



The anticancer effects of the vitamin E isoform,  $\gamma$ -tocotrienol, and vitamin D3 act synergistically to inhibit MDA-MB-231 triple negative breast cancer (TNBC) cell proliferation and viability *in vitro*.

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- **Introduction and Background:**

- **Triple Negative Breast Cancer:** Triple negative breast cancer (TNBC) is the most difficult type of breast cancer to treat among all breast cancer subtypes. MDA-MB-231 was the TNBC cell line used in these studies.
- **Problems with the Conventional Therapies:** Generation of side effects, drug resistance after prolonged treatment, as well as relapse and disease progression.
- **Natural Product Based Therapy:** Natural products are an important source of therapies. In the period of 1981 to 2014 the FDA approved 1,562 drugs, which 38% were natural products or their derivatives.
- **Benefits of Combined therapy:** There are many benefits in use of combination therapy. Often times each drug can be used at a lower dose and results in less side effects and enhance efficacy, as well as having a lower probability of drug resistance.
- **Background Information of  $\gamma$ -Tocotrienol:** It is a Natural form of Vitamin E. It is found in palm oil and other natural resources. Studies showed high dietary intake of palm oil suppressed carcinogen induced mammary tumor growth.  $\gamma$ -Tocotrienol is responsible for the suppression of various types of cancer signaling pathways in many types of breast cancer like: PI3K/Akt/mTOR, MAPK, and so on.
- **Background Information of Vitamin D3:** Vitamin D is acquired through both sun exposure and dietary sources.  $1\alpha,25(\text{OH})_2\text{D}_3$ , the active form of vitamin D. It is found in any many studies that  $1\alpha,25(\text{OH})_2\text{D}_3$  has potential anticarcinogenic effects, including regulation of cell growth and proliferation, stimulation of apoptosis, and down-regulation of estrogen receptors.

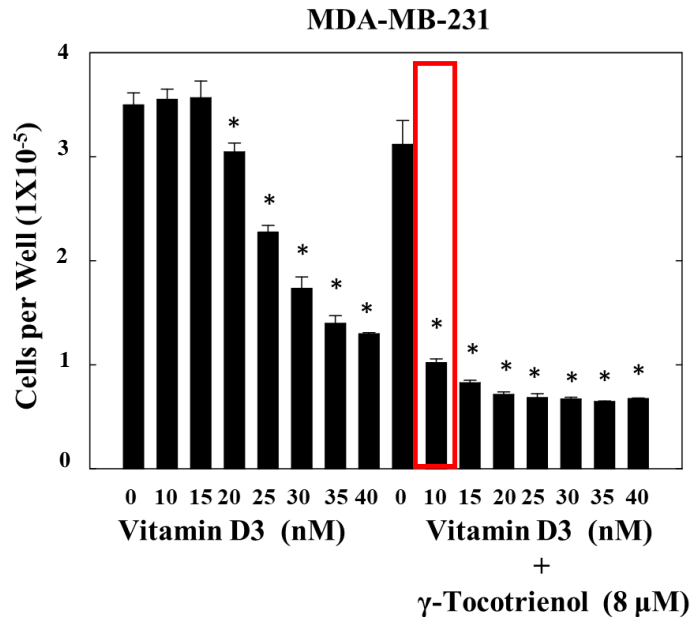
- **Hypothesis:**

- Combination of  $\gamma$ -Tocotrienol and Vitamin D3 may show a synergistic anticancer effects against MDA-MB-231 TNBC cells.

