# Identification of cyto PELP1-induced pathways that promote breast cancer invasion

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## Abstract

As of 2021, breast cancer is the most common cause of mortality in 20-50 year old women and accounts for 10.4% of malignancy across all cancers.[1] Recurrence and resistance that is more common in cancers that have metastasized is thought to be linked to the potential biomarker proline, glutamic acid, and leucine-rich protein 1 (PELP1), which exhibits elevated expression levels in about 70% of breast cancers.[2] Previous studies found that inhibition of cytoplasmic PELP1 target genes PFKFB3 and PFKFB4 led to decreased tumorsphere formation, even in models that exhibited hormone therapy resistance.[3] Unpublished preliminary research from the Ostrander lab confirmed overexpression of cytoplasmic PELP1 promotes invasion and migration in ER+ breast cancer. To identify which cyto PELP1-induced signaling pathway contributed to breast cancer invasion, the Ostrander lab sent samples off for Reverse Phase Protein Array (RPPA). In the RPPA, 4 different cell lines were analyzed: pCW without doxycycline (doxy), pCW with doxy, pCW cyto PELP1 without doxy, and pCW cyto with doxy. In this cell line model cyto PELP1 is only expressed in the pCW cyto cells in the presence of doxy. The RPPA heatmap data prompted us to probe for Cyclin E, and PFKFB4, neither of which did not exhibit any significant differences in expression across the 4 cell lines. However, GR was found to be downregulated in the pCW cyto with doxy cells. Looking into the raw RPPA data did not yield any significant results either, as $\beta$-catenin was probed for because of its high fold change values. These results validated the RPPA data, however did not highlight any novel cytoplasmic PELP1 induced signaling pathways that potentially promote breast cancer invasion.

## Model

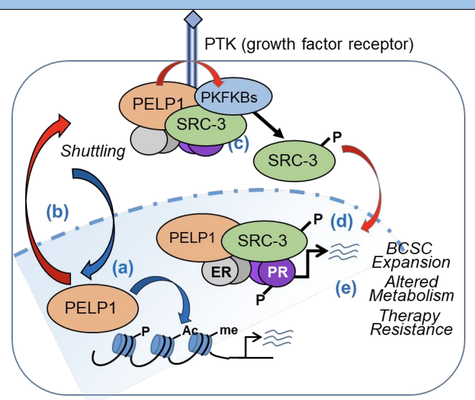


**Figure 1:** Diagram of the transcriptional and signaling complex between PELP1 and SRC-3.

## Hypothesis

**Cytoplasmic PELP1 expression induces signal transduction pathways that promote breast cancer invasion and metastasis.**

## Methods

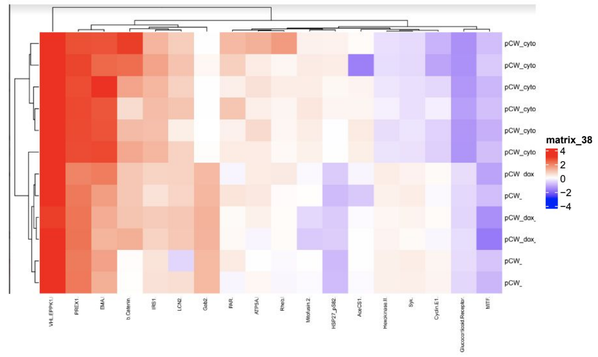


**Figure 2:** RPPA heatmap highlighting protein expression across 4 cell lines

### RPPA Analysis

The RPPA data was received in the form of a heatmap (Figure 2). After validating the heatmap by Western blotting with Cyclin E, PFKFB4, and GR (Figure 3), the raw data was investigated. This was done by calculating the p-values for all antibodies tested and fold change values. Analysis of the raw data led us to probe for b-Catenin.

### Western Blot

The procedure shown in Figure 3 was performed with GAPDH as a loading control. PELP1 was also probed for as another control.

