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Potential Integrin Switch in NIH/3T3 Cells in Response to High Concentrations of Ascorbic Acid

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Abstract

NIH/3T3 (ATCC ® CRL-1658) is an adherent, fibroblastic cell line used as a model in *in vitro*experiments due to the high survivability and short transition through the cell cycle. A high concentration of Vitamin C (ascorbic acid) has been shown to increase collagen production, which is essential for extra cellular matrix formation and skin integrity. In other cell lines, researchers have seen that upregulation of an integrin protein results in the down regulation of another, also known as an integrin switch. NIH/3T3 cells treated with ascorbic acid may lead to an integrin switch, potentially upregulating Itgb1, a gene that regulates collagen processing, while downregulating other integrin genes involved in cell differentiation and proliferation. This research will investigate this possible integrin switch and how it is associated with changes in collagen production and the cell cycle. High concentration of ascorbic acid is becoming a common cancer treatment for some individuals, and NIH/3T3 cells may serve as a model to gain a better understanding of how integrin expression on cancer-associated fibroblasts may play a role in cancers such as leukemias and sarcomas.

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High Concentration Ascorbic Acid May Adversely Affect Breast Cancer Metastasis and Disease Progression

INTRODUCTION

NIH/3T3 (ATCC® CRL-1658) cells are adherent embryonic mouse fibroblast cells, used as a model in *in vitro* experiments due to their high survivability and short transition through the cell cycle.

Integrins are heterodimeric transmembrane proteins found in many cell types, including NIH/3T3 cells, and are involved in extracellular matrix adhesion and cell to cell signaling. They are composed of alpha (α) and beta (β) subunits that can form 24 different heterodimer combinations, each being characterized by their individual ligand binding properties. For example, $\alpha 2\beta 1$ binds to collagen, $\alpha 3\beta 1$ binds to laminin, and $\alpha 4\beta 1$ binds to fibronectin. In other cell lines, researchers have seen that upregulation of an integrin protein results in the down regulation of another, also known as an integrin switch. NIH/3T3 cells treated with ascorbic acid may lead to an integrin switch in genes involved in cell differentiation and proliferation. In this experiment, we explored the effects of high ascorbic acid treatment on NIH/3T3 cells, and the possibility of a resulting integrin switch.

High concentration of ascorbic acid is becoming a common cancer treatment for some individuals, and NIH/3T3 cells may serve as a model to gain a better understanding of how integrin expression on cancer-associated fibroblasts may play a role in deleterious cancers, such as breast cancer. Still, the mechanisms behind this treatment remain unclear. Additionally, contradictory evidence shows that high concentration ascorbic acid may lead to an increased expression of laminins bound to integrins, which has been found in patients with malignant breast cancer. This research will further elucidate the cellular response to high concentration ascorbic acid by exploring integrin switches, as well as possible changes in laminin expression.

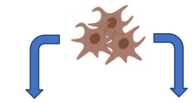
HYPOTHESIS

NIH/3T3 cells treated with high ascorbic acid concentration may cause an integrin switch, seen by an upregulation of certain integrins, and a downregulation of others.

METHODS

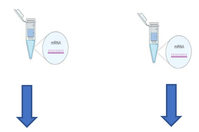
1. Culture cells

Control | **Experimental** 100 mg/mL Ascorbic Acid

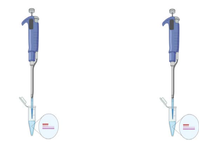


2. Divide and apply treatment

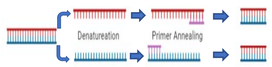
3. RNA Purification



4. Add nucleotides to build cDNA



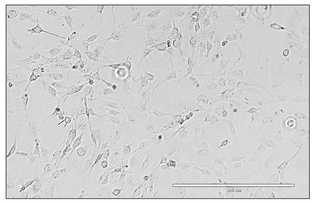
5. Polymerase Chain Reaction (PCR)
Applied to each treatment group



