The individual and additive effects of Paclitaxel and Verteporfin on breast cancer stem cells

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Triple negative breast cancer
Most breast cancer cell proliferation is prevented by the inhibition of endocrine receptors for estrogen (Er), progesterone (PgR), and HER2. Triple negative breast cancer (TNBC) cells are characterized by their lack of the aforementioned receptors, thus TNBC is most commonly targeted using chemotherapy. However, chemotherapeutic methods enrich cancer stem cells, resulting in cell metastasis and patient relapse. This study observes the use of Verteporfin and Paclitaxel in conjugation to target the uncontrolled proliferation of TNBCs (MDA-MB-231) and their resistance to chemotherapy.

Background

The Hippo pathway and YAP in cancer
Yes-associated protein (YAP) is an oncoprotein that is part of the Hippo signaling pathway. Inactivation of the Hippo pathway causes YAP to move into the nucleus and suppress the function of another protein, TEAD, by displacing its co-factor VGLL4. TEAD is responsible for the suppression of cell division[1].
In cancer, YAP is present in unusually high quantities. Through TEAD, YAP overexpression leads to unregulated tumor proliferation[1].

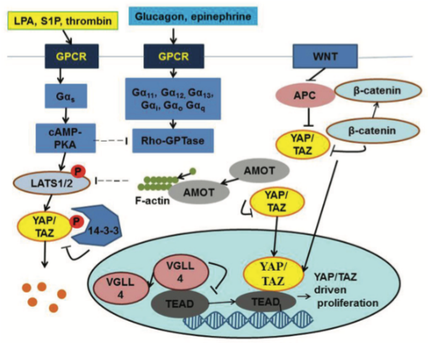

Figure 1. The Hippo Pathway[1]

Verteporfin
Verteporfin is a drug that causes YAP to stay in the cytoplasm, thus VGLL is not displaced and TEAD can resume its regular function: suppression of tumor proliferation[2].

Paclitaxel
Paclitaxel is a chemotherapeutic drug that stabilizes tubulin, preventing microtubule disassembly[3]. Previous work has shown that Paclitaxel is effective in decreasing tumor cell proliferation. However, its chemotherapeutic effects also lead to cancer cell resistance, as well as the enrichment of cancer stem cells[4].

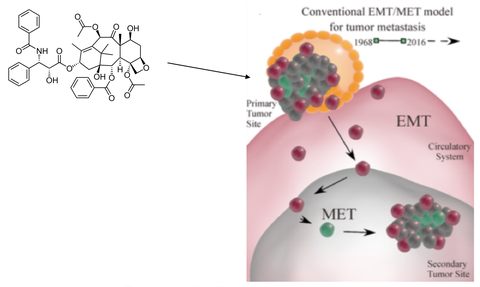

Figure 2. Paclitaxel induces tumor cell migration[5,6]

The expectation for using both drugs in combination was to kill TNBCs, while using Verteporfin to decrease the upregulation of YAP by Paclitaxel.

Results

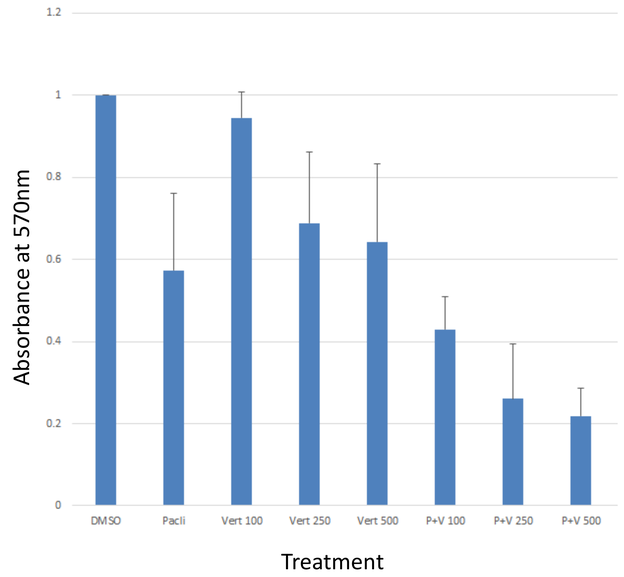

Figure 3. MDA cell viability in response to treatment with Paclitaxel and Verteporfin. The MTT assay used DMSO as a control, Paclitaxel (5nM), and Verteporfin (100nM, 250nM, and 500nM).

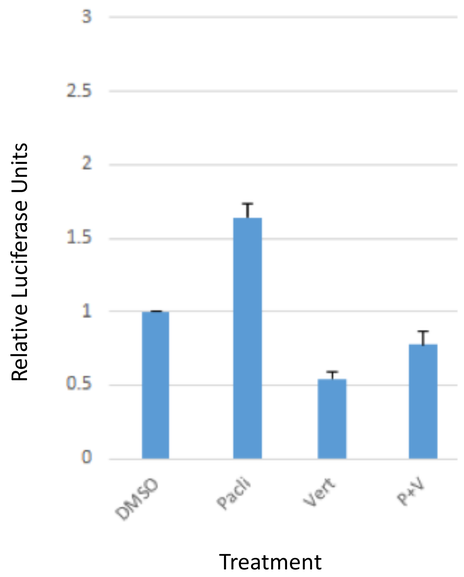

Figure 4. Detection of YAP target gene expression after treament of MDA cells with Paclitaxel and Verteporfin. Cells were treated with DMSO, Paclitaxel (5nM), and Verteporfin (100nM, 250nM, 500nM). The Luciferase assay was run following the Dual-Glo Assay protocol by Promega[7].

Methods

Cell Treatment
50 000 MDA-MB-231 cells were cultured in a 24 well plate with media (DEME with Phenol red, 5% fetal calf serum, 5% newborn calf serum, and Penstrap).

Cells were treated with:
- DMSO (control)
- Paclitaxel (5nM)
- Verteporfin (100nM, 250nM, and 500nM)
- A combination of Paclitaxel and Verteporfin

Subsequently, the cells were incubated in 37°C and 5% CO_2 atmosphere for 5 days.

MTT assay
To detect the effect of the drugs on cell viability, an MTT assay was run in a 96 well plate, and the absorbance of each well was read using a spectrophotometer at 570nm.

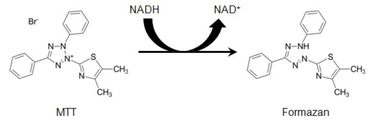

Figure 5. MTT reaction.[8]

Luciferase assay
To look at the the effect of the drugs on the expression of YAP target genes, a Dual-Glo luciferase assay was run.

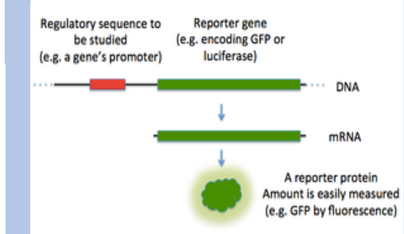

Figure 6. Luciferase Assay[9]

Conclusion

MTT assay
The absorbance levels for both Paclitaxel and Verteporfin were decreased when compared to that of the control, DMSO. This confirms that both drugs are capable of killing TNBCs. An additive effect was observed when the two drugs were administered in conjugation, suggesting that both drugs are capable of eliminating cancer and do not interfere with each other's activity.

Luciferase assay
When compared to bioluminescence with DMSO, Paclitaxel caused upregulation of YAP target gene expression, whereas Verteporfin depicted a decrease. When the two were used in combination, Verteporfin's inhibition of YAP activity compensated for the enrichment of YAP caused by Paclitaxel. The overall gene expression remained lower than that of the control.

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