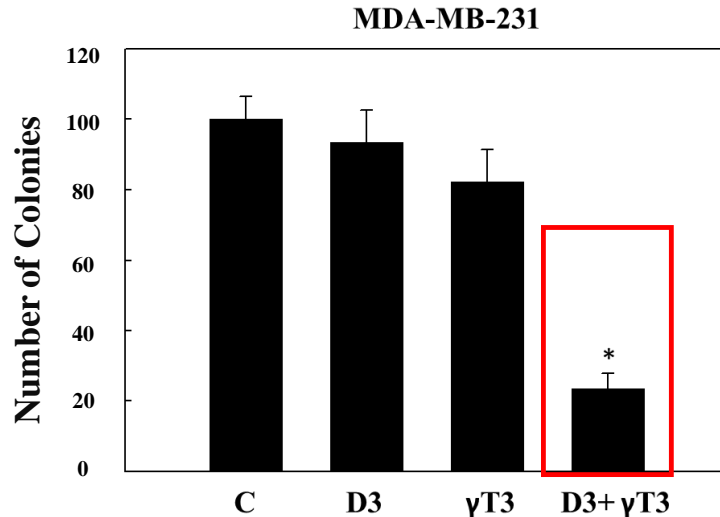
- **Aims:**

**1.** To determine the anti-proliferative dose of  $\gamma$ -tocotrienol and Vitamin D3 on MDA-MB-231 cell line. **2.** To determine the anti-colony formation effects of the combined doses by colony formation assay. **3.** To determine the anti-invasiveness and migratory effects of the combined doses by Matrigel invasion and scratch wound healing assay. **4.** To determine the effect of combined effect on cell cycle by flow cytometry **5.** To determine the effect of combined effect on apoptosis by flow cytometry **6.** To determine the effect combined dose on p-MAPK, and other cell cycle-apoptosis markers by western blotting.

# Methods and Results: Cell Viability Assay & Colony Formation Assay

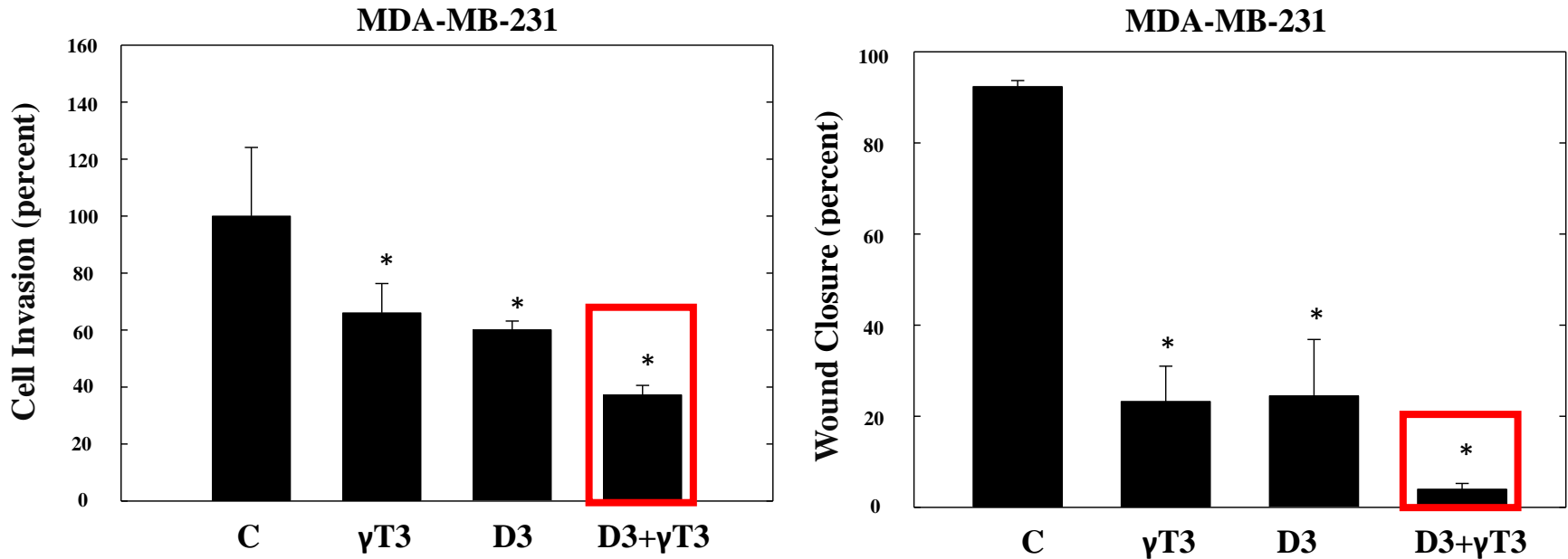


**Fig:** Effects of various doses of Vitamin D<sub>3</sub> treatment alone or in combination with 8 μM (subeffective dose) γ-Tocotrienol on growth of human MDA-MB-231 cells.



