Effects of Ceramide Concentration on Yeast Cell Proliferation

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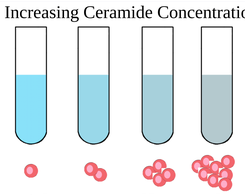
Problem

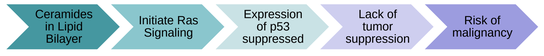


Does ceramide concentration contribute to malignancy?

Ceramides are cell membrane lipids that regulate the cell-cycle, and they are found in common skin-care products, chemotherapeutic agents, and in illegal recreational drugs. Ceramides contribute to a variety of processes in cell-cycle regulation, which is why their overall effect on cell proliferation has not yet been determined. Due to the fact that the contribution of ceramide concentration to malignancy has not yet been researched, ceramides can not be used in the process of developing cancer therapy.

Background

Ceramides are lipids that initiate an increase in Ras signaling, or signaling by a protein that initiates suppression of the p53 gene. The p53 gene, a tumor-suppressor, initiates a wide range of regulation pathways in order to prevent malignancy, or unregulated cell proliferation. Ceramides, which initiate Ras signaling, consequently increase levels of the dmtf1 protein, a phenomenon which has been shown to increase cell-proliferation levels in primary cells(Inoue, Mallakin, and Frazier, 2007).



Ceramides have been shown to contribute to the initiation of apoptosis, cell death, and excessive proliferation, all of which are contradictory functions of the regulation of malignancy while their overall effect on cell proliferation has not been determined. It follows logically that ceramides can initiate a pathway of signaling that can cause a decrease in the regulation of cell proliferation, leading to oncogenic stress in eukaryotes.

Hypothesis

To determine the effect of ceramides on cell proliferation in yeast cells (*Saccharomyces cerevisiae*), differing concentrations of dissolved ceramide were inserted into solid and liquid yeast cultures. The following experiments test the following hypothesis:

If the ceramide content in a yeast cell's external environment is increased, then proliferation in the culture will increase.

Methods & Materials

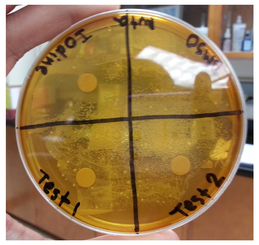
- YPD Agar Plates
- Highly Active Baker's Yeast
- Incubator for plates
- Incubator for culture tubes
- Sterile filter paper discs
- Eppendorf tubes
- Culture tubes
- Tweezers
- Iodine
- Dimethyl sulfoxide (DMSO)
- Micropipette and tips
- Deionized water (DI water)
- CeraVe© Moisturizing Lotion with Ceramides and Hyaluronic Acid
- Bunsen burner
- Ethanol to sterilize spreader
- Metal spreader for agar plates

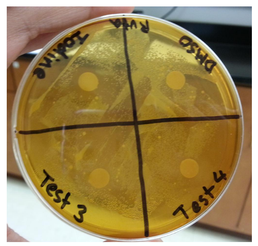
Different concentrations of ceramide lotion in a ceramide-DMSO solution were used to determine changes in growth based on ceramide concentration.

For each consecutive test, a more concentrated solution of ceramide and DMSO was created. A filter paper disc was dipped in each solution and placed on an agar plate to compare to a positive and negative control through the Kirby-Bauer disc-diffusion method of growth inhibition analysis. A disc dipped in iodine was a positive control (inhibited proliferation) while a disc dipped in DMSO was a negative control (did not inhibit proliferation).

Experimentation/Data

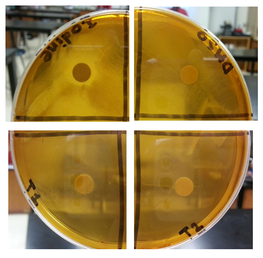
Trial 5 - Concentration 0.02 to 0.08 g/mL

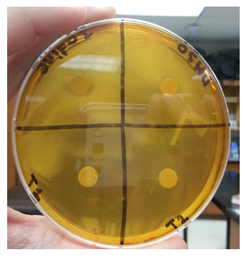




- T1 = 0.0209 g ceramide / mL DMSO
  - Zone of inhibition = 0.2 mm
- T2 = 0.0418 g ceramide / mL DMSO
  - Zone of inhibition = 0.2 mm
- T3 = 0.0621 g ceramide / mL DMSO
  - Zone of inhibition = 1.2 mm
- T3 = 0.0823 g ceramide / mL DMSO
  - Zone of inhibition = 1.0 mm

