0.638 | 120.9 |
| CXCL10 | 0.724 | 117.7 |

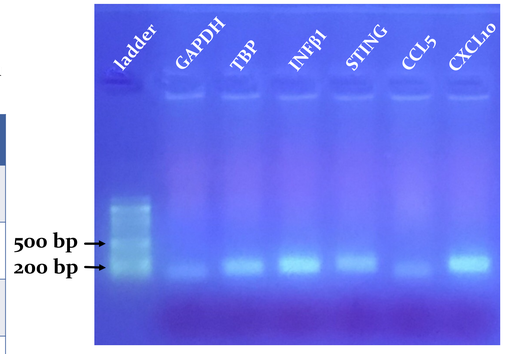



**Figure 2: A single amplicon is detected for each primer pair after qPCR**. qPCR product derived from cDNA from HeLa treated with 10 µg/ml cGAMP. 2% agarose gel electrophoresis.

### STING expression and induction of inflammatory signals induced by cGAMP stimulation of OSA cells:

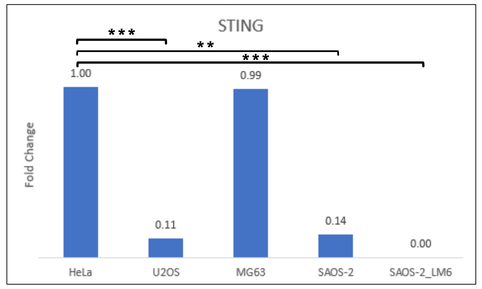
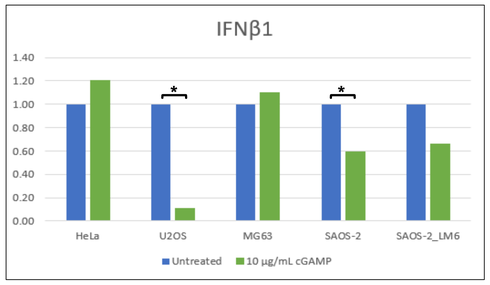



**Figure 3: OSA cell lines exhibit variably downregulated STING expression compared to HeLa cells.** Expressed as fold change in RNA expression relative to HeLa. ANOVA with Tukey's post hoc multiple comparison's test. ** = p<0.005, *** = p<0.001

**Figure 4: OSA cell lines with decreased STING expression exhibit impaired IFNβ1 production after cGAMP stimulation.** Expressed as fold change in RNA expression relative to each cell line's untreated control. Paired t-test. * = p<0.05



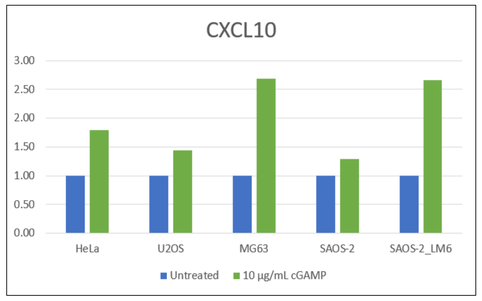




**Figure 5: OSA cell lines with decreased STING expression generally exhibit impaired CXCL10 production after cGAMP stimulation.** Expressed as fold change in RNA expression relative to each cell line's untreated control.

## Conclusions & Future Studies

- STING is significantly downregulated in some OSA cell lines.
- STING deficiency is associated with decreased cGAMP-induced IFNβ1 and CXCL10 upregulation in OSA cells.
- STING downregulation may therefore be a mechanism of immunoevasion by OSA cells.
- Further studies are needed to determine whether STING downregulation in OSA cells can be exploited to target oncolytic virus therapy.
- Further studies will determine whether STING expression may be a biomarker of radiation therapy response in OSA.

## Methods & Materials

1. Maintain OSA cell lines (U2OS, MG63, SAOS-2, SAOS-2_LM6)
2. Plate and incubate cell lines overnight
3. Stimulate STING pathway using cGAMP (10 µg/ml)
4. Incubate for 4 hours
5. Isolate RNA from each cell line
6. Quantify RNA & test for impurities via Nanodrop
7. Verify integrity of RNA via fragment analysis
8. Reverse transcription
9. qPCR
10. Relative quantification

## References & Acknowledgements

1. Mirabello, L., et al. *Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program.* Cancer. 2009;115(7):1531-1543.
2. Li, T., et al. *The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer.* Journal of Experimental Medicine. 2018, Vol 215, 1287-1299.
3. Deschamps, T., et al. *Impaired STING Pathway in Human Osteosarcoma U2OS Cells Contributes to the Growth of IPC0-Null Mutant Herpes Simplex Virus.* Journal of Virology. 2017, 91, 1-14.
4. Sokolowska, O., et al. *STING Signaling in Cancer Cells: Important or Not?* Arch. Immunol. Ther. Exp. 2018, 66, 125-132.

LSU School of Veterinary Medicine, Department of Veterinary Clinical Sciences

*Research reported in this publication was supported by an Institutional Development Award from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 5 P20 GM103424-17 – Louisiana Biomedical Research Network*