

Therapeutic testing of a novel PKC inhibitor GAP-107B8 on ovarian cancer cells

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Introduction

- In 2009, an estimated 23,896 new cases of ovarian cancer will develop in North America, while 15,240 women will have succumbed to the disease (1).
- Current treatments are limited to surgery or chemotherapy, but toxicity is an issue; development of novel cancer therapeutics targeting specific cellular pathways remains important.
- The protein kinase C (PKC) family of serine/threonine kinases is involved in cellular proliferation, differentiation, apoptosis and cell polarity.
- One PKC isoform, PKC ι (i), has recently been identified as a human oncogene and has been shown to be overexpressed in serous epithelial ovarian cancers and is thus a potential therapeutic target for ovarian cancer (2,3).
- Among 11 different solid cancers, PKC ι was most highly expressed in ovarian cancer (3).
- An array-based comparative genomic hybridization on human ovarian cancer specimens showed that PKC ι DNA copy number exhibited a 43.8% gain, the largest of any PKC isoform (4).
- A novel PKC inhibitor has been developed (GAP-107B8 by PharmaGap) that has potent inhibitory actions on several PKC isoforms.
- This project investigates the effects of this inhibitor on several biological functions of ovarian cancer cells.

Objective

- To evaluate the effect of GAP-107B8 on cell proliferation, colony formation, and migration on a panel of ovarian cancer cell lines.

Hypotheses

- GAP-107B8 will lead to decreases in several malignant characteristics of ovarian cancer cells *in vitro*.

Materials and Methods

- Ovarian cancer cell lines:** A2780cp, A2780s, C13, OV2008, ES-2, HEY, OVCA 429, SKOV-3, OVCAR-3
- GAP-107B8:** PKC inhibitor (PharmaGap)
- Invitrogen CyQUANT Cell Proliferation assay kit:** cell proliferation assay in adherent cultures
- Cell Biolabs Cytoselect 96-well cell transformation assay:** cell proliferation assay in anchorage independent cultures
- Cell Biolabs Cytoselect 96-well cell migration kit:** cell motility assay
- Fluorimeter:** Fluoroskan Ascent FL (Thermo Labsystems)
- TUNEL Assay kit:** In Situ Cell Death Detection Kit, Fluorescein (Roche)
- Flow Cytometer:** Beckman Coulter Epics XL Flow Cytometer
- Nine ovarian cancer cell lines were treated with three different concentrations of GAP-107B8 and then screened using high throughput assays to measure the proliferation of cells in adherent and anchorage independent cultures. The ability of cells to migrate in the presence of GAP-107B8 was also determined.
- Cells were treated with GAP-107B8 labeled by dUTP nick end labeling (TUNEL) assay kit and counterstained with 4',6-diamidino-2 phenylindole (DAPI).
- Cells were fixed in ethanol and stained with propidium iodide (PI) prior to being analyzed by flow cytometry.

Results

LC₅₀ of ovarian cancer cell lines treated with carboplatin for 48 hours

Cell Line	Carboplatin LC ₅₀ (μ M)
A2780cp	200
A2780s	40.6
C13	resistant
OV2008	44.1
ES-2	ND
HEY	ND
OVCA 429	301
SKOV-3	ND

Table 1: Two cell lines (A2780cp, C13) are derivatives of A2780s and OV2008 and were generated to become resistant to platinum by continuous exposure. The LC₅₀ for the ES-2 and HEY cell lines has not yet been determined (ND).

Proliferation of ovarian cancer cell lines in adherent cultures in the presence of GAP-107B8

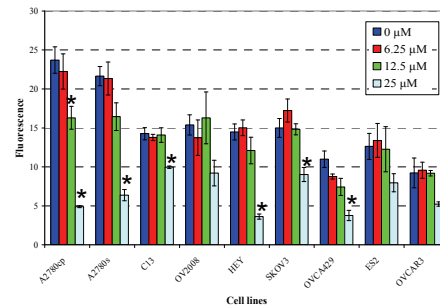


Figure 1: Nine ovarian cancer cell lines were incubated with the indicated concentrations of GAP-107B8 for 48 hours. All data bars represent the means of three experiments performed in triplicate. Error bars show the standard error of the mean (SEM). Bars indicated with an asterisk (*) are treated groups that are significantly different ($p < 0.05$) from their respective untreated control cells. Significant reduction in cell proliferation in adherent conditions ranging from 30% to 79% was observed in six of nine ovarian cancer cell lines, including two cell lines resistant to the standard chemotherapy.

Proliferation of A2780cp, A2780s and HEY cells in soft agar following treatment with GAP-107B8

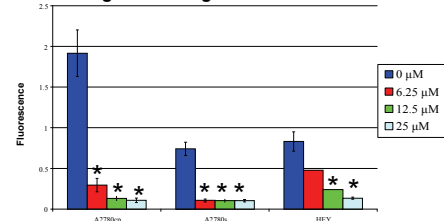


Figure 2: Cell numbers were measured following five (A2780cp and A2780s) or seven (HEY) days of growth in soft agar in the presence of the indicated concentrations of GAP-107B8. All data bars represent the means of three experiments performed in triplicate. Error bars show the standard error of the mean (SEM). Bars indicated with an asterisk (*) are treated groups that are significantly different ($p < 0.05$) from their respective untreated control cells. Significant reduction in cell proliferation in anchorage independent conditions ranging from 50% to 93% was observed in seven of nine ovarian cancer cell lines, including a cell line resistant to the standard chemotherapy.

