The Effect of Calcium Signaling on Cell-Cell Fusion

By Saloni Somia

All photos courtesy of researcher

Research Question

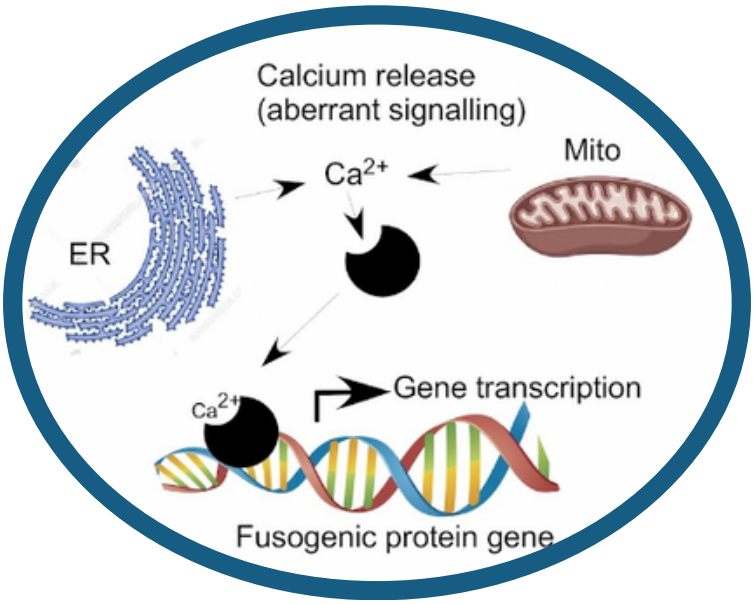
Background: This project was preceded by observations that cancer cells can fuse and create new cells with an increased number of chromosomes. These cells which are polyploid can go on to make aggressive metastasis (Lobo, 2008).

Most normal cells don't fuse (with a few exceptions), and repel each other due to their charges. However, cancer cells have deregulated many processes in the cell. Past research reported that calcium signaling was used to direct intracellular organelle fusion (Burgoyne, 2003). So I wondered if calcium signaling might be involved in extracellular cell-cell fusion. This led me to my research question.

Purpose: The mechanisms involved in cell-cell fusion in cancer are not well understood (Sieler et al. , 2021). This study aims to look at this knowledge gap in the field of cancer. Knowing the signal that initiates cell-cell fusion in cancer cells could help enhance current cancer treatments. The purpose of this study is to investigate if calcium signaling can increase cell-cell fusion in cancer cells.

Hypothesis: The proposed hypothesis is that an increase in calcium signaling in cancer cells will lead to the expression or activation of proteins that lead to cell-cell fusion.

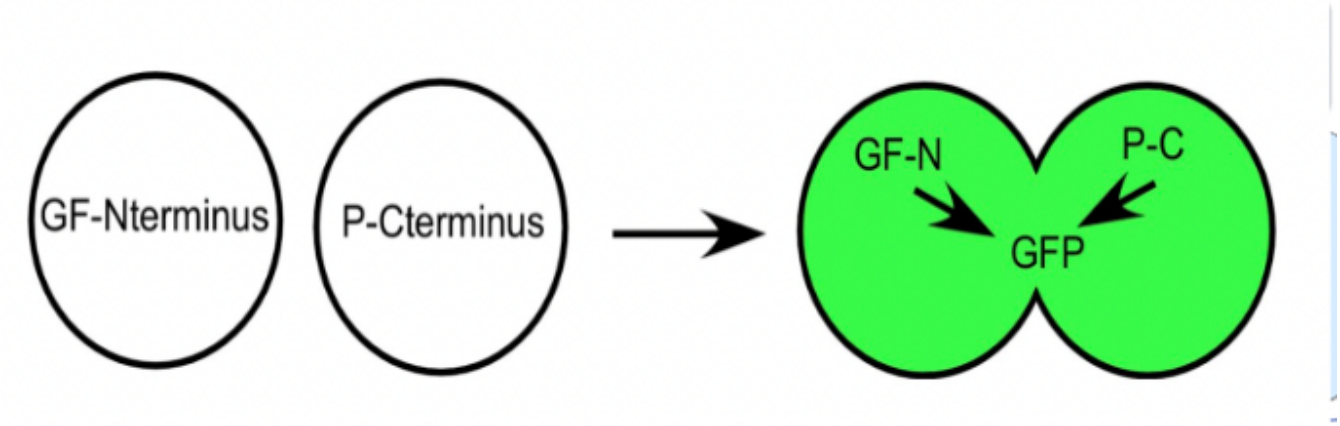
Methodology



My proposed model is that calcium is aberrantly released in the cancer cells from stores in the endoplasmic reticulum or the mitochondria and can result in a signal that affects transcription of genes, and production of proteins that cause cell-cell fusion.

