The Roles of ENPP1 Activity and 2'3'-cGAMP Degradation in Ovarian Cancer Cell Lines

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KEY FINDING

Our cell-based ENPP1 activity assay correlates with protein expression levels and was used to gauge the efficacy of an ENPP1 inhibitor in cells.

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. Due to the lack of early diagnostic tools, the prognosis is mainly poor, as most cases are typically identified in advanced stages. Thus, a more thorough understanding of the inherent complexities of the disease, in terms of its biochemical etiology, will allow for identification of specific molecular and genetic profiles that could lead to more effective treatment plans.[1]

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's substrates include mononucleotides and cyclic dinucleotides such as 2'3'-cGAMP.[2-4]

Objectives

- Investigate whether ENPP1 activity and its hydrolysis of 2'3'-cGAMP correlate with ENPP1 expression levels in ovarian cancer using cell lines such as CaoV-3, SK-OV-3, and PA-1.
- Test whether published ENPP1 inhibitors can sufficiently inhibit ENPP1 activity in both cell-free assays and *in vitro* cell culture models.
- Delineate whether inhibition of ENPP1 affects the viabilities of different ovarian cancer cell lines.

MATERIALS & METHODS

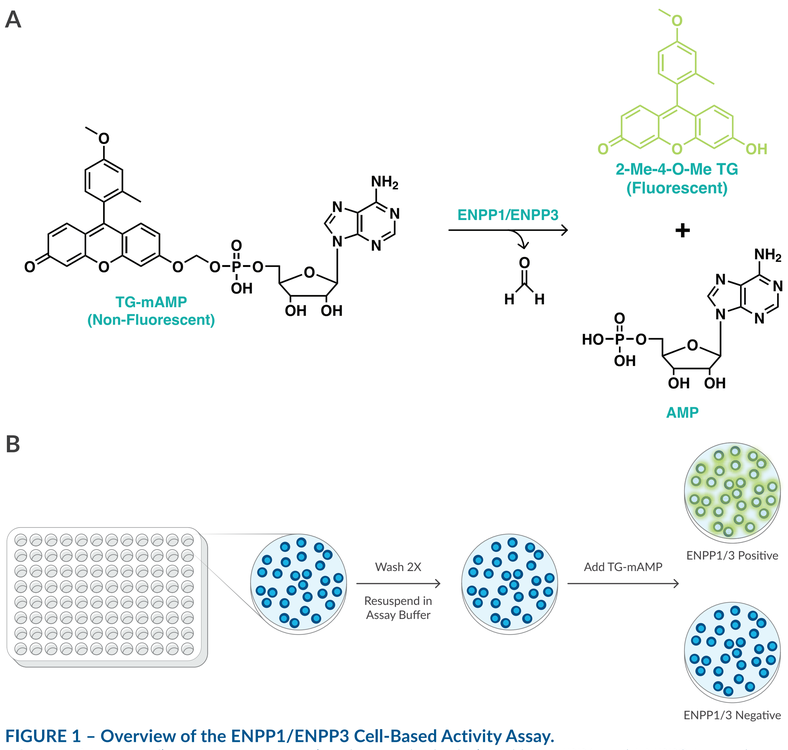

FIGURE 1 – Overview of the ENPP1/ENPP3 Cell-Based Activity Assay.
TG-mAMP is a non-fluorescent, AMP analog that can be hydrolyzed by ENPP1 and ENPP3 to produce a fluorescent molecule and AMP **(A)**. A summary of the cell-based assay procedure to measure ENPP1/ENPP3 activity in ovarian cancer cells is shown in **(B)**.[5]

RESULTS

ENPP1 Enzymatic Activity in Ovarian Cancer Cells can be Inhibited by ENPP1 Inhibitors

ENPP1/3 activities were measured using Cayman's ENPP1/ENPP3 Cell-Based Activity Assay Kit (Item No. 702080).

