# Chemotherapy induces $PGE_2$ production from cancer cells

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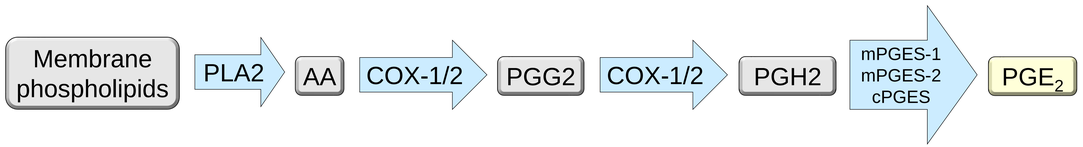
## Abstract

**Introduction**: $PGE_2$ presence in cancer progression appear to support tumor health, leading to increased metastasis and angiogenesis, decreased apoptosis, and pro-tumor immunoregulation [1]. Thus, methods that inhibit $PGE_2$ production have potential to negatively impact cancer growth.
**Objective**: It is hypothesized that chemotherapy treatment induces $PGE_2$ production in cancer cells. This project examines $PGE_2$ levels in mammary (4T1) and Luis Lung Carcinoma (LLC) mouse cancer cells that have been exposed to varying levels of chemotherapy drug cisplatin. Platinum-based chemotherapy drugs are widely used to crosslink DNA, which should induce the most cell death among rapidly proliferating cells, such as cancer [3].
**Methods**: LLC and 4T1 cells were plated with control and cisplatin treated groups. $PGE_2$ ELISA was used to measure $PGE_2$ concentration of supernatant. PTGES genes were knocked down in 4T1 cells using lentiviral shRNA technology.
**Results**: Our results show that cisplatin-treated cancer cells produce significantly higher levels of $PGE_2$ in comparison to vehicle controls, with the peak $PGE_2$ production occurring in LLC cells 48 hours after the 25μM cisplatin treatment. In addition, shPTGES knockdown 4T1 cells exhibit lower PTGES protein levels in western blots and produce significantly less $PGE_2$ when compared to normal 4T1 cells. When these 4T1 cells with PTGES knockdown are treated with cisplatin, $PGE_2$ production increases, albeit less than shScr control cells.
**Conclusions:** Chemotherapy drug cisplatin appears to induce significantly more $PGE_2$ production in 4T1 and LLC cancer cells. Cisplatin induces more $PGE_2$ production through the PTGES genes.

## Background

$PGE_2$ (prostaglandin E2) is a lipid molecule that can be secreted by various cell types in our body. Its G-protein coupled receptors, EP1-4, contain inhibitory and stimulatory subunits, causing downstream signaling cascades. Due to the ubiquitous expression of EP receptors and formation of $PGE_2$ in most organ systems, $PGE_2$ presence regulates a large range of biological effects, such as inflammation [4].
The $PGE_2$ synthesis pathway first begins when membrane phospholipids are cleaved by phospholipase A2 (PLA2), producing arachidonic acid (AA). Cyclooxygenase activity from the COX enzymes then produce intermediate PGG2, which is reduced into alcohol PGH2. From here, a variety of prostaglandins can be synthesized. PGH2 is rapidly converted into $PGE_2$ by three terminal synthases: mPGES-1, mPGES-2, and cPGES. In humans, these enzymes are encoded by the genes PTGES, PTGES2, and PTGES3 respectively.