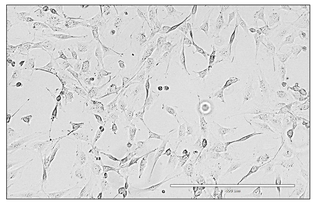
6. Extracellular Matrix Assay

7. Data Analysis

IMAGES



IMG 1: NIH/3T3 adherent control group under EVOS imaging (48 hours)



IMG 2: NIH/3T3 adherent experimental group under EVOS imaging
- Treated with 100 mg/mL Ascorbic Acid (48 hours)

RESULTS

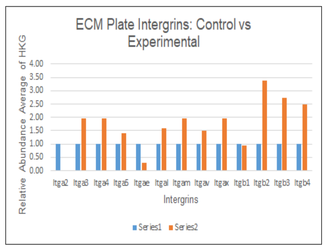


Fig 1: All integrins from ECM plate. This graph compares the control group to the experimental group with normalized results. Itga2 was 0.01 which is why there is no bar showing. There were two integrin genes that significantly downregulated: Itga2 and Itgae. The following genes were significantly upregulated: Itga3, Itga4, Itgam, Itgax, Itgb2, Itgb3 and Itgb4. The rest of the genes had little to no change. For the y axis: "Relative abundance $2^{(-\Delta CD)*}$. For the title, you don't need to include "ECM Plate". Maybe just: Integrin genes: Control vs Experimental".

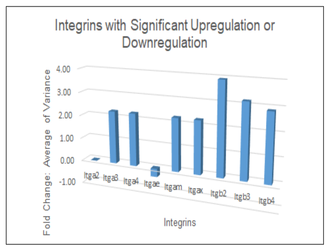


Fig 2: Genes that showed upregulation or downregulation with fold change normalized for control. Upregulated genes : Itga3, Itga4, Itgam, Itgax, Itgb2, Itgb3 and Itgb4. Downregulated genes: Itga2 (-0.01) and Itgae.

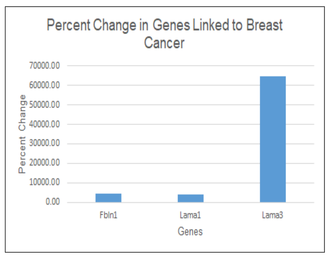


Fig 3: Basement membrane proteins linked to Breast Cancer. Fibulin and Laminin are upregulated by treatment of high concentration of ascorbic acid.

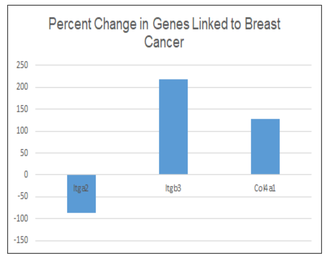


Fig 4: Collagen is part of the basement membrane and is linked to Breast Cancer. Integrin alpha 2 and integrin beta 3 showing an integrin switch in high concentration of ascorbic acid treatments

DISCUSSION

Ascorbic acid is a common cancer treatment that has been utilized since the early 70's. Our study intended to analyze the efficiency of this treatment in breast cancer. What we found after treating NIH/3T3 cells was that there was an integrin switch which has been identified as a vital player in metastatic diseases such as breast cancer. Additionally we analyze our data to identify other ECM genes that may support our finding that ascorbic acid may be a detrimental treatment option for breast cancer.

Extracellular Proteins that were Regulated in the Presence of Ascorbic Acid

Protein	Percent change	Past Research Findings
Integrins $\alpha 2$ $\beta 3$	99% ↓ regulation 293% ↑ regulation	Integrin switches have shown to play a role in metastasis and large upregulation has been shown to advance the disease progression leading to higher morbidity rates
Laminin $\alpha 1$ $\alpha 3$	4167% ↑ 64931% ↑	Expression enhances significantly in breast cancer and interactions with MMP-2 degrade the ECM and BM
Collagen IV	127% ↑	Considered a marker for angiogenic activity
Fibulin-1	4379% ↑	Identified as an immunogenic breast cancer indicator protein

FUTURE DIRECTION

- Repeat experiment in triplet
- Further study the relationship between ITGA2, ITGB3, and Laminin
- Continue research on ascorbic acid treatments and how they affect ITGA2, ITGB3, Laminin, and breast cancer metastasis

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