Research Plan:

A) Rationale:

Approximately 38.4 percent of people in the United States will be diagnosed with cancer at some point during their lifetime, which translates to around 125.6 million people. However, a proper treatment for them remains to be seen. Although there have been advances in terms of treatment, cancer is still among the leading causes of death worldwide. Thus, it is necessary to continue research to discover the optimal treatment for cancer. Many current cancer researchers focus on the immune response to cancer, however, there may be other windows to tackle solid tumors. Death induced by survival gene elimination (DISE) is a type of cell death that involves the stimulation of more than one apoptotic pathway. This is achieved by the knockdown of multiple survival genes simultaneously. Recent studies suggest that DISE may be caused by the uploading of trinucleotide repeat siRNAs into the RISC complex. Thus, the transfection of dinucleotide siRNAs into cancer cells may be beneficial in determining whether or not DISE can play a role in cancer treatment.

B) Hypothesis:

Since trinucleotide repeats have been shown to decrease the proliferation of cancer cells and cause DISE, certain dinucleotide repeat sequences should decrease cell proliferation as well.

Research Questions:

1. Will the dinucleotide sequences decrease cancer cell proliferation?
2. Which sequences will be the most toxic to the HeyA8 cells?
3. Do the dinucleotide sequences cause a decrease in survival gene expression?

Expected Outcome:

Dinucleotide sequences should decrease survival gene expression, but it is unlikely that these sequences will be as effective as siCAG in killing the cancer cells.

C) Procedure, Risk and Safety, Data Analysis

Procedure:

Cell Culture:

- HeyA8 cells will be defrosted from a supply, and grown in Roswell Memorial Park Institute (RPMI) medium with 10% Fetal Bovine Serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine.

siRNA Transfection:

- Reverse siRNA transfection:

  siRNAs will be mixed with optiMEM in specified concentrations. 50 microliters of reagents will be plated into each used well of a 96-well plate. The siRNAs will include the dinucleotide repeats siCA, siAG, siGA, siUC, siCU, siUG, siGU, siCAG, siNT1. siNT1 will be the positive control, along with the mock transfection which will contain RNAiMAX but no siRNA, cells with optiMEM and the untreated cells. siCAG will act as a negative control because its known toxicity is very high, especially when compared to siNT1.

- Forward siRNA transfection:

  HeyA8 cells will be seeded into two 6 well plates at a concentration of 50,000 cells per well and will be cultured overnight before the transfection reagents are added. The siRNAs will be siCU, siUC, and siNT1. Three wells will be allotted per treatment.

IncuCyte Imaging:

After transfection, plates will be monitored using the IncuCyte imaging system, which will be contained in an incubator. A 96-well plate will be monitored by the machine, with the different treatments to the cells in three wells each. Two images of each well are taken every four hours and at the starting point. Confluency of each well will be calculated by IncuCyte software, and graphs will be generated by the software as well.

CellTiter-Glo Viability (ATP) Assay:

The CellTiter-Glo viability (ATP) assay will be performed after the cells are grown for either 72 hours or 120 hours. Old media will be replaced with 70 microliters of new media, then 70 microliters of CellTiter-Glo reagent (Promega) will be added to each well. Plates will be covered, shaken for 5 minutes and left at room temperature for 15 minutes. Plates will then be inserted into the BioTek Cytation 5, where luminescence is measured.

qPCR:

After a period of 72 hours, cells will be lysed with QIAzol. mRNA will be isolated using the Qiagen miRNeasy mini kit. Then, cDNA will be made using the cDNA reverse Transcription Kit (Applied Biosystems) of highly expressed survival genes in HeyA8 cells that contained the reverse complement repeat sequence (a AG/GA repeat) in the mRNA for that gene. These genes will include SLC20A1, MYO10, CCND1, and LPP. The control will be GAPDH. qPCR will be performed, and data will be generated by the qPCR machine.

Risk and Safety:

1. Human Subjects: N/A
2. Vertebrate Animals: N/A

3. Potentially Hazardous Biological Agents (PHBAs): HeyA8 cells will be obtained from Dr. E. Lengyel of the University of Chicago, originally established at MD Anderson Center Houston, and will be disposed of in designated biohazard waste bins for proper disposal.
4. Hazardous Chemicals/Activities/Devices: Throughout the duration at the laboratory, protective gear, including goggles, lab coats, and gloves, along with closed-toed shoes, and pants will be worn. Hazardous materials will be disposed of in designated waste bins. Proper supervision will be present at all times.

- Roswell Park Memorial Institute **Medium** (RPMI)- May cause eye irritation with susceptible persons. May cause skin irritation in susceptible persons. May be harmful by inhalation. May be harmful if swallowed.
- Fetal Bovine Serum (FBS)- May cause eye irritation with susceptible persons. May cause skin irritation in susceptible persons. May be harmful by inhalation. May be harmful if swallowed.
- optiMEM-May cause eye irritation with susceptible persons. May cause skin irritation in susceptible persons. May be harmful by inhalation. May be harmful if swallowed.
- RNAiMAX- May cause eye irritation with susceptible persons. May cause skin irritation in susceptible persons. May be harmful by inhalation. May be harmful if swallowed.
- RNAse- May cause eye irritation with susceptible persons. May cause skin irritation in susceptible persons. May be harmful by inhalation. May be harmful if swallowed.
- Phosphate Buffered Saline (PBS) - May cause eye irritation with susceptible persons. May cause skin irritation in susceptible persons. May be harmful by inhalation. May be harmful if swallowed.
- Trypsin- May cause allergy or asthma symptoms or breathing difficulties if inhaled

Data Analysis:

1. IncuCyte Imaging: After transfection cells will be monitored using the IncuCyte ZOOM live-cell imaging system (Essen BioScience), which will be contained in an incubator at 37 °C with 5% CO_2. Two images of each well are taken every four hours and at the starting point. Confluency of each well will be calculated by IncuCyte software.
2. CellTiter-Glo Viability (ATP) Assay: The CellTiter-Glo viability (ATP) assay will be performed after the cells are grown in 96-well plates for either 72 hours or 120 hours. Old media will be replaced with 70 microliters of new media, then 70 microliters of CellTiter-Glo reagent (Promega) will be added to each well. Plates will be covered, shaken for 5 minutes and left at room temperature for 15 minutes. Plates will then be inserted into the BioTek Cytation 5, where luminescence is measured, and data is collected. Subsequent graphs will be student generated.

3. qPCR: After a period of 72 hours, cells will be lysed with QIAzol. mRNA will be isolated using the Qiagen miRNeasy mini kit. Then, cDNA will be made using the cDNA reverse Transcription Kit (Applied Biosystems) of highly expressed survival genes in HeyA8 cells that contained a AG/GA repeat. These genes will include SLC20A1, MYO10, CCND1, and LPP. The control will be GAPDH. qPCR will be performed, and data will be generated by the qPCR machine. Subsequent graphs will be student generated.

Bibliography

1. Murmann, Andrea E., et al. "Small Interfering RNAs Based on Huntingtin Trinucleotide Repeats Are Highly Toxic to Cancer Cells." 2018, doi:10.1101/247429.

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4. Peter, M.e. "164 A Novel Role of the Death Receptor CD95 as a Tumor Promotor." *Lung Cancer*, vol. 54, 2006, doi:10.1016/s0169-5002(07)70240-4.

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6. Putzbach, William, et al. "DISE: A Seed-Dependent RNAi Off-Target Effect That Kills Cancer Cells." *Trends in Cancer*, vol. 4, no. 1, 2018, pp. 10–19., doi:10.1016/j.trecan.2017.11.007.

NO ADDENDUM EXIST