Targeted oleuropein and hydroxytyrosol treatment for breast cancer cells, MCF-7 and MCF-10A, using integrated PDX-conditioned media paired with time-lapse microscopy

Research Proposal - Matthew Li

Introduction

OLE/HT - phenolic compounds derived from the olive plant which have been shown to exhibit antioxidant and anticancer behaviors. These behaviors include tumor growth inhibition, pro/anti apoptotic expression amongst different cells, and altercations amongst p53 pathways in cell lines.

MCF-7/10A - MCF-7 is a cancerous cell line while MCF-10A is a non-cancerous mammary epithelial cell line.

PDX media - refers to patient-derived xenograft media and is a tumor-derived environment rich in the growth factors, cytokines, and secreted molecules that actual cancer tissues release. The PDX media aims to simulate certain biochemical conditions of a tumor's microenvironment that are only present only in an in vivo environment. The MCF-7 and 10A will both be seeded with the PDX media to replicate an in vivo environment.

Among women, breast cancer is one of the leading causes of cancer deaths worldwide. Current treatment options, including chemotherapy and targeted therapeutic treatment, are lacking in several fields and are often coupled with significant side effects. Therefore this proposal attempts to address these issues and pose better-mediated cancer therapeutics involving OLE and HT.

Hypothesis

OLE and HT will exert selective apoptotic effects against MCF-7 cells by modulating p53 pathways, inducing Bax upregulation, lowering Bcl-2 expression, and affecting ROS with minimal impacts on MCF-10 cells

Methods and materials

Cell lines

- MCF-7: will be cultured in Eagle's Minimal Essential Media with 5% fetal bovine serum, and 50 nh/ml insulin
- MCF-10A: will be cultured in Mammary Epithelial Basal Medium (MEBM) with the addition of bullet kit supplements
- Cell lines MCF-7 and MCF-10A will be incubated at 37°C to ensure stability
- A hemocytometer/trypan blue exclusion assay will be used to quantify viable cells

Cell line assay

- Both MCF-7 and MCF-10 will be seeded into a 96-well plate before either the ethanol or OLE/HT is added
- OLE/HT treatment
- OLE and HT will be taken in purified form and diluted to the final concentration of 200 µg/ml each

PDX conditioned media

- Tumor fragments from consenting breast cancer patients will be extracted and grafted to immunodeficient mice to be cultured. The resulting growth factors and cytokines will be collected from the mice and added to the 96-well plate that will be augmented with MCF-7 or 10A.

Time-lapse fluorescence microscopy

- Both MCF-7 and MCF-10A will be parallel cultured in the 96-well plate and fluorescent probes of Annexin V-FITC will be used to constantly track apoptosis
- Imaging will occur every 30 minutes for 72 hours

Molecular Analysis:

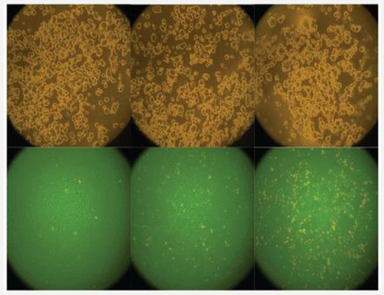
- Western Blot will be used for Bax, Bcl-2, and p53
- Taqman Real-Time PCR Assays will be used for p53 target genes and peroxiredoxin isoforms (Prdx)

Predicted Results

Cell/Model	Treatment	Concentration	Key Findings
MCF-7	Oleuropein + HT	50 μM each	Viability ↓ 55% at 72h
MCF-7	Oleuropein	100 μM	Viability ↓ 70%; ROS ↑ 40% vs. control
MCF-10	Oleuropein + HT	100 μM each	Viability ↓ 10% at 72h
PDX-based ex vivo	HT (20 day study)	25–75 μM	Tumor volume ↓ 30–50%; Enhanced p53 expression

Cell model	Treatment	Concentration	Findings
MCF-7 + PDX graft	Oleuropein + HT	50 µg/ml	Cell viability decreased by 55% ±10 at the 72-hour mark
MCF-7 + PDX graft	Oleuropein	100 µg/ml	Cell viability decreased by 70% ±10 at the 72-hour mark
MCF-10A + PDX graft	Oleuropein + HT	100 µg/ml	Viability decreased by 10% ± 5. Tumor volume decreased by 30-50% ±10 at 72-hour mark

Compound	MCF-7 effect (OLE/HT addition)	MCF-10A effect (OLE/HT addition)
p53	1.5 to 2-fold increase in total p53 protein expression	≤10% difference in p53 protein expression
Bax & Bcl-2 expression	Bax protein increases by roughly 2.5 fold and Bcl-2 levels decrease by 40%	≤15% difference in Bax & Bcl-2 levels
ROS/Prdx expression	Roughly 95-100% difference equating to apoptosis	No significant changes



End Time-lapse Fluorescence microscopy predicted results:

- Annexin V staining and fluorescence microscopy used to visualize cells
- Demonstrates oleuropein induced apoptosis on varying oleuropein

Conclusion

The effect of OLE/HT on Pdx media infused with traditional MCF-7 and MCF-10A cell lines has been predicted to yield a decrease in cellular viability in the cancerous MCF-7 cells. This proves the hypothesis to ultimately be true, and demonstrates OLE/HT's predicted ability to trigger apoptosis via p53 pathways, BAX/Bcl-2 expression, and altercations in ROS levels.

Discussion

Based on preliminary literature, the hypothesis for this proposal has been proven to be true. Therefore, this validates OLE/HT's ability to selectively target breast cancer cells while leaving non-cancerous cells unharmed. Further research in this field is needed to clarify the exact differences that Pdx media will exert in comparison to traditional cancerous cell lines. In addition, this study does have certain limitations, mainly the fact that PDX media mimics the growth factors and cytokines of an in vivo environment, it can vary greatly between different patients, thus results yielded may be hard to generalize.

Review of Literature

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