To test this model, I used a human embryonic kidney cancer cell line (HEK-293) and green fluorescent, GFP.

The GFP molecule is split into two portions: the N-terminal and C-terminal. The rationale for the assay is if cells containing either the N-terminal or the C-terminal half of the GFP are mixed then if the cells fuse the GFP protein will be recombined and the cells will fluoresce green. This also helps with quantifying the fusion.



Methodology Part 2

PEG assay (positive control)

- A 50% Poly ethylene glycol (PEG) 1500 solution, a known fusogen, was added to the mixed cells.
- This assay showed that the cells can fuse properly.

Calcium Toxicity Assay

- Calcium can be toxic to cells at high concentrations
- Cells with calcium above 12.5 mM range looked abnormal.
- Experiments were done at concentrations well below 12.5 mM.

Calcium + Fusion Experiment

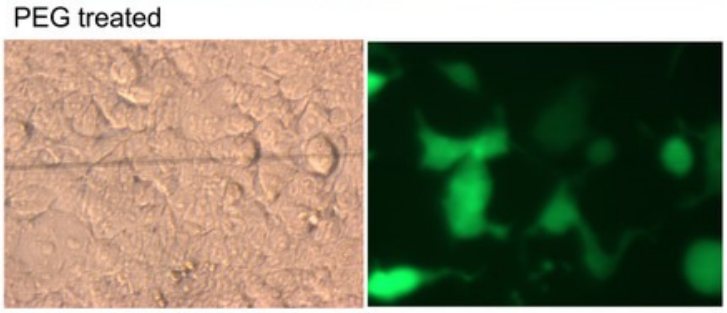
- A million cells were distributed into each well of a 12 well plate, and exposed to different concentrations of a calcium precipitate.
- The cells were observed under a fluorescent microscope, and fluorescence was measured in a fluorometric plate reader.

Negative Controls

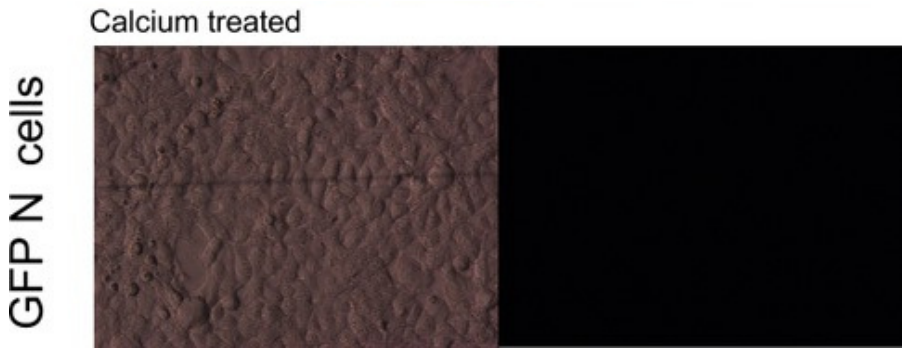
- To test to see if the cells would fluoresce with a chemical similar to calcium, I added a precipitate of magnesium chloride to the mixed cells
- I added calcium to the N terminal and C terminals independently to control for fluorescence occurring without fusion

Results

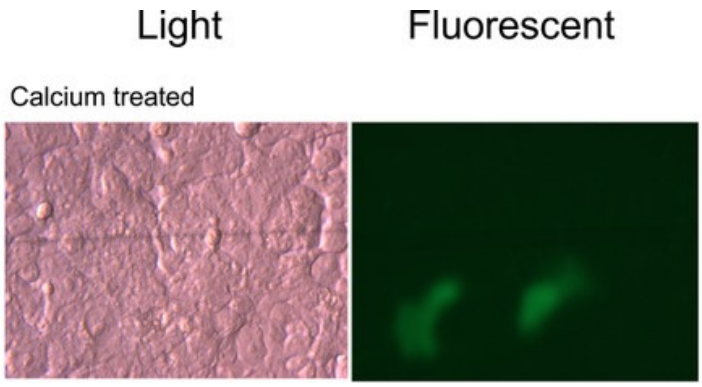
In the positive control PEG treated wells I observed fluorescence meaning that the cells could fuse properly.



