



Background

- Cancer arises when mutated cells become unresponsive to cell cycle signaling that regulates cell growth. These uncontrolled cells can invade and spread to other parts of the body.³
- As the cancerous cells spread, Myeloid-derived suppressor cells (MDSC) are formed and can prevent our body's defense mechanisms from limiting the spread of cancer.^{2,4}
- Due to estrogen signaling, MDSC are able to proliferate and reduce T cell response.¹
- Studies have shown phytoestrogens, specifically Lycopene and Trans-Resveratrol, have chemopreventive potential.²

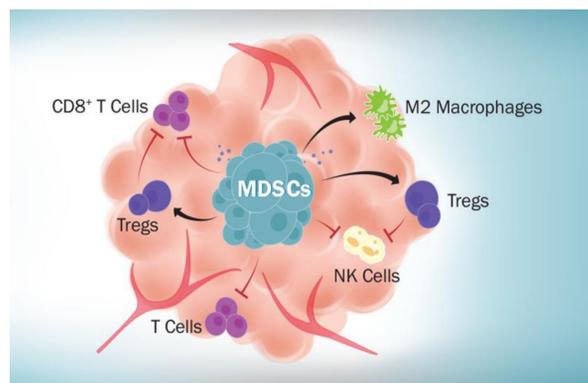


Figure 1: Imaging depicting MDSC blocking many of the body's defense-killing tumor cells

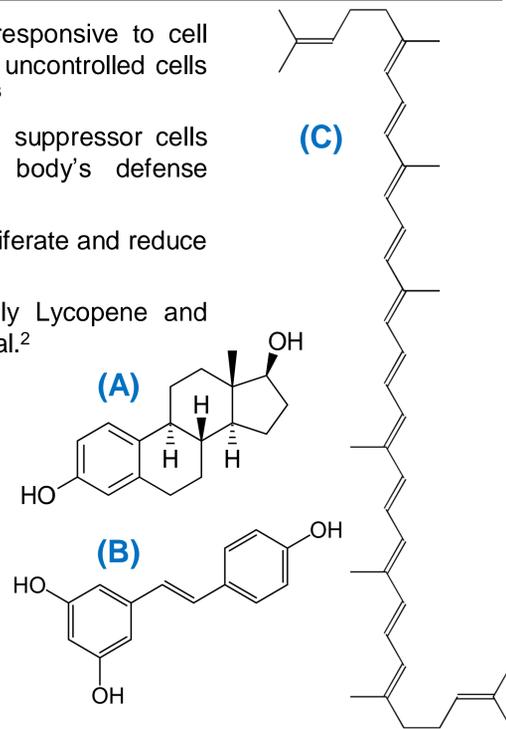


Figure 2: Chemical Structures of (A) Estrogen, (B) Trans-Resveratrol, and (C) Lycopene

Specific Aim

Research Question: We will investigate the role these phytoestrogens play in reducing estrogen from binding to myeloid precursor cells and the role they play in decreasing tumor growth.

Hypothesis: We hypothesize that Lycopene and Trans-Resveratrol will bind to the estrogen receptors and block all estrogenic activity, decreasing the production of MDSC cells.

Methods

Cultured

Bone marrow derived dendritic cells were incubated with media, estrogen, trans-resveratrol, and lycopene for 24 hours.

Flow Cytometry

Fluorescent monoclonal anti-CD80-FITC and anti-MHC II-PE antibodies. Cells were run on Accuri C6 Flow Cytometer.

T-cell Isolation and Cell Culture

Isolated splenic CD4 T cells were cultured with bone-marrow derived dendritic cells and compounds for seven days. Three replicates were done for each compound.

T-cell Proliferation

MTT assay used to measure the viable T cells in culture.

ELISA T-cell Cytokines

IFN- γ and IL-4 specific antibodies to analyze the amount of cytokine produced by the CD4 T-cells cultured with compounds.

Statistical Analysis

Paired T-tests used to measure statistical significance

Results

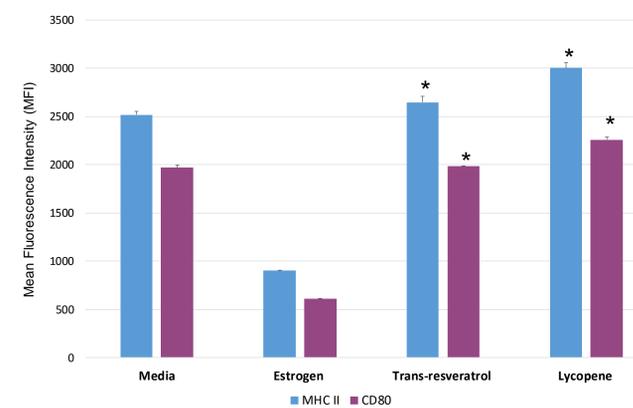


Figure 3: MHC II and CD80 expression on cells incubated with phytoestrogen compounds lycopene and trans-resveratrol. Compounds mean fluorescence intensity was analyzed using flow cytometry. Anti-MHC II and anti-CD80 antibodies bound to MHC II and CD80 respectively to measure the amount of each receptor present in the incubated samples. *Indicates a statistical significance, $p < 0.05$, when compared to MHC II and CD80 expression in estrogen.

