Testing the viability of chemotherapy and vitamin E on triple negative breast cancer stem cells

Tommy Han, Dr. Wang, Andrew Sulaiman
Department of Biochemistry, Microbiology and Immunology

Introduction

What is Triple Negative Breast Cancer stem cells

Triple negative breast cancer (TNBC) is considered one of the most aggressive and rare forms of breast cancer subtypes. TNBC stem cells, that this study will focus on, are a population of cells that can initiate and maintain tumor growth and is believed to be the main cause of cancer reoccurrence after undergoing treatment.

Characteristics of TNBC

This cancer is uniquely characterized by its lack of estrogen receptors, progesterone receptors and human epidermal growth factor receptor 2. These receptors are normally the root sources for other forms of breast cancer.

Current target therapy and hormonal therapy treatments, that specifically target these receptors, are ineffective on TNBC. The growing concern is for the lack of effective treatments available for TNBC patients.

Objective

Micronutrients are believed to be useful in breast cancer treatment but this claim is currently inconclusive. Thus, the objective of this study is to:

1) Determine if a combination of Vitamin E and chemotherapy drugs will have a greater effect on reducing triple negative breast cancer stem cell growth than chemotherapy drugs alone.

Testing chemotherapy drugs with micronutrients will be important towards developing better systematic treatments.

Results

The combination of Paclitaxel and vitamin E have roughly the lowest absorbance's in both MTT and Luciferase Assays

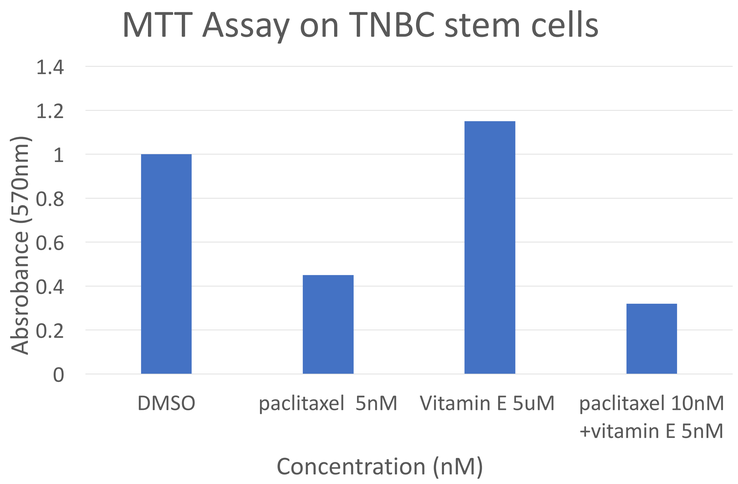

Figure 3: MTT assay of TNBC stem cells in 4 different conditions with DMSO as a control. MTT reagent was thawed and 40uL of MTT was put into a 24 well plate that contained the CSCs and the different reagents. The plate was incubated for 3 hours. 100uL from each well were taken and put into 96U standard R wells. A spectrophotometer measured absorbance's at 570nm.

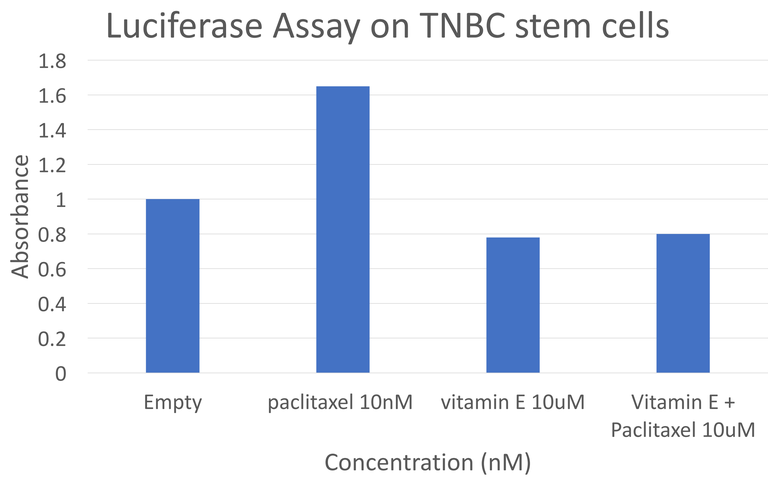

Figure 4: Luciferase Assay of TNBC cells is performed in 4 different conditions with "empty" as a control. 100uL of Dual-Glo Reagent was a added to a 96 well plate that contained the CSCs and the different reagents. After 10min and measure the fire firefly luminescence in a luminometer. 100uL of Dual-Glo Stop and Glo reagent are added to the 96 well. After 10min Renailla luminescence is measured. A spectrophotometer measured absorbance.

