# NUCLEAR LOCALIZATION OF THE PHOSPHOETHANOLAMINE-CYTIDYLYLTRANSFERASE PROTEIN (PCYT2) IN BREAST CANCER CELLS

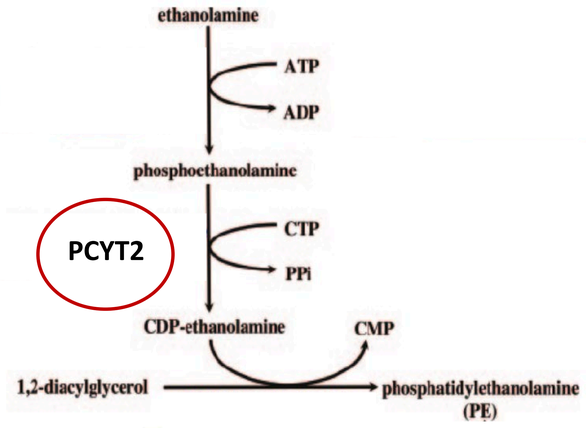
Youstina Laban, Roya Iraji, Marica Bakovic

University of Guelph, Department of Human Health and Nutritional Science

## Abstract

The rapid proliferation of cancer cells demands a continuous supply of cell membranes, highlighting the importance of an understanding of their biosynthetic pathways. The Kennedy Pathway occurs in the cytoplasm and rough endoplasmic reticulum of cells for the production of phospholipids. CTP: phosphoethanolamine cytidylyltransferase-2 (PCYT2) is the rate-limiting enzyme in the CDP-ethanolamine branch of the Kennedy pathway for the production of phosphatidylethanolamine (PE). Recent studies have demonstrated the presence of PCYT2 in the nucleus of monkey myoblast cells, with serum deprivation causing increased cytoplasmic localization. This study aims to examine PCYT2 nuclear localization under serum deprivation in non-invasive, estrogen-dependent **MCF-7** breast cancer cells, and **MDA-MB-231** aggressive, triple negative breast cancer cells and **MCF10-A** mammary non-cancerous epithelial controls cells.

## PCYT2 in The Kennedy Pathway

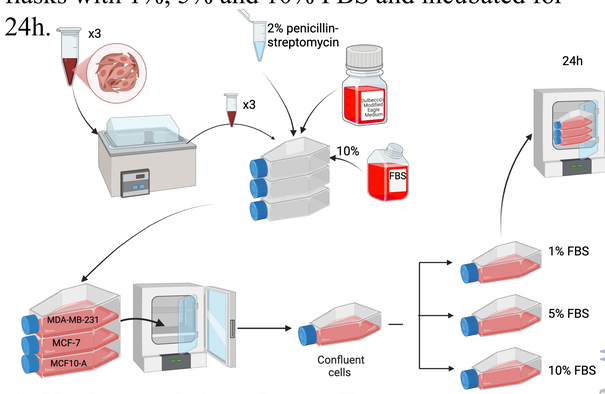



(Gustin *et al*, 2008)

**Fig 1**. The CDP-ethanolamine branch of the Kennedy Pathways for PE biosynthesis. The regulatory step of this pathway is the exchange of the pyrophosphate group of phosphoethanolamine for cytidine diphosphate (CDP) via PCYT2 and cytidine triphosphate (CTP) to produce CDP-ethanolamine.

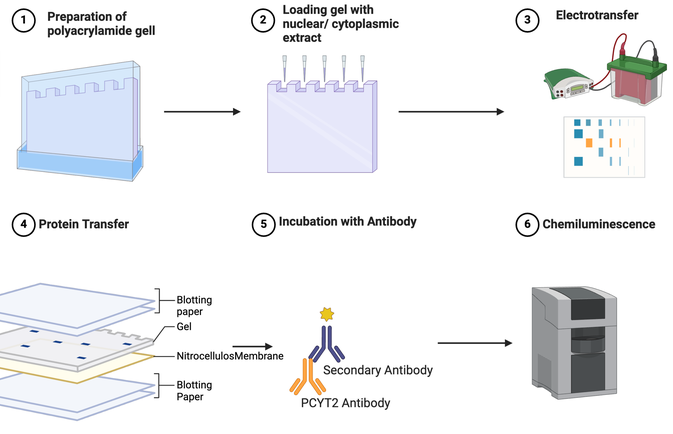
## Methodology

**1. Cell Culture:** Control cells (MCF-10A) and two cancer cell lines were cultured until confluent at 10% fetal bovine serum (FBS), then split into 3 different flasks with 1%, 5% and 10% FBS and incubated for 24h.




**2. Nuclear and Cytoplasmic Extraction:** cell compartments were separated using the NE-PER™ Nuclear and Cytoplasmic Extraction Reagents

**3. Western Blotting:** proteins were separated by weight then transferred onto a nitrocellulose membrane, incubated with a PCYT2total antibody (Ab) and a secondary HRP-linked Ab , then imaged.




