# Modulation of ENPP1 Activity and 2’3’-cGAMP Degradation in Ovarian Cancer Cell Lines *via* Loadable Preformed Lipid Nanoparticles

David Taylor, Phil Rzeczycki, and Aileen Ariosa

Assay R&D, Cayman Chemical Company, 1180 E. Ellsworth Road, Ann Arbor, MI 48108 USA

Abstract No.
488

## KEY FINDING

**Preformed loadable SM-102 lipid nanoparticles enabled effective delivery of nucleic acid cargo to ovarian cancer and macrophage cell lines to modulate ENPP1 activity and STING activation.**

## INTRODUCTION

Ovarian cancer is a complex and heterogeneous disease with various subtypes and diverse molecular mechanisms of pathogenesis. Among different forms, epithelial ovarian cancer is the most common, representing up to 90% of total reported cases. Recent studies have shown that ENPP1 may play a role in cancer cell proliferation, migration, and invasion in ovarian and other types of cancer. ENPP1 is a glycosylated, type II transmembrane protein acting as a pyrophosphatase and phosphodiesterase with broad specificity. ENPP1 substrates include cyclic dinucleotides such as 2’3’-cGAMP, which binds to and activates STING, leading to the activation of the STING-dependent type 1 interferon (IFN) pathway and stimulation of antitumor activity.[2-4] ENPP1 is frequently upregulated during tumorigenesis where its cGAMP hydrolytic activity contributes to an immunosuppressive tumor microenvironment.[5] In this study we used preformed lipid nanoparticles (LNPs) to modulate ENPP1 activity and STING activation in ovarian cancer cell lines and THP-1 macrophages, respectively.

### Objectives

- Knockdown ENPP1 in PA-1, Caov-3, and SKOV3 ovarian cancer cell lines *via* loadable SM-102 LNPs.
- Assess whether reduced ENPP1 activity leads to an increase in 2’3’-cGAMP production.
- Test whether ENPP1 is present on extracellular vesicles (EVs) in ovarian cancer cell lines.
- Demonstrate utility of loadable, lyophilized SM-102 LNPs as a research tool in delivering nucleic acid cargo to different cell types.

## MATERIALS & METHODS

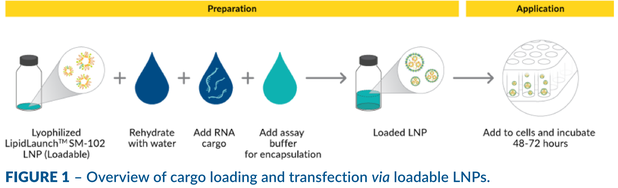


**FIGURE 1** – Overview of cargo loading and transfection *via* loadable LNPs.

PA-1, Caov-3, and SKOV3 cells were seeded into 96- or 24-well plates at densities of 4,000 or 20,000 cells per well, respectively, 24 hrs prior to treatments. ENPP1 siRNA (50 to 250 ng per well) was loaded into preformed SM-102 LNPs, and cells were transfected for up to 72 hrs prior to assay.

ENPP1 activity was determined using Cayman’s ENPP1/ENPP3 Cell-Based Activity Assay Kit (**Item No. 702080**). For 2’3’-cGAMP quantitation, PA-1 cells were refreshed with serum-free media 72 hrs after siRNA treatment and incubated for 24 hrs prior to detection of 2’3’-cGAMP in the conditioned media using Cayman’s 2’3’-cGAMP ELISA Kit (**Item No. 501700**). EV fractions were prepared from conditioned media using Cayman’s Extracellular Vesicle Isolation Kit for tissue cultures (**Item No. 702630**).

## MATERIALS & METHODS CONTINUED

2’3’-cGAMP (**Item No. 19887**) or Cy5-labeled 2’3’-cGAMP (Biolog) was delivered to THP-1 macrophages *via* preformed loadable LNPs. Activation of STING was assessed after 24 hrs *via* immunoblotting (STING mAb; **Item No. 17856**). Uptake of labeled 2’3’-cGAMP was determined *via* fluorescence imaging (Cytation™ 5, Agilent). IL-1β and IL-6 were quantified in THP-1 conditioned media using Cayman’s Interleukin-1β (human) ELISA Kit (**Item No. 583311**) and Interleukin-6 (human) ELISA Kit (**Item No. 501030**).

