Promoting BAX Production in Breast Cancer Cells via CRISPR Cas 9 Gene Editing

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Abstract

In addition to producing energy for a cell, mitochondria produce signals induce or inhibit apoptosis by transcribing the Bcl-2 BAX. Our study aims to induce apoptosis, or cell death, to cancer cells by inserting the Bcl-2 BAX gene to the mitochondrial DNA of a BT-20 human breast cancer cell via CRISPR Cas 9 gene editing techniques. Before designing the CRISPR cassette, we selected a version of the BAX gene that induces apoptosis and transfected cancer cells with a plasmid containing our gene of choice to verify the concept of our study. Our preliminary data suggests that overexpression of the BAX protein in cancer cells committed the cells to apoptosis. Future experiments will be performed to confirm our data and to create a CRISPR Cas 9 cassette and delivery system. This will allow us to consistently place the BAX gene in a precise location of the mitochondrial DNA and to direct our treatment exclusively to cancer cells. Successful design and implementation of this project can be further developed into advanced cancer therapeutics that avoid toxic solutions such as radiation and chemotherapy.

Introduction

Bcl-2 associated (BAX) protein is a apoptosis promoter that binds to mitochondrial membrane opening channels. This interaction allows for cytochrome c to release into the cell and trigger apoptosis by reducing ATP production and, therefore, halting the cell's metabolic process. This mechanism is critical to recycle old or defective cells. Cancer cells lose their ability to induce cell death and consequently metastasize across the body. By introducing additional Bcl-2 BAX genes into the mitochondria, we aim to produce a substantially larger quantity of the BAX protein that will override a cancer cell's faulty apoptosis mechanism.