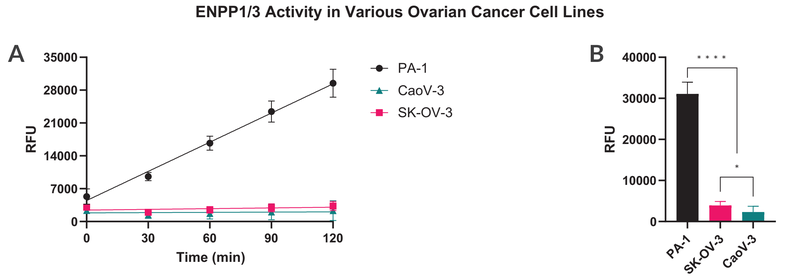

FIGURE 2 – ENPP1/3 Activity Observed in Various Ovarian Cancer Cell Lines.
ENPP1 and/or ENPP3 activities were observed in various cancer cell lines over a period of 2 hours as shown in **(A)**. The endpoints after the 2-hour incubation are shown in **(B)**. N=3, ****p<0.0001, *p<0.05.

Known inhibitors were tested against recombinant ENPP1 (Figure 3) and ENPP3 (data not shown). Inhibitor C specifically inhibited ENPP1 and has superior solubility compared to others, making it most suitable for cell culture experiments.

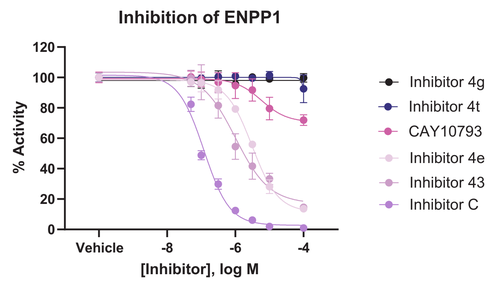

FIGURE 3 – ENPP1 Inhibitors C, -43, and -4e are Potent Attenuators of ENPP1 Activity.
Inhibition of recombinant ENPP1 was assessed using Cayman's ENPP1 Fluorescent Inhibitor Screening Assay Kit (Item No. 702090). The IC_{50}s for inhibitors C, -43, and -4e are 120, 1,010, and 3,107 nM, respectively.[5-7]

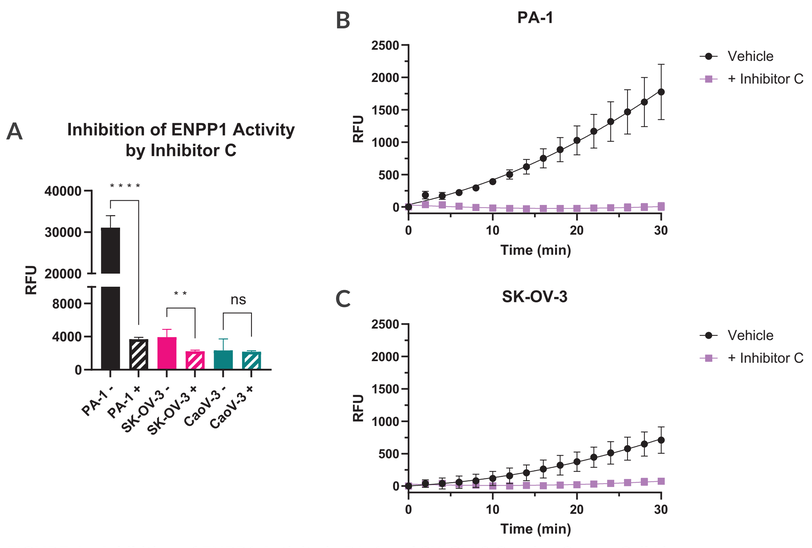

FIGURE 4 – Inhibition of ENPP1 Activity in PA-1 and SK-OV-3 Ovarian Cancer Cell Lines.
ENPP1 activities were significantly reduced by the presence of 30 µM Inhibitor C in PA-1 and SK-OV-3 cells but not in CaoV-3 cells **(A)**. Time course of inhibition for PA-1 and SK-OV-3 cells are shown in **(B)** and **(C)**, respectively. N=3, ****p<0.0001, **p<0.01.