## RESULTS

### ENPP1 mRNA levels, protein levels, and enzymatic activity are suppressed in PA-1, SKOV3, and Caov-3 cells following loadable LNP-mediated siRNA knockdown

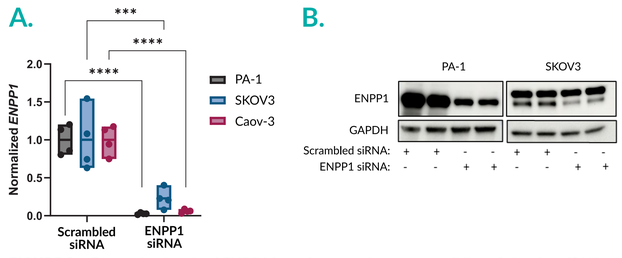


**FIGURE 2** – Expression levels of ENPP1 in various ovarian cancer cell lines following siRNA-mediated knockdown.
The mRNA levels of ENPP1 in PA-1, SKOV3, and Caov-3 cell lines is significantly reduced following transfection with siRNA-loaded *vs.* scrambled siRNA-loaded SM-102 LNPs **(A)**. Protein expression was determined *via* Western blotting **(B)**. N=4, ****<0.0001.

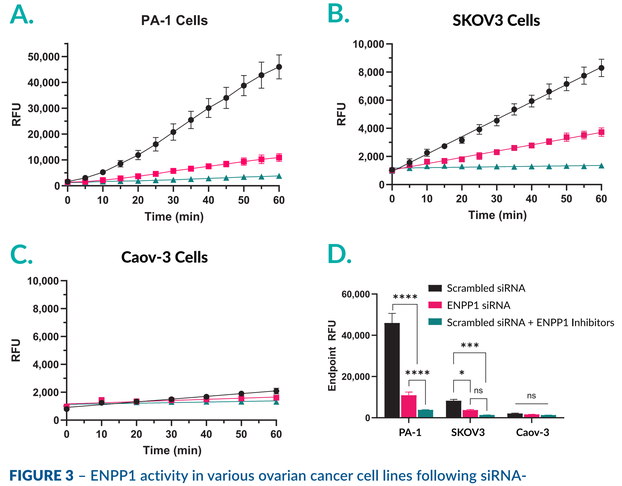


**FIGURE 3** – ENPP1 activity in various ovarian cancer cell lines following siRNA-mediated knockdown.
ENPP1 activities were observed over a period of 60 minutes for PA-1 **(A)**, SKOV3 **(B)**, and Caov-3 **(C)**. The endpoints after the 1-hr incubation are shown in **(D)**. N=5, ****p<0.0001, ***p<0.001, *p<0.05.

## RESULTS CONTINUED

### Extracellular 2’3’-cGAMP levels are increased in ENPP1-depleted PA-1 cells

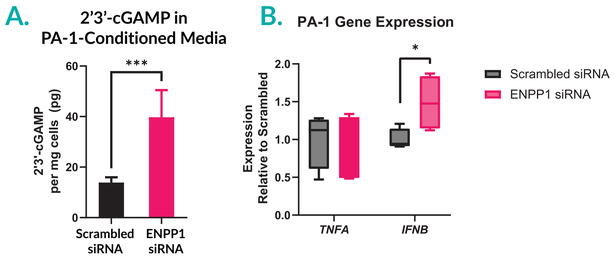


**FIGURE 4** – 2’3’-cGAMP levels and cytokine transcript expression in PA-1 cells following siRNA-mediated knockdown of ENPP1.
2’3’-cGAMP released into media from scrambled siRNA-transfected *vs.* ENPP1 siRNA-transfected PA-1 cells was quantified **(A)**; N=5, ***p<0.001. ENPP1 knockdown led to increased IFNB expression **(B)** determined *via* RT-PCR; N=4, *p<0.05.

