Continuation/Research Progression Projects Form (7)

Required for projects that are a continuation/progression in the same field of study as a previous project.

This form must be accompanied by the previous year's abstract and Research Plan/Project Summary.

Student's Name(s) Hannah Farley

To be completed by Student Researcher: List all components of the current project that make it new and different from previous research. The information must be on the form; use an additional form for previous year and earlier projects.

Components	Current Research Project	Previous Research Project: Year: 2018
1. Title	Characterization of Gxq Inhibitors for Uveal Melanoma Treatment	NR2F1 is a Key Regulator of Quiscence in Uveal Melanoma
2. Change in goal/ purpose/objective	The goal of evaluating mapping out gene relationships says the same, however the impact of Gxq inhibitors on NR2F1 in relation to Uveal Melanoma treatment is now being studied.	The goal was to determine NR2F1's importance in Uveal Melanoma and to map out its relationship to other factors invoved in the change between the dormant and proliferative phenotype of the cell.
3. Changes in methodology	In this study, an inhibitor of the mutation Gxq, one of the causes of Uveal Melanoma, will be administered to cells and changes to their phenotype measured by quantifying changes in dormancy and proliferative factors using the programs imagej and cell profiler.	SiRNA and qPCR were previously used to induce changes in NR2F1 and then measure the changes in expression of genes with known functions in NR2F1 to determine its overall function and importance in Uveal Melanoma.
4. Variable studied	The average number of Prame per cell and the number of cells that were positive for NR2F1 after the drug treatment.	Levels of tumor growth genes and tumor surpressor genes in response to the decrease in NR2F1 levels that were instigated by the small interfering RNA (siRNA).
5. Additional changes	None	None

Attached are:

☑ Abstract and Research Plan/Project Summary, Year 2018

I hereby certify that the above information is correct and that the current year Abstract & Certification and project display board properly reflect work done only in the current year.

Hannah Farley	Hannah Farley	07/07/19
Student's Printed Name(s)	Signature	Date of Signature (mm/dd/yy)

Hannah Farley

1. Research Plan

a. **Rationale**: Uveal Melanoma is a cancer that arises from the melanocytes in the iris. This particular cancer has a tendency to metastasize to the liver where it then becomes dormant. The genes involved in the activation of cancer quiescence vary within cell types. Nuclear Receptor Subfamily 2 Group F (NR2F1) is a gene that has shown to be involved in inducing dormancy through the activation of different pathways, particularly in Uveal Melanoma. In this study Uveal Melanoma metastases will be exposed to siRNAs that block the dormant phenotype produced by the NR2F1 gene. By measuring the levels of cell growth after siRNA exposure, the importance of NR2F1 and its down stream targets in Uveal Melanoma dormancy can be confirmed and an understanding of how cancer cells go into dormancy and escape detection can be formed. This knowledge may someday be useful in creating a therapy against cancer reoccurrence.

b. **Question/Problem**: Metastases often go into dormancy once they reach their destination, and begin growing again some time later, even if the primary tumor is destroyed. What activates this quiescence? Is NR2F1 signaling is related to cell dormancy?
Goal: Identify NR2F1 as an important regulator of Uveal Melanoma dormancy.
Hypothesis: If the NR2F1 genes is blocked in the dormant metastases, then the dormant cells will die because they no longer receiving any signals to stay alive.
Expected Outcomes: The cultures in which NR2F1 is inhibited will have fewer cells.

c. **Procedure**:

1. Providing cells Fresh Media/Seeding the cells
    1. Have confluent cell culture already growing within a proper container.
    2. Remove media and clean with 5mLs of PBS.
    3. Add 1mL of Trypsin to dislodge cells. Incubate for approximately 2-3 minutes or until cells are no longer attached to the substrate.
    4. Inactivate the Trypsin with 10mLs of media + 10% FBS
    5. Transfer into a 15mL tube and centrifuge the tubes for 6 minutes at 10,000 rpms.
    6. Suspend the cell pellet in 4.5mLs of new media, and homogenize using a pipet.
    7. Set aside 4 50mL tubes with for Days 1-4. Each tube will contain 535mL of fresh media + 10% FBS and 1mL of cell seed.
    8. Using these tubes prepare 3 dishes for each group, Si-C, SiNR2F1-1, and SiNR2F1-2, and for each day. (In total 36 dishes are need, 9 for each day 1-4)
    9. Any excess cells/media solution place in 3 dishes for matrigel.
2. Transfect the cells
    1. Remove the media and place 1mL of media + 10% FBS into dishes.
    2. There are a total of 13 wells for each group between the days. To account for possible loss of solution. Materials will be prepared for three extra slides for each group. (Prepare samples for 16 per group)
    3. Label 6 tubes A'B'C' and A"B"C" and add 800uL of OptiMEMmed and 60uL of RNAiMAX to A'B'C'. Then in A"B"C" add 800uL of OptiMEMmed +1.6uL (50uM siRNA)

4. Add A' to A", B' to B", and C' to C". Incubate at room temperature for 5 minutes.
5. Add 100uL to each well (3 wells for each letter, 9 wells in total).
6. Leave for 6 hours, and then change to the media to new media + 5% FBS 3mL/well.