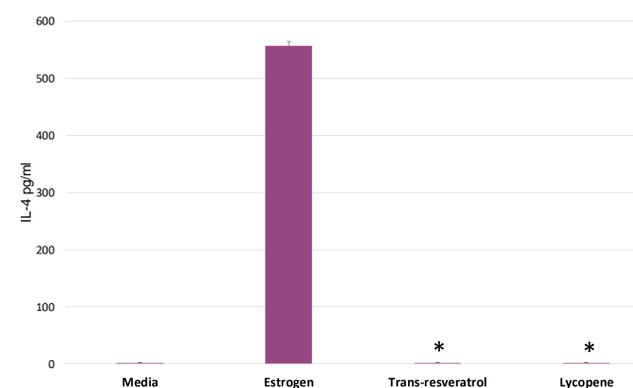


Figure 5: Average IL-4 cytokine production measured in CD4 T cells cultured with lycopene and trans-resveratrol. CD4 T cell IL-4 production was measured using IL-4 specific antibodies in ELISA. CD4 T cells in Trans-resveratrol and lycopene had diminutive IL-4 production, whereas in estrogen, CD4 T cells produced 500 pg/ml of the cytokine. *Indicates statistically significant difference, $p < 0.05$, of the amount of IL-4 produced in lycopene and trans-resveratrol compared to estrogen and media.

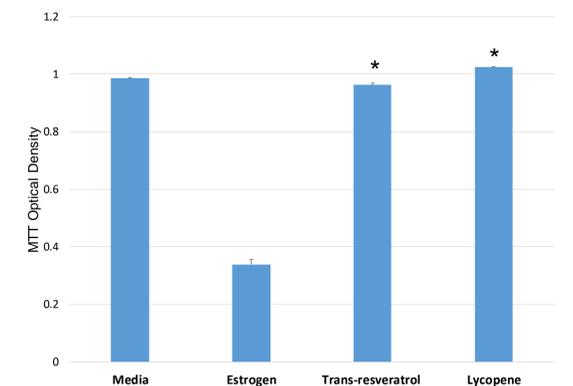


Figure 4: Average T-cell proliferation in the presence of lycopene and trans-resveratrol. The optical density of T-cells in each compound was measured using MTT assay. T cell proliferation was significantly decreased in the presence of estrogen. *Indicates statistically significant difference, $p < 0.05$, of the T cell proliferation in media and estrogen compared to lycopene and Trans-resveratrol.

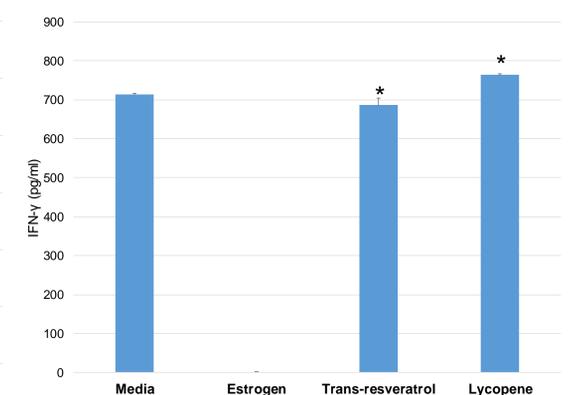


Figure 6: Average IFN- γ cytokine production measured in CD4 T cells cultured with lycopene and trans-resveratrol. CD4 T cell IFN- γ production was measured using IFN- γ specific antibodies in ELISA. IFN- γ production was significantly higher in CD4 T cells in trans-resveratrol and lycopene when compared to estrogen and media. *Indicates statistically significant differences, $p < 0.05$, in IFN- γ cytokine production when compared to the IFN- γ production of CD4 T cells in estrogen and media.

Conclusion

- T-cell proliferation significantly increased in the presence of these phytoestrogens suggesting that lycopene and trans-resveratrol can increase the body's defense mechanisms in combating tumor growth by decreasing MDSCs affect on T cells.
- IL-4 is a cytokine that promotes tumor growth. IL-4 was significantly higher in the presence of estrogen compared to these two phytoestrogens. This suggests the decrease of T-cell proliferation (Fig. 3) was caused by estrogen.
- IFN γ increases T-cell activation which decreases tumor growth. In the presence of trans-resveratrol and lycopene, IFN γ was significantly higher than estrogen. This provides evidence that these phytoestrogens can bind to estrogen receptors and in turn decrease estrogenic effects on MDSCs.

Future Direction

- Investigate other phytoestrogens and introduce them to those genetically predisposed for cancer.
- Examine other cells that aid in the growth of cancerous tumors and observe different cytokines, chemokines, and signals produced.

Citations

- 1 Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., and Gustafsson, J. A. (2007). Estrogen receptors: how do they signal and what are their targets. *Physiological reviews*, 87(3), 905-931.
- 2 King-Batoon, A., Leszczynska, J., & Klein, C. (2008). Modulation of gene methylation by genistein or lycopene in breast cancer cells. *Environmental And Molecular Mutagenesis*, 49(1), 36-45.
- 3 National Institute of Health. (2016). Help me understand genetics: how genes work. *Genetics Home Reference*. Retrieved from NIH.gov
- 4 Umansky, V., Blattner, C., Gebhardt, C., & Utikal, J. (2016). The role of myeloid-derived suppressor cells (MDSC) in cancer progression. *Vaccines*, 4(4), 36.