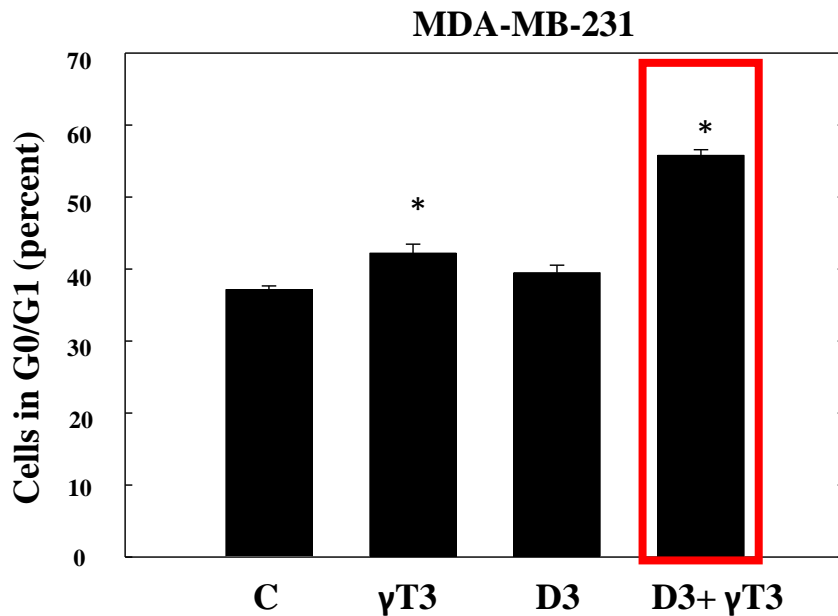
**Fig:** Effects of untreated control, 8 μM γ-tocotrienol, 10nM vitamin D<sub>3</sub>, and combined treatment of 8 μM γ-tocotrienol and 10nM vitamin D<sub>3</sub> on clonogenicity of MDA-MB-231 cells as detected by colony formation assay.

# Effects of Mono and Combined Therapy on Invasion and Migration

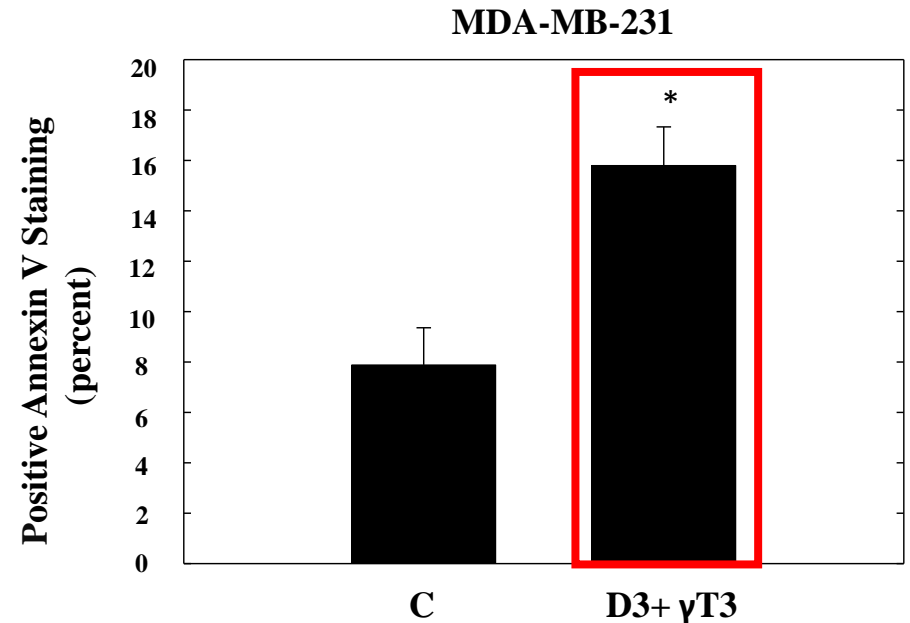


**Fig:** Effects of untreated control (C), 8  $\mu$ M  $\gamma$ -tocotrienol ( $\gamma$ T3), 10nM vitamin D3 (D3) and combined 8  $\mu$ M  $\gamma$ -tocotrienol and 10nM vitamin D3 ( $\gamma$ T3+D3) treatments on invasiveness and migration of MDA-MB-231 cells as detected by matrigel invasion assay and scratch wound healing assay.

