Investigating the Role of Ca^{2+} and the Acto-myosin Mechanism on the Human MCF-7 Breast Cancer Cell Line

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ABSTRACT

Cancer cells have shown to exhibit enhanced potential to metastasize via increased actin-myosin crossbridge formation, which requires increased Ca^{2+} influx via the voltage-gated calcium channels.
This then leads to the activation of myosin light chain kinase (MLCK). MLCK then phosphorylates myosin light chain and forms the actin-myosin cross bridges

Additionally, the PI3-Kinase/Akt pathway is critical for cell survival and division, which works from the activation of Akt through phosphorylation of PI,4,5BP by PI-3 kinase.

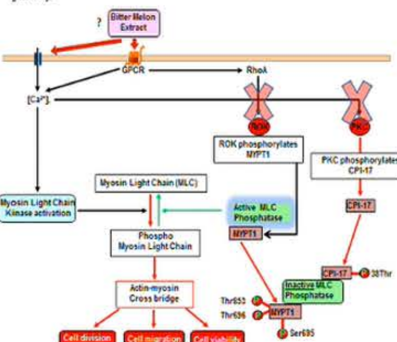
These two critical cell survival pathways have been extensively studied in the field of breast cancer cell research and my research is primarily focused on investigating the role of VGCC, MLCK and PI-3 kinase on MCF-7 cell viability by using pharmacological inhibitors to assess significant changes in morphology and cell survival via microscopy and MTT assays.

AIMS & HYPOTHESES

- Investigate the role of Ca^{2+}-dependent pathway leading to acto-myosin cross-bridge formation in MCF-7 cells
  - The inhibition of the Ca^{2+}-dependent pathway will decrease viability of MCF-7 cells due to the prevention of actin-myosin crossbridge formation.
- Investigate thr role of PI-3 kinase pathway (cell survival pathway) in MCF-7 cells
  - The inhibition of PI-3 kinase pathway will decrease viability of MCF-7 cells due to the inhibition of Akt activation necessary for cell survival and division

PREVIOUS RESEARCH OBTAINED

Prior to investigating the Ca^{2+}-dependent pathway, we investigated the Ca^{2+}-independent pathway and didn't find a significant effect when assessing the role of ROK and PKC on MCF-7 cell viability.
Our focus now is to investigate the Ca^{2+}-dependent pathway alongside the PI-3 kinase pathway.

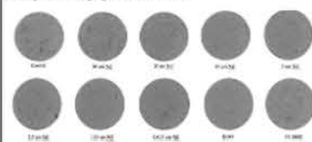


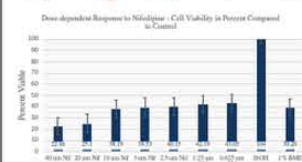
METHODS

MCF-7 cells were cultured on 96 well plates with 100 ul of media (comprised of DMEM, fetal bovine serum, and antibiotic/antimycotic) in each well and were cultured for 24 hours to allow the cells to adhere to the plate. Solutions of various concentrations of the drugs were prepared and administered to the wells accordingly, with 1% BME (bitter melon extract) being the positive control and EtOH being the negative control. Cells were then allowed to culture for 48-72 hours. Cell viability was assessed via MTT assay and morphology via microscopy and compared to the three controls.

NIFEDIPINE RESULTS

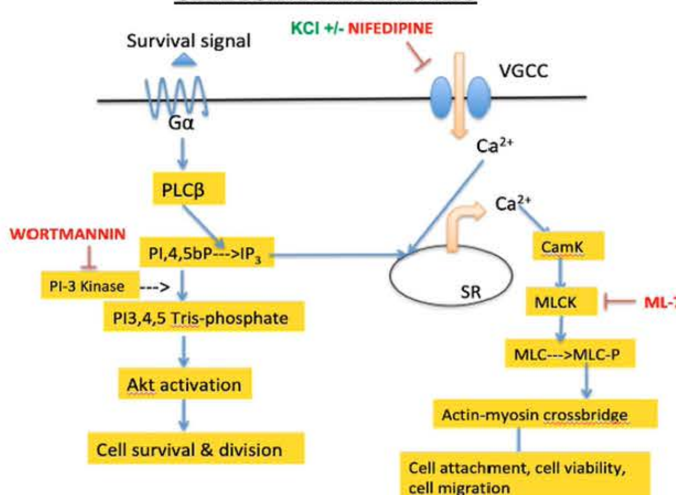
Nifedipine – Voltage-gated Ca^{2+} blocker





Nifedipine is a VGCC inhibitor, and as the concentrations increase, the cell viability decreases. The dose-dependent response with the most significant effect was at 40 um.

CELL SURVIVAL PATHWAYS



TARGETED HYPOTHESES OF PHARMACOLOGICAL AGENTS

Nifedipine is a VGCC inhibitor.

Application of Nifedipine with varying doses will decrease cell viability because calcium is necessary for the formation of the actin-myosin crossbridge and closing the channels deprives the cells of the necessary calcium for cell attachment, viability, and migration.

ML-7 is an MLCK inhibitor

Application of ML-7 with varying doses will decrease cell viability through the inhibition of MLCK preventing the phosphorylation of myosin light chain necessary for the actin-myosin crossbridge.

KCl is an indirect VGCC stimulator

Application of varying doses of KCl, an indirect VGCC stimulator will allow for the channels to open and simultaneous administration of Nifedipine will result in a significant reduction of KCl's effect.

Wortmannin is a PI-3 Kinase inhibitor

Application of Wortmannin with varying doses will decrease cell viability of the MCF-7 cells through the inhibition of PI-3 Kinase and restriction of Akt activation necessary for cell survival and division.

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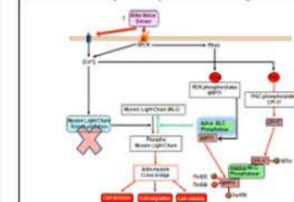
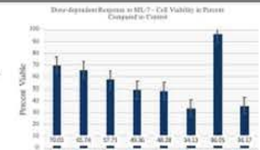
ML-7 RESULTS

ML-7 – Myosin Light Chain Kinase Inhibitor

ML-7 is an MLCK inhibitor, and as the concentrations increase, the cell viability decreases. The dose-dependent response with the most significant effect was at 16 um.

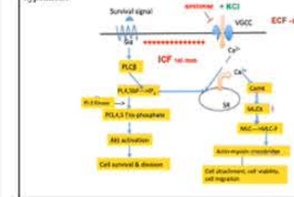


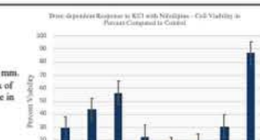


NIFEDIPINE + KCl RESULTS

Nifedipine – VGCC Blocker
KCl – Indirect VGCC Stimulator

KCl exists naturally in the extracellular fluid around 5.5 mm and intracellularly at 140 mm. Increasing the amount of KCl extracellularly induces the VGCC to open with an influx of Ca^{2+}. Simultaneous administration with Nifedipine should ensure a significant decrease in the effect of the calcium.
Results seen in the MTT assay and morphology assessment is compatible with the hypothesis.





FUTURE AIMS

Analyze the KCl with Nifedipine experiment again with smaller doses of KCl compatible with increased cell proliferation

Analyze the effects of Wortmannin – a PI-3 Kinase inhibitor on MCF-7 cells

Transfection of MCF-7 cells with siRNA for VGCC knockout for further verification

Proceed with PCR and Western blotting