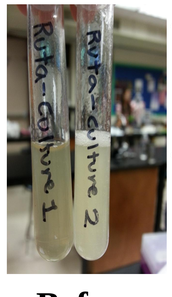
Trial 7 - Concentration Increase by 10 times





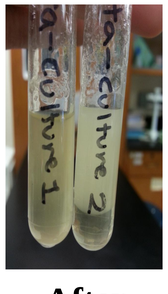
- T1 = 0.4117 g ceramide / mL DMSO
  - Zone of inhibition = 7.0 mm
- T2 = 0.8204 g ceramide / mL DMSO
  - Zone of inhibition = 9.1 mm

Liquid Cultures – Concentration 0.8 g/mL



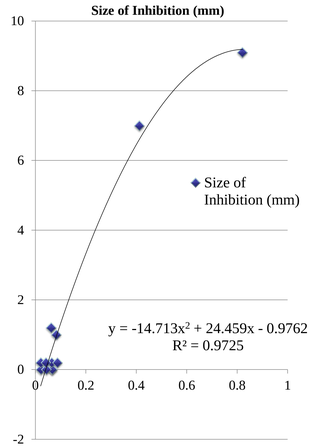
Before

- Culture 1 = Yeast, growth broth, DI water
- Culture 2 = Yeast, growth broth, DI water, ceramide / DMSO solution

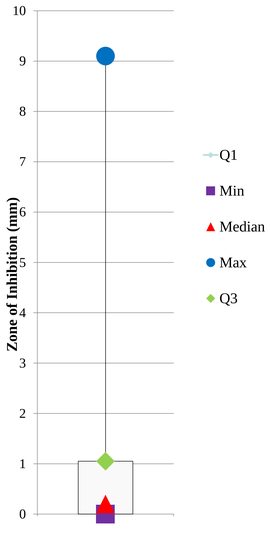


After

Results



The polynomial function above describes the relation between the concentration of ceramide and the size of the zone of inhibition.



The box plot above describes the spread of data that documents the sizes of zone of inhibition based on ceramide concentration.

Conclusions

The documented experiments rejected the hypothesis that an increase in ceramides would lead to an increase in yeast cell proliferation because the actual results described an increase in growth inhibition rather than an increase in proliferation. The zones of inhibition resulting from an increase in the ceramide content in the external environment increased in size as the concentration of ceramide placed in the environment was increased. The source of ceramides used, CeraVe© brand moisturizing lotion, contained Ceramides 3, 6-II, and 1, as well as hyaluronic acid and lipids that function similarly to ceramides, allowing the overall conclusion that increased ceramide content in the external environment led to an increase in activity that inhibited the cell cycle in *Saccharomyces cerevisiae.*

Discussion and Data Interpretation

Ceramide generation is induced by a variety of substances, including chemotherapeutic agents used in cancer therapy, Cannabinoids, found in the drug Cannabis, and endotoxins, released by bacteria. The sources of the agents that induce an increase in ceramide generation can be studied further to determine their exact contributions to the inhibition of cell proliferation. These sources can be classified as substances that contribute to cell-cycle inhibition by cell death through an ceramide generation due to the fact that an increase in ceramides inhibited proliferation.

The results of the documented experimentation confirm that ceramides contribute to cell-cycle inhibition in yeast, while their effect on proliferation in humans has yet to be researched. The increase of ceramides in the external environment affected the cell-cycle regulation of the yeast cells through interaction with the cell membranes of the yeast cells. The increase of ceramides in the external environment led to overall inhibition of cell proliferation.

Further Research

Further research can be done to determine if ceramides play the same role in human cells as they do in yeast cells. The effects of increased ceramide concentration may differ based on cell differentiation in multicellular organisms. The exact interaction that occurred between the ceramide and the yeast cell membrane can be researched further, and the contribution of chemotherapeutic agents, Cannabinoids, and endotoxins as contributors to malignancy can also be researched.