# Cell Cycle and Apoptosis Analysis with Flow Cytometry

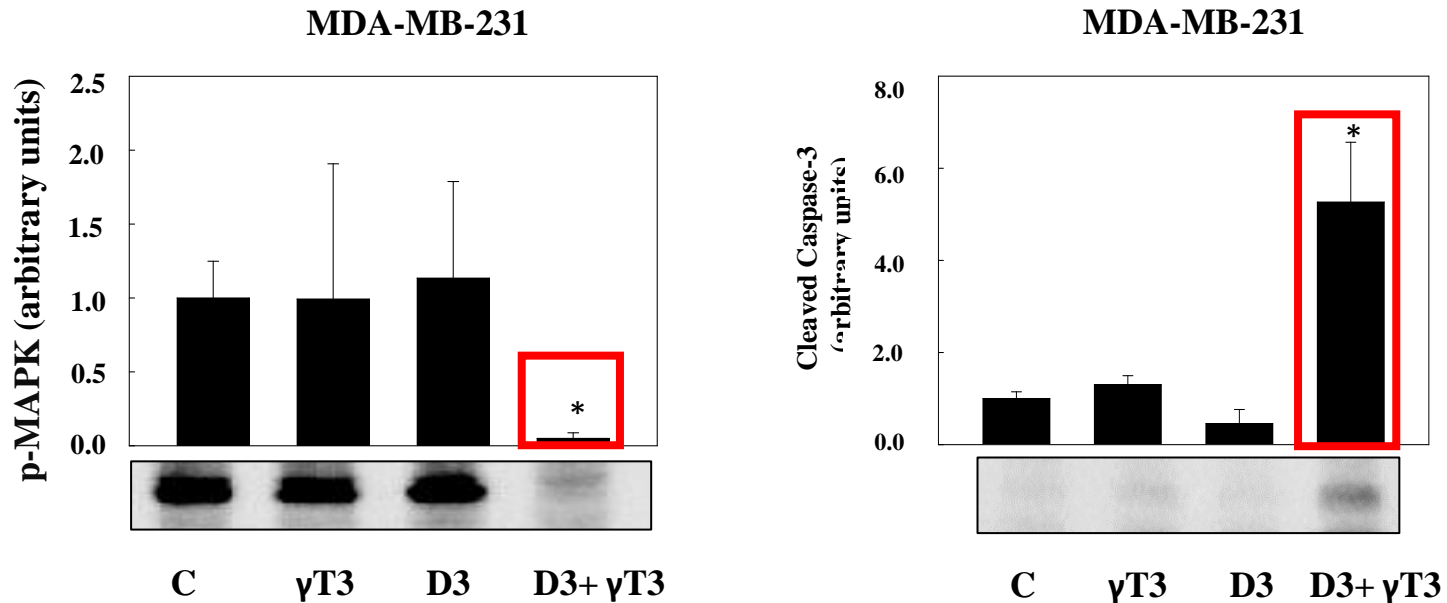


**Fig:** Percent of cells in G0/G1 phase of cell cycle following a 4-day treatment with 8 μM γ-tocotrienol (γT3), 10 nM vitamin D3 (D3) or the combination of γT3+D3 as compared to vehicle treated control cells (C).



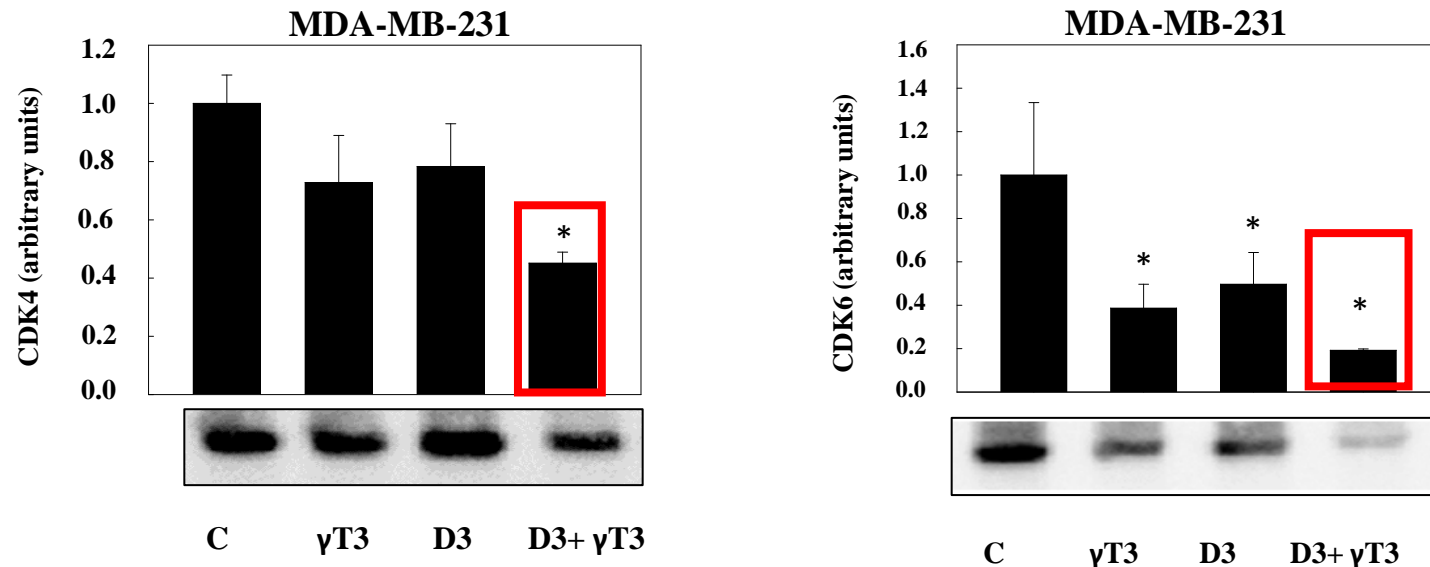
**Fig:** Annexin V positive staining in MDA-MB-231 cells following a 4-days treatment with 8 μM γ-tocotrienol (γT3) and 10 nM vitamin D3 (D3) as compared to untreated group.