In my negative controls with the N-terminal or the C-terminal cells alone, no green cells were observed when treated with 0.1 mM calcium which was the highest concentration added to the mixed cells. In addition, no fluorescence was observed when 0.1 mM of magnesium was added to the mixed cells.



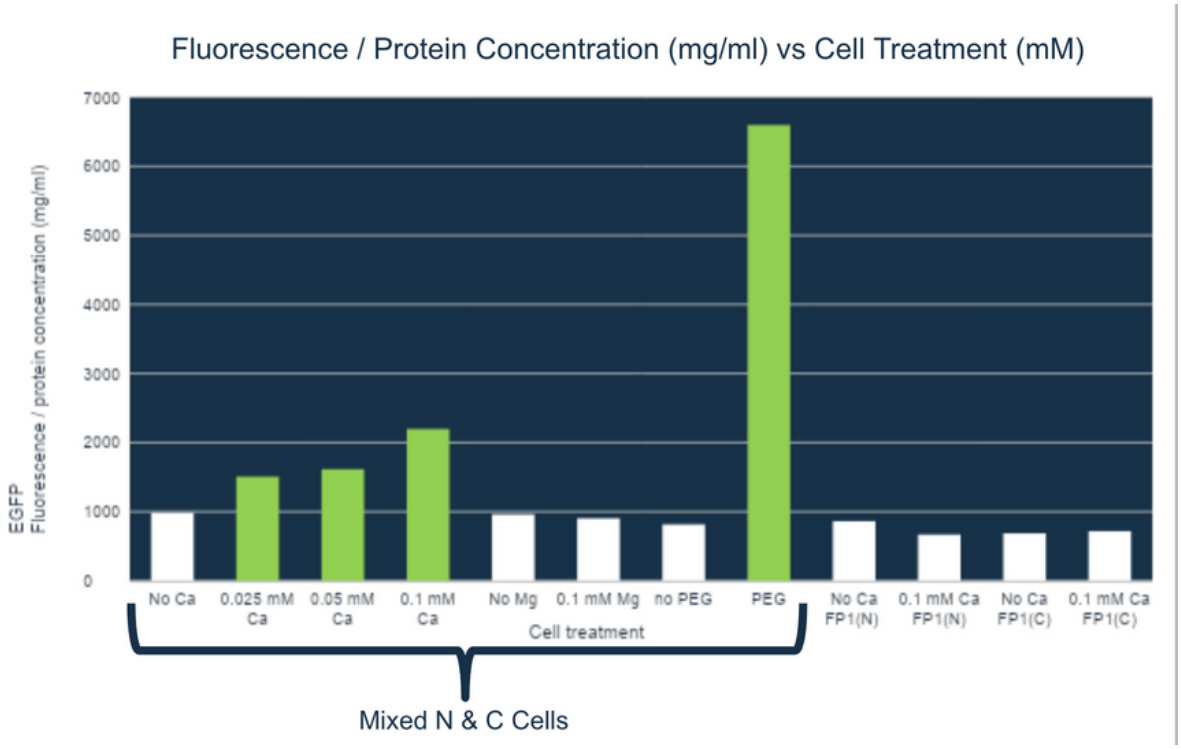
Green cells were observed for the N and C mixed cell population, in wells where the calcium precipitated was added.



The results show that fusion required both the N and C terminal cells as well as calcium.

Conclusion & Impact

The graph represents the average of three wells for each treatment. There was an increase in fluorescence with increased calcium concentration.



In conclusion my experiments here support my hypothesis that “Calcium signaling induces cells to fuse”. It can be seen through the graph that increasing intracellular calcium concentrations in HEK cells induces fusion of cells. The work implicates calcium signaling to cancers becoming polyploid and metastatic, and suggests that inhibiting calcium signaling early in the treatment of cancer may hinder the emergence of metastasis.

The main questions that I want to address next are:

1) Are calcium stores in the cell enough to cause fusion?
2) Is this true for other cancer cell lines?
3) which protein does the calcium trigger
4) does inhibiting calcium signaling inhibit fusion