RESULTS CONTINUED

ENPP1 Enzymatic Activity Correlates with Protein Expression Levels in PA-1 and SK-OV-3 Cells

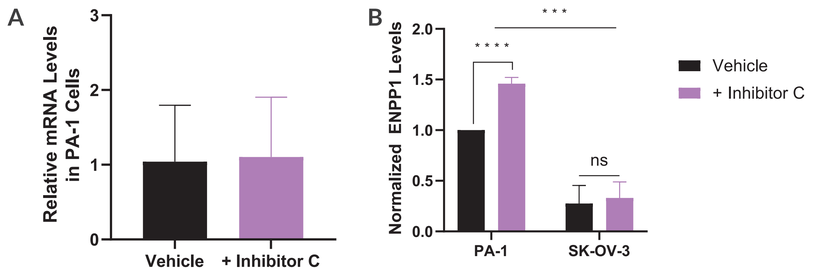

FIGURE 5 – Expression Levels of ENPP1 in Ovarian Cancer Cell lines.
The mRNA levels of *ENPP1* in PA-1 cells were not affected by treatment with ENPP1 Inhibitor C **(A)**. However, protein levels increased after treatment in PA-1 cells **(B)**. Protein levels in SK-OV-3 cells were ~60% lower compared to PA-1 levels. Treatment with Inhibitor C did not affect ENPP1 protein levels in SK-OV-3. N=6, ****p<0.0001, ***p<0.001.

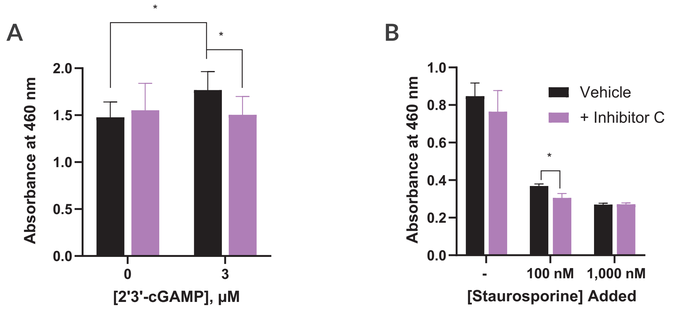

FIGURE 6 – Viability of PA-1 cells.
PA-1 cells were treated with DMSO (Vehicle) or 30 µM ENPP1 Inhibitor C in the presence or absence of spiked 2'3'-cGAMP **(A)**. Cell death was induced in PA-1 cells using staurospaurine in the presence or absence of ENPP1 Inhibitor C **(B)**. Cell proliferation was measured using Cayman's WST-8 Cell Proliferation Assay Kit (Item No. 10010199). N=6, *p<0.05.

CONCLUSIONS

- ENPP1 activity was observed in two of three ovarian cancer cell lines tested: PA-1 and SK-OV-3.
- The extent of ENPP1 activity correlates with the protein expression levels as observed through SDS-PAGE/Western blotting.
- ENPP1 Inhibitor C is a potent inhibitor of recombinant ENPP1. It was also able to reduce ENPP1 activity *in vitro* in PA-1 and SK-OV-3 ovarian cancer cells.
- Addition of 2'3'-cGAMP resulted in a slight increase in viability, but co-treatment with ENPP1 Inhibitor C reduced viability of 2'3'-cGAMP spiked PA-1 cells.
- Supplementation of ENPP1 Inhibitor C resulted in slightly greater cell death upon overnight treatment with 100 nM staurosporine PA-1 cells.
- In future experiments, we plan to test how the addition of ENPP1 Inhibitor C affect primary ovarian cancer cell lines as they typically have not lost the capability to produce 2'3'-cGAMP.

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