3. RNA collection with TRIzol
    1. Remove media and wash with PBS.
    2. Add 1ml TRIzol. After thoroughly washing the plate, collect the TRIzol into an tube.

4.Cell Quantification
    1. Collect siRNA-NR2F1 (RNA exp) cells and transfer them to chamber slides.
    2. Take 500uL trypsin + 1mL FBS and add to chamber slides.
    3. Centrifuge for 6 minutes at 1000 rpm. Then resuspend the solution.
    4. Add 20uL of Trypan Blue to the slides.
    5. Quantify cells in each of the groups (Si-C, Si-Nr2F1-1, SiNR2F1-2)
    6. Matrigel slides accordingly.

4.RNA extraction
    1. Pellet the cells by centrifugation and discard the supernatant
    2. Add .75mL of TRIzol reagent per .25mL of sample to the pellet
    3. Pipet the lysate up and down several times to homogenize.
    4. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
    5. Add .2mL of chloroform per 1mL of TRIzol reagent used for lysis. Make sure the cap of the tube is secure.
    6. Centrifuge the sample for 15 minutes at 4 degrees Celsius and 12,000 rpm.
    7. The mixture is separated into a lower pink phenol chloroform, interphase, and a colorless upper aqueous phase. Transfer the colorless solution into a new tube.
    8. Add 250uL of isopropanol to each tube.
    9. Incubate them at room temperature for 10 minutes.
    10. Centrifuge at 14,000 rpm, 4 degrees Celsius for 10 minutes.
    11. Remove colorless solution and add 1mL of 75% ethanol, vortex for 30 seconds.
    12. Centrifuge at 14,000 rpm, 4 degrees Celsius for 10 minutes.
    13. Repeat steps 11-12 two more times for a total of 3 washes.
    14. After the third wash remove the ethanol and let the tubes air dry for 20 minutes,
    15. Then add 20uL of water and vortex them 30 seconds to homogenize them.
    16. Give each sample a quick spin to gather the entire sample at the bottom.
    17. Place the samples on ice and proceed to next step.

5.RNA quantification +dilution
    1. Using NanoDrop machine, prepare the machine with the designated buffer.
    2. Quantify the RNA using the machine.
    3. Add the correct levels of RNA and water based on measurements.

6. Measure levels of cDNA/Perform quantitative PCR
    1. Using cDNA and qPCR machinery measure levels of the RNA produced genes to determine overall activity of the genes in the cells.
    2. Relate these findings to make conclusions about how NR2F1 effects its knockdown targets and relate the gene activity to the overall dormancy of the cell.

Risk and Safety: Mount Sinai health and safety courses were completed to ensure that materials and machinery are going to be used properly. Safety equipment such as gloves, goggles, or facemask, will be used when handling certain materials that require greater protection. Mentor will perform any procedures in the protocol that require the use of potentially hazardous chemical (ex: use of chloroform), but student will participate in all other laboratory activities that are deemed safe.

Data Analysis: data gathered from quantitative PCR with SYBR will determine the expression level of NR2F1 and its down stream targets after NR2F1 has been silenced. The number of cells in the cultures will also be quantified to determine the effect the silencing of NR2F1 has on dormancy. Student t-test will be performed on data sets from control SiRNA groups and the SiNR2F1 group.