### Preformed loadable SM-102 LNPs enable enhanced 2’3’-cGAMP uptake into THP-1 cells, leading to STING activation

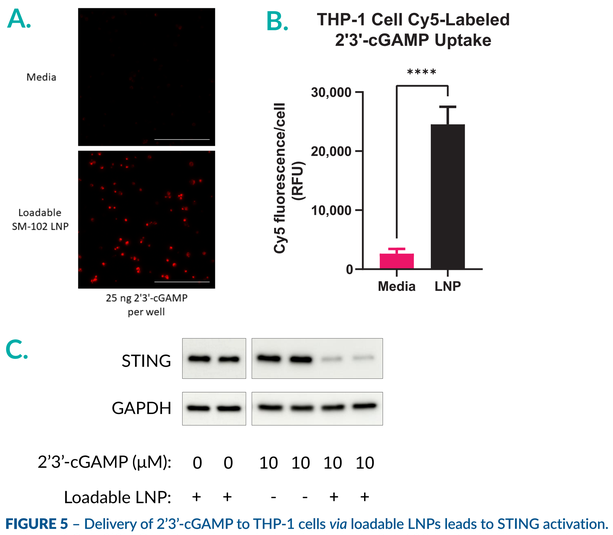


**FIGURE 5** – Delivery of 2’3’-cGAMP to THP-1 cells *via* loadable LNPs leads to STING activation.
Uptake of Cy5-2’3’-cGAMP is enhanced when loaded into SM-102 LNPs **(A)**. The quantification is shown in **(B)**. STING is activated in THP-1 cells following treatment with 2’3’-cGAMP loaded LNPs at 10 μM **(C)**. *p<0.05. Bar size = 200 μm.

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## RESULTS CONTINUED

### PA-1 cells secrete EVs containing ENPP1, which suppressed IL-6 release in 2’3’-cGAMP-stimulated THP-1 macrophages.

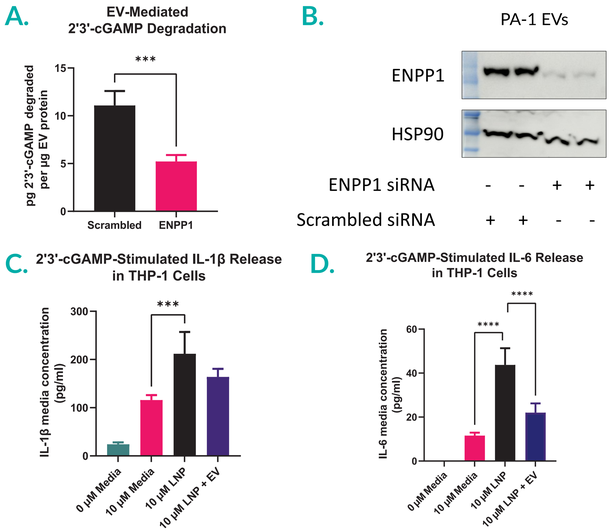


**FIGURE 6** – PA-1 cells secrete EVs containing ENPP1, which can modify immune responses.
Degradation of extracellular 2’3’-cGAMP by EVs isolated from scrambled and ENPP1 siRNA-transfected PA-1 cells **(A)**; N=4, ***p<0.001. EV ENPP1 protein levels were reduced following ENPP1 knockdown in PA-1 cells **(B)**. 2’3’-cGAMP stimulated IL-1β and IL-6 production by THP-1 cells was enhanced by LNP-mediated cGAMP delivery **(Panels C & D)**; IL-6 release was blunted by PA-1 cell-derived EVs, N=4, ****p<0.0001.

## CONCLUSIONS

- ENPP1 activity was detected in PA-1, Caov-3, and SKOV3 cell lines. Preformed loadable SM-102 LNPs facilitated effective siRNA-mediated ENPP1 knockdown in all three cell lines.
- Among the tested lines, PA-1 cells exhibited the highest ENPP1 activity, which following siRNA-mediated ENPP1 knockdown, led to an increase in extracellular 2’3’-cGAMP levels.
- cGAMP delivery to THP-1 macrophages and activation of STING was enhanced by the use of preformed loadable SM-102 LNPs.
- PA-1 cells secreted EVs containing ENPP1, which blunted 2’3’-cGAMP-stimulated cytokine release in THP-1 macrophages.

**References**

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