## $PGE_2$ synthesis pathway




## Results

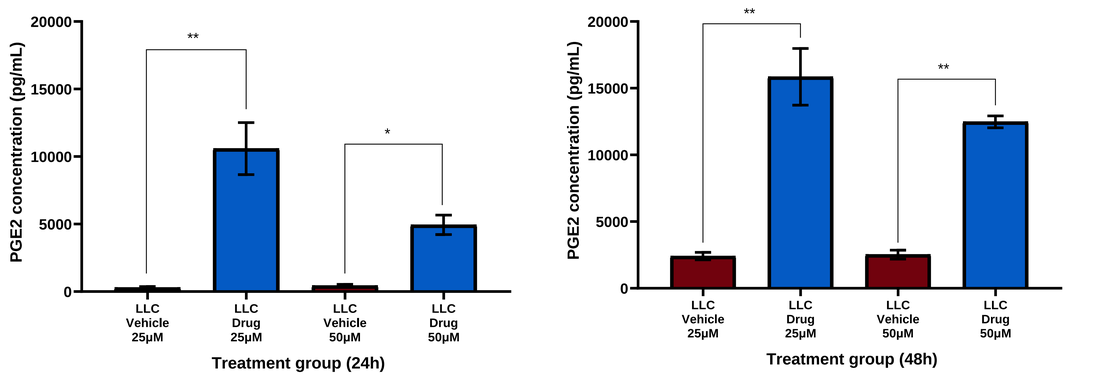



**Figure 1. $PGE_2$ production in cisplatin treated LLC cells in 24h and 48h.** Cisplatin treated cells produced significantly higher $PGE_2$ concentration than the vehicle treated cells in both 24h and 48h.

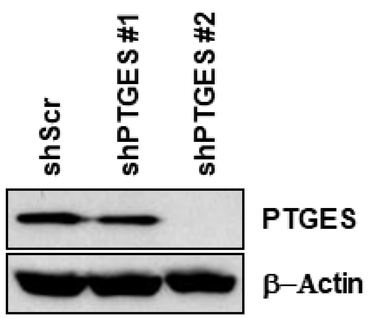



**Figure 2. Western blot of PTGES in ShPTGES and ShScr 4T1 cell lysates.** shPTGES 4T1 cells had significantly lower PTGES expression than the shScr 4T1 cells.

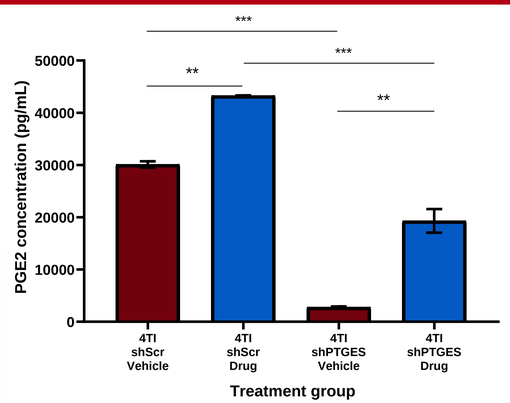



**Figure 3. $PGE_2$ production in cisplatin treated shPTGES and shScr 4T1 cells.** PTGES knockdown cells showed significantly lower $PGE_2$ production than the control cells. As shown in LLC cells, cisplatin treated ShPTGES and shScr 4T1 cells produced more $PGE_2$ than the vehicle treated, even though PTGES genes were knockdown in shPTGES 4T1 cells.

## Methods

**Culture**: LLC and 4T1 cells (shPTGES and shScr) were cultured in complete media (10% FBS, 1% L-glutamine, 1% penicillin/streptomycin in DMEM) and incubated at 37°C in 5% CO2.

**Treatment**: 0.3 million cells/well plated and cultured for 24h. Plated LLC cells treated with 25μM and 50μM cisplatin and 25μM and 50μM vehicle control (NaCl) solution. Supernatant was collected after 24h and 48h and frozen. Cell lysates were collected for western blotting.

**$PGE_2$ ELISA**: Following the manufacturer protocol $PGE_2$ concentration was measured in supernatants.

**PTGES Knockdown:** PTGES genes were knocked down in 4T1 cells using lentiviral shRNA technology.

## Conclusions and clinical implications

- Chemotherapy drug cisplatin appears to induce significantly more $PGE_2$ production in 4T1 and LLC cancer cells and cisplatin induces more $PGE_2$ production through the PTGES genes.
- Further research can aid in designing more efficient chemotherapy drugs

## Future directions

- Use CRISPR Cas-9 to completely knockout all enzymes that directly synthesize $PGE_2$ and observe $PGE_2$ production of these knockout cells in presence of cisplatin
- Determine mechanistic pathways responsible for how cisplatin induces higher $PGE_2$ expression in cancer cells
- Provide stronger evidence to solidify higher $PGE_2$ presence through methods such as:
  - Western blot to determine protein levels of the enzymes that produce $PGE_2$
  - PCR to determine gene expression rates of genes that code for $PGE_2$ producing enzymes
- Extend these research methods to other cell lines or chemotherapy drugs and observe if any distinct shift in $PGE_2$ levels occur
- Determine viable methods of targeting $PGE_2$ receptors (EP1-4) that inhibit its binding with $PGE_2$

## References

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