The effect of genetic modification of p53 uptake and Bcl-2 inhibition in the apoptosis of MCF-7 cells treated with oleuropein

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Introduction

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 CRISPR/Cas9 . OLE refers 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. Amplification of OLE through genetic modifications from CRISPR is hypothesized to increase apoptotic expression. Moreover, this study aims to utilize the MCF-7 breast cancer cell for experimentation.

Review of literature

Elmore, S. et al. (2021). Apoptosis: Review of programmed cell death mechanisms and markers. Toxicologic Pathology, 49(3), 456–468. https://doi.org/10.1177/0192623321997210

Idres, Y. M., Lai, A. J., McMillan, N. A. J., & Idris, A. (2023). Hyperactivation of p53 using CRISPRa kills human papillomavirus-driven cervical cancer cells. Virus Genes, 59(2), 312–316. https://doi.org/10.1007/s11262-023-01988-5

Junkins, R. D., Li, Y., & Zhao, Y. (2023). Oleuropein induces cytotoxicity and peroxiredoxin overexpression in MCF-7 human breast cancer cells. Anticancer Research, 43(10), 4333–4339. https://doi.org/10.21873/anticanres.17264

Youle, R. J., & Strasser, A. (2021). The BCL-2 protein family: Roles in cell survival, apoptosis, and cancer. Nature Reviews Molecular Cell Biology, 22(1), 1–19. https://doi.org/10.1038/s41580-020-00389-9

Problem statement/purpose

Which cancerous pathways should be altered in MCF-7 cancerous cells to yield the highest rates of apoptosis through select markers.

Hypothesis

CRISPR/Cas9 genetic modifications to upregulate the p53 pathway while inhibiting Bcl-2 in the MCF-7 cancerous cells will ultimately yield the highest levels of apoptosis as measured through Annexin V/propion flow cytometry, Caspase - 3/7 Assay, and western blot analysis.

Proposed Methods/Materials

1. MCF-7 Human breast cancer cells will be cultured in DMEM at 10% FBS and antibiotics at 37°C

2. CRISPR/Cas9 modifications:
- p53 upregulation ~ TP53 targeted
- Bcl-2 knockout ~ sgRNA targeted exon2 in Bcl-2 gene

3. Separation into proper experimental groups with 3 trials and additional sub trials.
- Ensure cell treatment with 100 µM oleuropein for 48 hours
- Ref image below

4. Apoptosis testing: Annexin V/PI flow cytometry, Caspase-3/7 activity assay, Western blot analysis

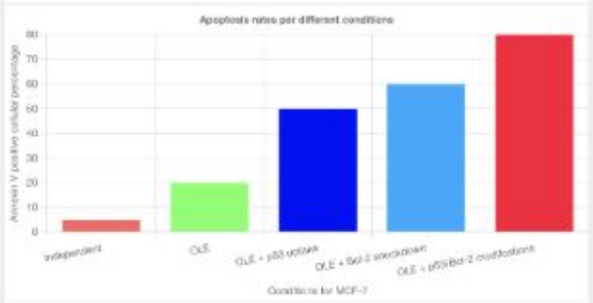


Proposed Methods/Materials continued.

Oleuropein will be purchased from a reputable vendor with a minimum of a 98% purity. The oleuropein will then be concentrated to with 100 µM with a solvent of DMSO and stored at -20°C. MCF-7 cells will be purchased from a reputable vendor and plated until it covers roughly 60% of the plate. Note: Experimental uncertainty will be reduced by repeating the experiment 3 times.

Expected Results

Annexin V expected results:



Expected results continued

Caspase 3-7 Assay predicted luminescence results:

Category	Luminescence output quantification
Independent	Nothing ~ 0
OLE	Moderate ~ 3
OLE/p53	High ~ 4
OLE/Bcl-2	High ~ 4
OLE/both	Very high ~ 8

Conclusion

The expected results indicate that the hypothesis is true, thus indicating that CRISPR/Cas9 is a viable method for cancer treatment. Ultimately, these findings can be applied to other modalities of cancer treatment, including a synergy between this and more conservative therapeutics such as chemotherapy. Future research in this field currently aims to integrate CRISPR/Cas9 into cancer treatment.

Discussion

The results indicate that CRISPR/cas9 modifications of the p53/Bcl-2 pathways are a viable option for inducing apoptosis in cancerous cells, which proves the hypothesis to be true. This inference was drawn from analysis of Annexin V staining in tandem with Caspase 3-7 results. Sources of error include that other tests such as Western Blot were not performed due to lack of relevant/recent literature. Moreover, this project proposal does not take confounding variables resulting from unforeseen interactions into account.

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