# Combinational Treatment of $\gamma$ -Tocotrienol and Vitamin D3 Causes Downregulation of p-MAPK and Apoptotic Marker



**Fig: I** Effects of untreated control (C), 8  $\mu$ M  $\gamma$ -tocotrienol ( $\gamma$ T3), 10nM vitamin D3 (D3) and combined 8  $\mu$ M  $\gamma$ -tocotrienol and 10nM vitamin D3 ( $\gamma$ T3+D3) treatments on p-MAPK and cleaved caspase-3 levels as indicated by their integrated optical density (arbitrary units) normalized to their respective corresponding total protein levels in each well.

# Combinational Treatment of $\gamma$ -Tocotrienol and Vitamin D3 Causes Downregulation of Cell Cycle Markers

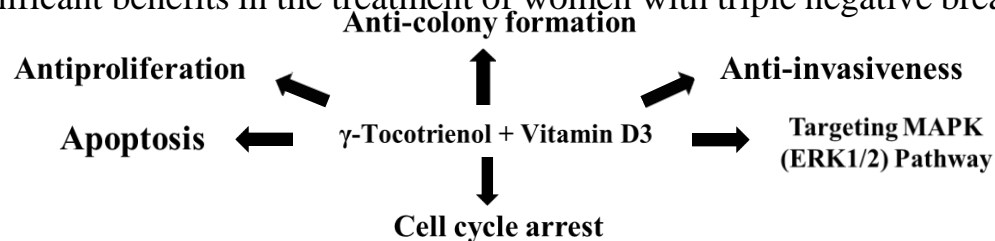


**Fig:** Effects of untreated control (C), 8  $\mu$ M  $\gamma$ -tocotrienol ( $\gamma$ T3), 10nM vitamin D3 (D3) and combined 8  $\mu$ M  $\gamma$ -tocotrienol and 10nM vitamin D3 ( $\gamma$ T3+D3) treatments on CDK4 and CDK6 levels as indicated by their integrated optical density (arbitrary units) normalized to their respective corresponding total protein levels in each well.

# Conclusion and Acknowledgement

- Combined treatment with  $\gamma$ -tocotrienol and vitamin D3 induces a synergistic antiproliferative, anti-metastatic and apoptotic effects in MDA-MB-231 TNBC cells.
- The antiproliferative effects induced by this combined treatment was associated with a significant increase in G<sub>0</sub>/G<sub>1</sub> cell cycle arrest as evidence by a reduction in CDK4, CDK6, and phosphorylated ERK1/2 (active form) mitogenic signaling.
- The anti-metastatic effects induced by this combined treatment  $\gamma$ -tocotrienol and vitamin D3 was evidenced by a significant increase in apoptosis as evidenced by elevations in positive Annexin V staining and increased levels of cleaved caspase-3.
- In summary, these findings strongly suggest that combined  $\gamma$ -tocotrienol and vitamin D3 therapy may provide significant benefits in the treatment of women with triple negative breast cancer.

- **Summary:**



- **Acknowledgement:**

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