Methodology

Cell Culture + Addition of Chemotherapy drugs and Vitamin E

MDA cell lines, which is a epithelia human breast cancer cell line, are used in this study. The cancer stem cells (CSCs) are cultured and treated with paclitaxel, Vitamin E, paclitaxel with Vitamin E and DMSO as a control.

MTT Assay

The MTT assay analyzes the cell viability of the CSCs. If the cancer cell is growing then the MTT will metabolize into the mitochondria, turning the cell purple, as demonstrated by figure 1.

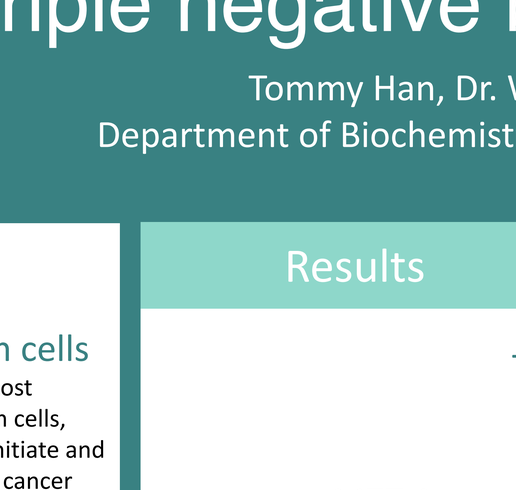

Figure 1: MTT Assay

Luciferase Assay

A Luciferase assay is performed to measure the expression of yes-associated proteins (YAP). A luciferase assay measures the light output from reporter gene (luciferase) to analyze the promotor activity; this is demonstrated by figure 2.

Measurements

The absorbance's from each assay were measured at a wavelength of 570nm with a spectrophotometer.

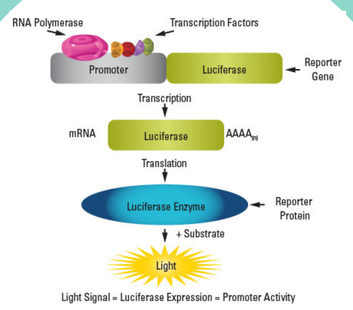

Figure 2: Luciferase Assay

Discussion

MTT and Luciferase Assay Results

Results from the MTT and Luciferase assay, relative to the control, indicate that:

- A combination of paclitaxel and vitamin E were significantly able to reduce the proliferation of TNBC cells more so than paclitaxel alone.
- Vitamin E is incapable of reducing the growth of the CSCs independently, but capable of reducing the YAP expression in TNBC cells independently
- Paclitaxel is capable of reducing both the proliferation and Yap expression of TNBC stem cells independently

Data Analysis

Vitamin E is able to enhance paclitaxel's chemotherapeutic effect because it can reduce paclitaxel induced YAP for viability.

- YAP regulates the Hippo pathways that promotes cell proliferation and organ growth. YAP activity is controlled by the SREBP/mevalonate pathway.
- In TNBC an oncogenic factor (ex. mutant p53) can cause the SREBP pathway to increase production of mevalonic acid; this triggers the over expression of YAP leading to increased cell proliferation and tumor growth.
- Vitamin E can inhibit the enzyme HMG-Coa which regulates the SREBP pathway and in turn decreases YAP activity. Through inhibiting YAP, CSC proliferation decreases.

When vitamin E's ability to inhibit YAP activity is combined with paclitaxel, it significantly decreases CSC proliferation.

Conclusion

The results suggest that vitamin E is capable of enhancing the chemotherapeutic effects of paclitaxel and reduces CSC proliferation to a greater extent than paclitaxel alone.

Acknowledgements

Thank you to Dr. Lisheng Wang, Andrew Sulaiman and the rest of the Wang lab for their continuous support throughout this project.

References

1. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. Cancer Biol Med. 2015;12:106–116.

2. Co SOrrentino G, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S, Manfrin A, Ingallina E, Sommaggio R, Piazza S, Rosato A, Piccolo S, Del Sal G. Metabolic control of YAP and TAZ by the mevalonate pathway. Nat Cell Biol. 2014;16:357–366. doi: 10.1038/ncb2936.