## Results

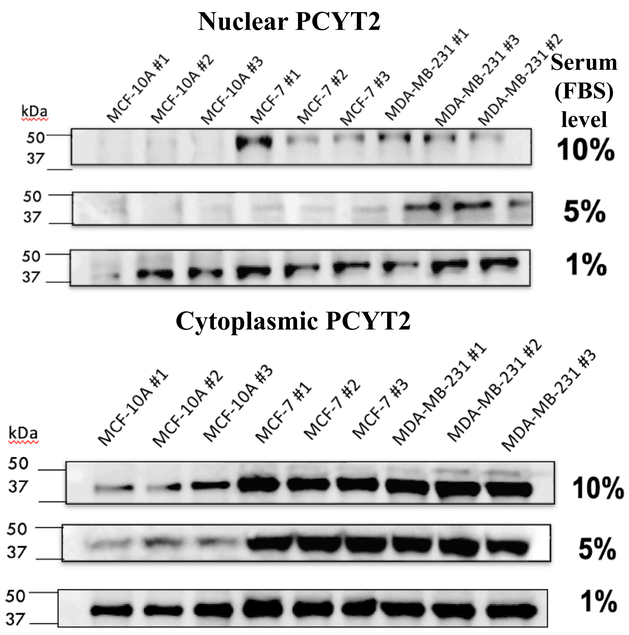



**Fig 2**. Western blotting results of cytoplasmic and nuclear PCYT2 protein.

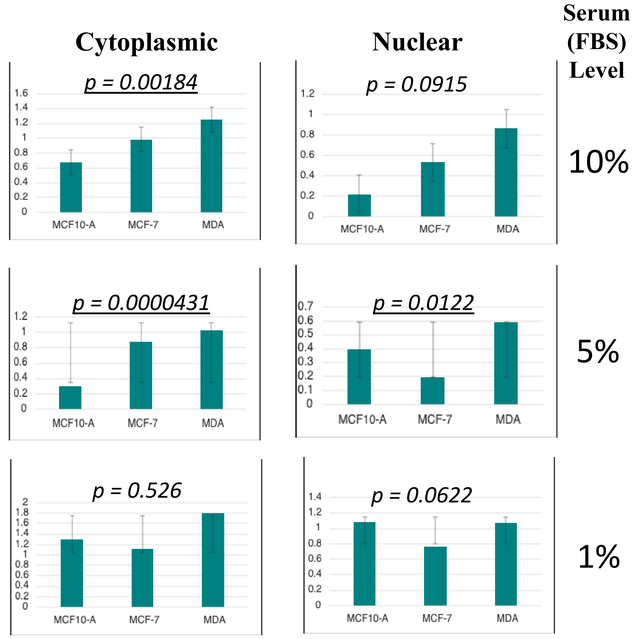


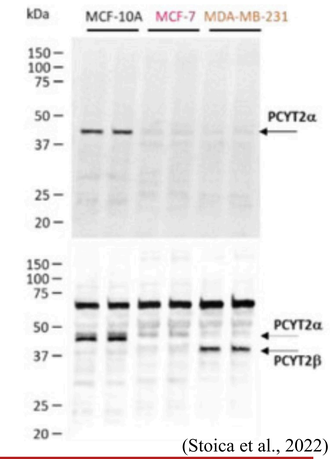

**Fig 3**. Comparison of quantified and standardized PCYT2 protein presence between cell lines at different serum levels ($p<0.05$).

## Conclusions and Discussion

- We found evidence of PCYT2 in the nucleus and cytoplasm of controls, MCF10-A and both cancer cell lines, MCF-7 and MDA-MB-231
- There was statistically significant evidence of different levels of PCYT2 between cell lines at the 10% serum level in both the cytoplasm and nucleus, and at 5% in the cytoplasm. No significant differences were found at the 1% serum level, due to higher protein levels in all cell lines.
- Meclizine is an example of a PCYT2 inhibitor (Gohil et al., 2013). Understanding the microenvironment of PCYT2 in cancer cells can aid in targeting the protein for halting PE production and cell proliferation.

## Splice variants of PCYT2

We take interest in the alpha isoform **(PCYT2α)** which is non-existent in cancer cells, and the beta isoform **(PCYT2β),** which is missing regulatory phosphorylation sites and is dominant in aggressive cancer cells (MDA-MB-231).




(Stoica et al., 2022)

## Next steps in the Bakovic Lab

→ Identification of PCYT2α and PCYT2β in the nucleus of cancer cells in varying serum levels

→ Co-Immunoprecipitation to identify PCYT2-protein interactions with confirmed nuclear proteins in cancer cells and to explore regulatory protein phosphorylation.

→ Confocal microscopy to view PCYT2 in the nucleus of live cancer cells and controls to verify western blot data.

## References

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