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Evaluating Synergism Between Vitamin D Metabolites and ω-3 Fatty Acids in Ovarian Cancer Cells

Introduction

Ovarian cancer is one of the most lethal cancers affecting women, with an estimated 22,000 newly diagnosed each year[1]. Of those diagnosed, only 15% are detected within the early stages, when the cancer is most treatable[1]. Both preventative and therapeutic measures need to be taken to increase the survival rate of this cancer. Research suggests vitamin D and ω-3 fatty acids have a positive effect on the prevention of multiple cancers, including ovarian cancer. Studies in breast cancer cell lines indicate these molecules act synergistically to inhibit growth of cells[2]. Our research project seeks to elucidate the effects of vitamin D metabolites (Calcidiol and Calcitriol) and the ω-3 fatty acid Docosahexaenoic acid (DHA) alone and in combination on the growth and gene expression of ovarian cancer cells.

Materials & Methods

The effect of these molecules on the growth of the ovarian cancer cell line OVCAR4 was measured by a Sulforhodamine B (SRB) assay[3]. OVCAR4 cells were cultured in RPMI 1640 media supplemented with 10% FBS (Fetal bovine serum), 2mM L-glutamine, and 100 U/mL of penicillin/streptomycin. Cells were stored at 37° C, in a humidified incubator containing 5% CO_2.

Experimental Methods

Day 1: OVCAR4 cells were plated a density between 2-5x10^3 cells/well in 96 well plates.

Day 2: Cells were treated with varying levels of DHA (docosahexaenoic acid), Calcidiol and Calcitriol alone and in combination.

Day 5: Following 72 hour incubation, cells were washed with 1X PBS then fixed to the plate with 20% trichloro acetic acid.

Day 6: Cells were washed with deionized water four times and allowed to air dry.

Day 7: Cells were stained with 0.04% SRB reagent for 30 minutes. After staining, the cells were washed 4 times with 1% acetic acid and allowed to dry.

Day 8: Cells were resuspended in 200 µL of 10 mM Tris buffer at pH 7.6 for 30 minutes. The absorbance of these plates were measured at 505 nm.

Results

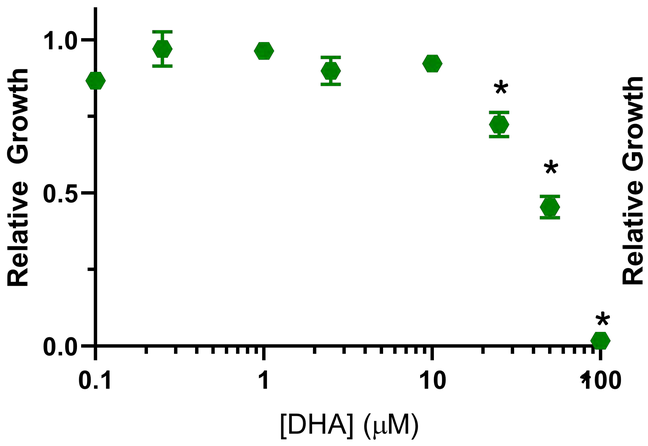

Figure 1: Treatment of OVCAR4 cells with DHA demonstrates a dose dependent decrease in relative cell growth. * indicates a significant difference from control treatment (0.1% ethanol) determined by 1-way ANOVA (25 µM DHA P<0.0149 n=9, 50 µM DHA P< 0.0036 n=5, 100 µM DHA P< 0.0001 n=2).