Results

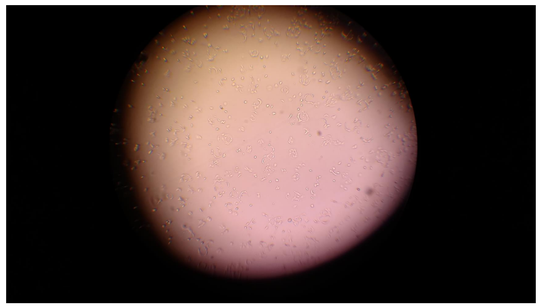

Figure 1: Negative Control before assay

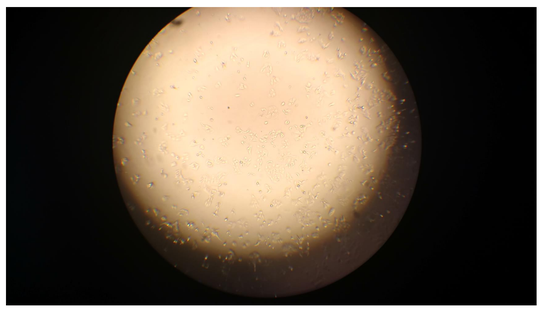

Figure 2: Negative Control after assay

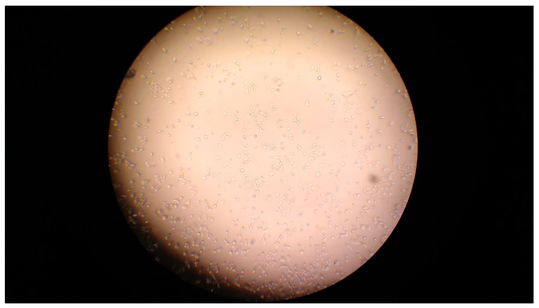

Figure 3: Negative control + cellfectin before assay

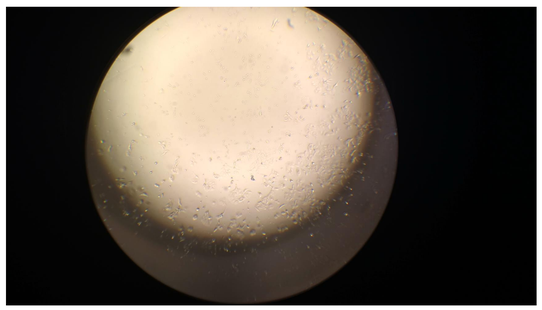

Figure 4: Negative control + cellfectin after assay

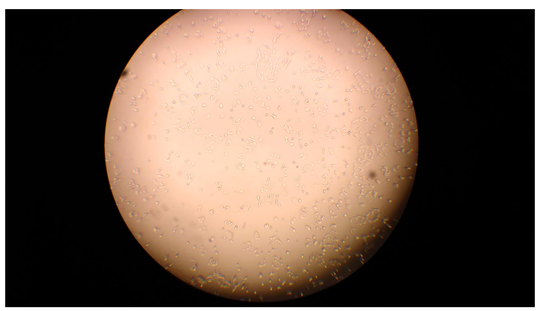

Figure 5: Negative control + GFP plasmid before assay

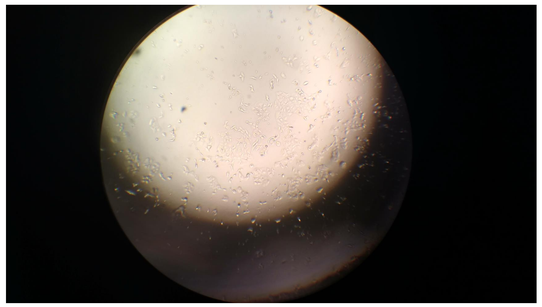

Figure 6: Negative control + GFP plasmid after assay

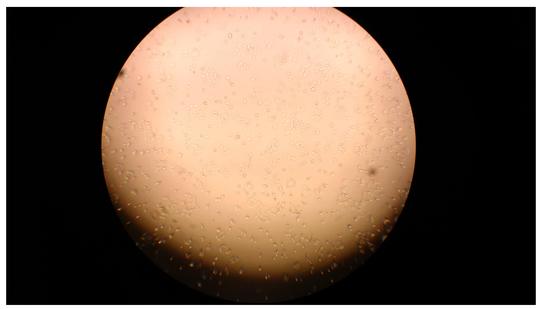

Figure 7: BAX transfection before assay

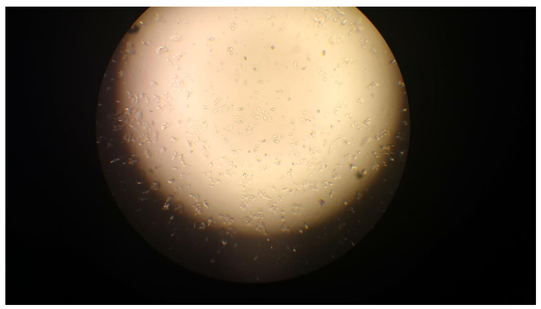

Figure 8: BAX transfection after assay

Methods

BT-20 breast cancer cells were cultured and prepared for a cellfectin transfection with 3534 pcDNA3 Bax plasmid (Addgene #8750) to insert Bcl-2 BAX gene (Genbank ID NM_177410). Cells were transfected and observed visually and by MMT assay for apoptotic activity.

Conclusion

Our preliminary qualitative assay suggests introducing the Bcl-2 BAX gene may cause BT-20 breast cancer cells to undergo apoptosis. The cellfectin reagent seemed to have a negligible effect on the cells, but the transfection of an empty GFP plasmid seems to have caused some cells to die due to stress. Per our qualitative assay, we can see that the GFP plasmid control has a more dense spread of cell than the cells transfected with the BAX gene. Further replicates will be performed to confirm this data in addition to quantitative assays by flow cytometry. Upon verification, design plans will ensue to develop a CRISPR Cas 9 cassette and a vector to deliver our BAX gene in a way that differentiates healthy cells from cancer cells.

References

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