Proliferation of ovarian cancer cell lines in soft agar following treatment with GAP-107B8

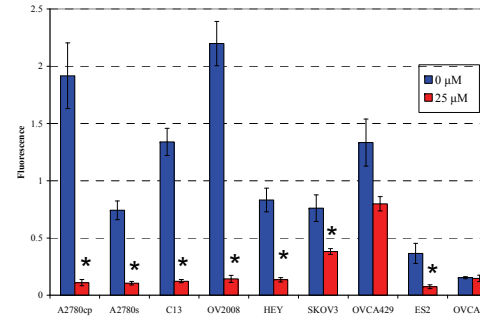


Figure 3: Cell numbers were determined following 5-14 days of growth in soft agar in the presence or absence of 25 μ M GAP-107B8. All data bars represent the means of three experiments performed in triplicate. Error bars show the standard error of the mean (SEM). Bars indicated with an asterisk (*) are treated groups that are significantly different ($p < 0.05$) from their respective untreated control cells. Significant reduction in cell proliferation in anchorage independent conditions ranging from 50% to 93% was observed in seven of nine ovarian cancer cell lines, including two cell lines resistant to the standard chemotherapy.

Motility of ovarian cancer cell lines following treatment with GAP-107B8

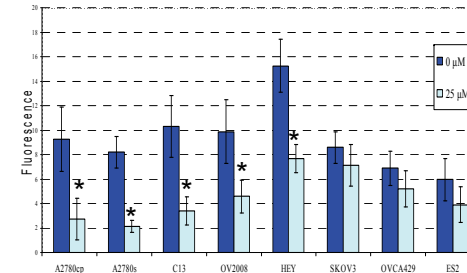


Figure 4: Bars represent the motility of the indicated cell lines treated with 25 μ M GAP-107B8 when compared to untreated cells. All data bars represent the means of three experiments performed in triplicate. Error bars show the standard error of the mean (SEM). Bars indicated with an asterisk (*) are treated groups that are significantly different ($p < 0.05$) from their respective untreated control cells. Significant reduction in motility ranging from 50% to 74% was observed in five of eight ovarian cancer cell lines, including two cell lines resistant to the standard chemotherapy.

Flow cytometry analysis of A2780cp cells treated with GAP-107B8

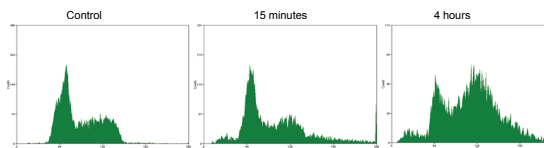


Figure 5: Cells were either untreated (control) or treated with 25 μ M GAP-107B8 and collected at 15 minutes and 4 hours after GAP-107B8 treatment. Treatment with GAP-107B8 appears to increase the proportion of sub-G1 (apoptotic) cells as well as the proportion of cells in G2/M stage of the cell cycle.

TUNEL and DAPI staining of GAP-107B8 treated A2780cp cells

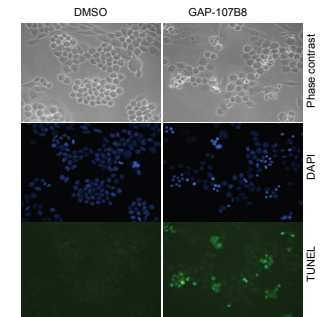


Figure 6: A2780cp cells were collected after 30 hours treatment with DMSO or 25 μ M GAP-107B8. 40X magnification was used. Treatment with GAP-107B8 increased the number of apoptotic cells.

Major oncogenic PKC ι signaling pathways

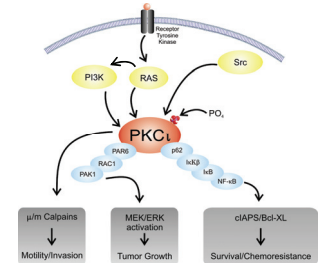


Figure 7: Many components of the PKC ι pathway are mutated in human tumours. Arrows indicate flow through signaling pathways; touching boxes indicate direct binding of signaling components.

Summary

- GAP-107B8 inhibits cell proliferation in adherent growth cultures in 6 of 9 ovarian cancer cell lines.
- GAP-107B8 inhibits cell proliferation in anchorage independent growth cultures in 7 of 9 ovarian cancer cell lines.
- GAP-107B8 inhibits cell motility in 5 of 9 ovarian cancer cell lines.
- GAP-107B8 appears to induce apoptosis in A2780cp cells as determined by flow cytometry and TUNEL staining.
- Results of *in vitro* studies suggest GAP-107B8 has an impact on several biological functions of ovarian cancer cells and thus should undergo further investigation as a potential treatment for ovarian cancer.

References:

- Canadian Cancer Society: www.cancer.ca; American Cancer Society: www.cancer.org
- Regala, RP *et al.* Cancer Research 2005; **65**: 8905-891
- Eder, AM *et al.* PNAS 2005; **102**: 12519-12524
- Zhang, L *et al.* Cancer Research 2006; **66**: 4627-4635