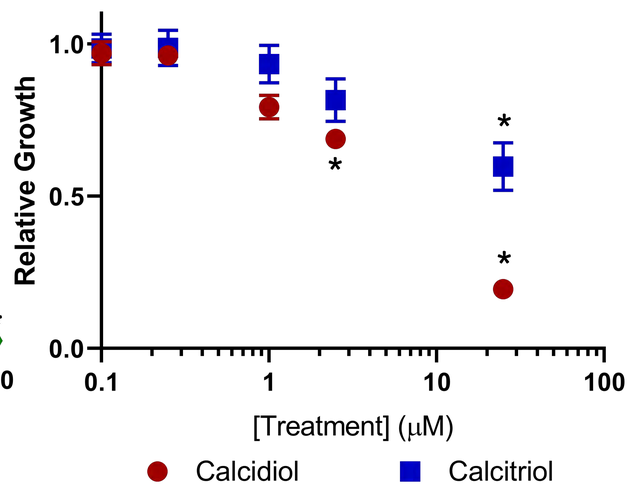

Figure 2: Treatment of OVCAR4 cells with Calcidiol or Calcitriol confers a dose dependent decrease in relative cell growth. * indicates significant difference from control treatment (0.1% ethanol) determined by 1-way ANOVA (2.5 µM Calcidiol P<0.001 n=8, 25 µM Calcidiol P<0.003 n=8, 25 µM Calcitriol P<0.003 n=7).

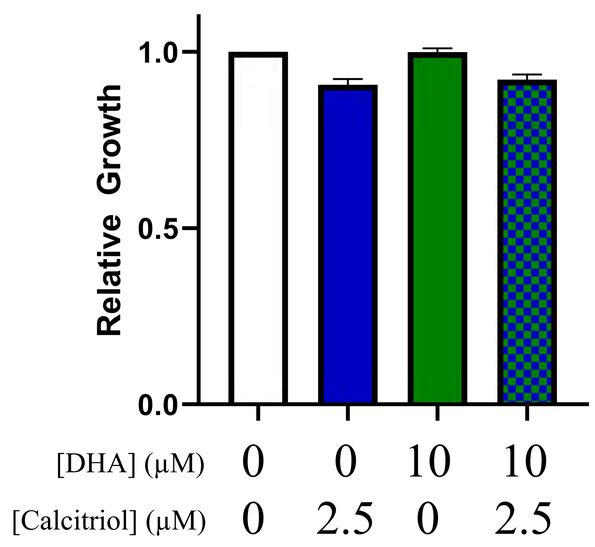

Figure 3: Cotreatment of OVCAR4 cells with DHA and Calcitriol shows no difference in relative growth compared to control or individual treated cells (n=2)

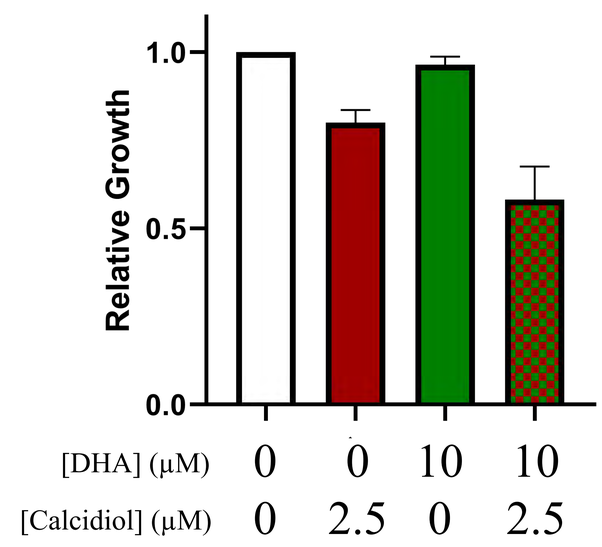

Figure 4: Cotreatment of OVCAR4 cells with DHA and Calcidiol shows a slight, albeit non-significant decrease in relative growth compared to control and individually treated cells (n=4).

Conclusions

- Individual treatments of DHA, Calcidiol and Calcitriol conferred a dose dependent decrease in relative cell growth of OVCAR4 cells.
- Treatment of OVCAR4 with both DHA and Calcitriol revealed no significant effect on relative cell growth at the concentrations tested.
- Treatment of OVCAR4 with both DHA and Calcidiol demonstrated a potential synergistic effect, although the results were not significant.
- Future studies will investigate the effects of single and combination treatments on gene regulation of downstream targets of vitamin D and DHA signaling pathways using qRT-PCR and Western blotting.
- Future studies will also include testing additional ovarian cancer cell lines to validate the observations.

Literature Cited

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3. Vichai, V, Kirtikara, K, Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening. *Nature Protocols* **2006**, *1* (3), 1112–1116.

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