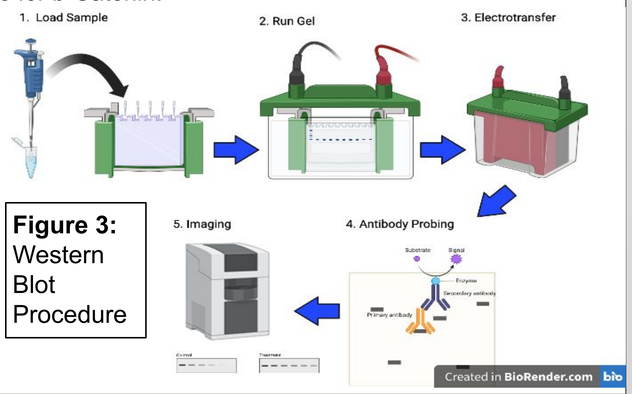


**Figure 3:** Western Blot Procedure

## Results

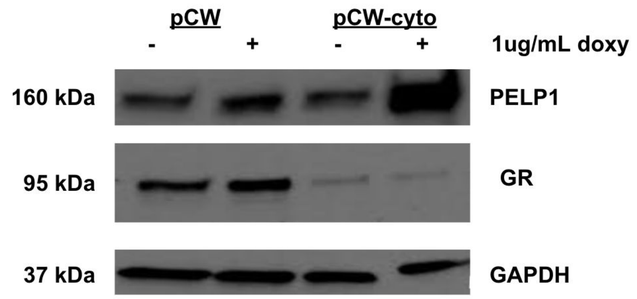


**Figure 4:** Western Blot showing expression of GR and PELP1 in T47D-CO cells

## Results

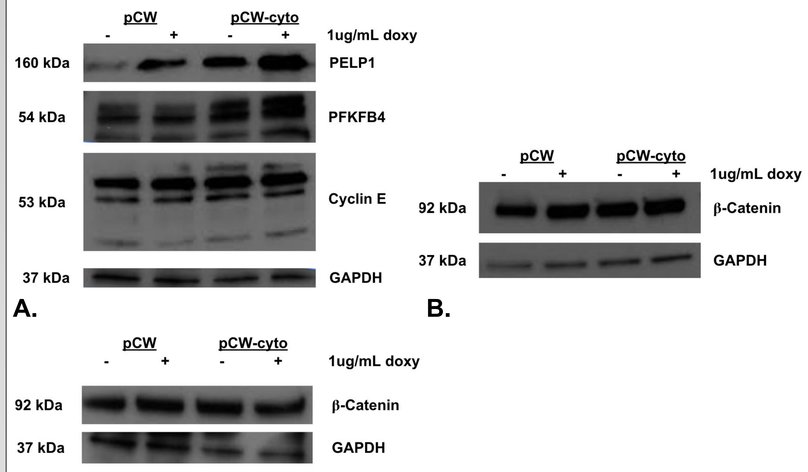


**Figure 5:** Western Blots showing expression of **(A)** PFKFB4, Cyclin E, and PELP1 **(B)** $\beta$-catenin in T47D-CO cells and **(C)** $\beta$-catenin in T47D-CO cells in MD Anderson lysis buffer

## Conclusion and Future Directions

- The results were inconclusive in regards to finding novel signaling pathways that promote breast cancer invasion induced by cytoplasmic PELP1.
- GR was downregulated in the pCW cyto with doxy cells.
- Future studies could involve examining the effect of PFKFB3 and PFKFB4 inhibition on cyto PELP1-induced breast cancer migration, as previous research has found a correlation between PELP1 and PFKFB3 and PFKFB4 gene knockdown in decreasing tumorsphere formation.
- Future experiments involving an RPPA could have cells in serum starvation conditions, as for this RPPA the cells sent in were in regular growth media. This could potentially allow for more significant results.

## References

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2. Wang, X., Tsang, J., Lee, M. A., Ni, Y. B., Tong, J. H., Chan, S. K., Cheung, S. Y., To, K. F., & Tse, G. M. (2019). The Clinical Value of PELP1 for Breast Cancer: A Comparison with Multiple Cancers and Analysis in Breast Cancer Subtypes. *Cancer research and treatment, 51*(2), 706–717. https://doi.org/10.4143/crt.2018.316.
3. Truong, T.H., Benner, E.A., Hagen, K.M., Temiz, N.A., Kerkvliet, C.P., Wang, Y., Cortes-Sanchez, E., Yang, C., Trousdell, M.C., Pengo, T., Guillen K.P., Welm, B.E., Dos Santos, C.O., Telang, S., Lange, C.A., Ostrander, J.H. (2021). PELP1/SRC-3-dependent regulation of metabolic PFKFB kinases drives therapy resistant ER+ breast cancer. *Oncogene(40)*, 4384-4397. https://doi.org/10.1038/s41388-021-01871-w.