d. **Works Cited**

1. Begley, Ulrike, et al. "A Human TRNA Methyltransferase 9-like Protein Prevents Tumor Growth by Regulating LIN9 and HIF1-α." *EMBO Molecular Medicine*, vol. 5, no. 3, 2013, pp. 366–383. doi: 10.1002/emmm.201201161.
2. Fluegen, Georg, et al. "Phenotypic Heterogeneity of Disseminated Tumor Cells Is Preset by Primary Tumor Hypoxic Microenvironments." *Nature Cell Biology*, vol. 19, no. 2, 2017, pp. 120–132., doi:10.1038/ncb3465.
3. Kim, Ryung S., et al. "Dormancy Signatures and Metastasis in Estrogen Receptor Positive and Negative Breast Cancer." *PLoS ONE*, vol. 7, no. 4, 2012, doi:10.1371/journal.pone.0035569
4. Sosa, Maria Soledad, et al. "ERK1/2 And p38α/β Signaling in Tumor Cell Quiescence: Opportunities to Control Dormant Residual Disease." *Clinical Cancer Research*, vol. 17, no. 18, 15 Sept. 2011, pp. 5850–5857., doi:10.1158/1078-0432.ccr-10-2574.
5. Sosa, Maria Soledad, et al. "NR2F1 Controls Tumor Cell Dormancy via SOX9- and RARβ-Driven Quiescence Programs." *Nature Communications*, vol. 6, no. 1, 2015, doi:10.1038/ncomms7170.

2.Potentially Hazardous Biological Agents Research

a. The tissue sample/cell line was obtained from the Animal Department of Mount Sinai. Biosafety level assessment is based on the fact that the cancerous tissue is not transmittable to humans and no hazardous chemicals are used in the culturing of these cells. Therefore, biosafety level was determined to be level one.

b. A fume hood is used to first seed the cells and proper gloves are used at all times when handling the cells. Once the study is complete, cell cultures will be disposed of in the proper biohazard containment bin and all tools that come in contact with the cells will be disposed of or autoclaved.

OFFICIAL ABSTRACT and CERTIFICATION

NR2F1 is a Key Regulator in of Quiescence in Uveal Melanoma

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There is little known about the mechanisms that control cancer dormancy and proliferation. Cancers often will go into a state of quiescence following metastasis, and these single cells spontaneously activate for unknown reasons, allowing for reoccurrence of the disease even after treatment. Uveal melanoma is one of these cancers which have a low survival rate after the cancer has melastasized, with 15% of patients alive after years of diagnosis (Houle, 2012), The need for effective treatments is dire, and dormancy needs to be investigated. In this study NR2F1, a gene that is associated with in many different cancer types, was silenced using siRNAS to determine the importance of the gene in uveal melanoma's growth cycle. After NR2F1 was successfully silenced, the expression of genes downstream of NR2F1 was also investigated to evaluate the relationship of the genes within the ERK/p38 pathway. The expression ol NR2F1 was also measured using immunohistochemistry on mouse liver models to see the gene's levels in metastasized cells, Genes CTGF, CYR61, and ANKRD1 had a clear pattern of upregulation after NR2F1's silencing. lmmunohistochemistry images revealed dormant single cells and small clusters demonslrated higher levels NR2F1 when compared to large, actively dividing tumors. These results suggest that NR2F1 if essential in uveal melanoma's dormancy. ln the future we plan to investigate the relationship of other genes in the ERK/p38 pathway with NR2F1 and the presence of these factors in the mouse model to gain a better understanding of the mechanisms of cancer quiescence.

Category
Pick one only — mark an "X" in box at right

- Animal Sciences
- Behavioral & Social Sciences
- Biochemistry
- Biomedical & Health Sciences
- Biomedical Engineering
- Cellular & Molecular Biology ■
- Chemistry
- Computational Biology & Bioinformatics
- Earth & Environmental Sciences
- Embedded Systems
- Energy: Sustainable Materials and Design
- Engineering Mechanics
- Environmental Engineering
- Materials Science
- Mathematics
- Microbiology
- Physics & Astronomy
- Plant Sciences
- Robotics & Intelligent Machines
- Systems Software
- Translational Medical Sciences

1. As a part of this research project, the student directly handled, manipulated, or interacted with (check ALL that apply):

   ☐ human participants ☐ potentially hazardous biological agents

   ☐ vertebrate animals ☐ microorganisms ☐ rDNA ■ tissue

2. I/we worked or used equipment in a regulated research institution or industrial setting: ■ Yes ☐ No

3. This project is a continuation of previous research. ■ Yes ☐ No

4. My display board includes non-published photographs/visual depictions of humans (other than myself): ☐ Yes ■ No

5. This abstract describes only procedures performed by me/us, reflects my/our own independent research, and represents one year's work only ■ Yes ☐ No

6. I/we hereby certify that the abstract and responses to the above statements are correct and properly reflect my/our own work. ■ Yes ☐ No

This stamp or embossed seal attests that this project is in compliance with all federal and state laws and regulations and that all appropriate reviews and approvals have been obtained including the final clearance by the Scientific Review Committee.