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Category: Cellular and Molecular Biology

Characterization of Gaq Inhibitors in Uveal Melanoma

1. Research Plan

a. **Rationale:** Cancer is the second leading cause of death, and as the world population ages, it is becoming more prevalent (Faststats, 2017). Most treatments are geared to the proliferative nature of cancer, for its metastatic nature is what makes it so deadly; depleting normal cells of their resources and displacing organs until patient death (ACHS, 2016). Due to this, researchers efforts have been mostly focused on the growth and development of cancer while it is in a metastatic state. Despite the progress made in cancer treatment with immunotherapies and less cytotoxic chemotherapies, the mechanisms by which cancers avoid detection by the immune system and develop acquired resistance have been neglected (ACHS,2016). Residual disease in the form of recurrences limits the effectiveness of cancer treatments, for the small metastases have already evaded the treatment or become resistant to it. The cancer is able to due this through a process called dormancy. Dormancy is a stage in cancer progression where the cells cease dividing but survive in a quiescent state while waiting for appropriate environmental conditions to begin proliferation again. These undesirable conditions may result from the introduction of treatment into the microenvironment or when the cell is introduced to a new environment when it metastasizes.

Patients that suffer from Uveal Melanoma often face these problems. Uveal Melanoma is a cancer (melanoma) of the eye involving the iris, choroid, and ciliary body, collectively called the uvea. With around 3,360 adults diagnosed annually, uveal melanoma remains one of the rarest cancers (ASCO, 2019). At the same time however, it has one of the highest mortality rates with stage IV melanoma having a survival rate of 20% after 5 years (ASC,2019). Along with

that, patients only have an average survival of 6 to 9 months after evidence of metastatic disease has been found. Currently, more than 90% of cases are driven by mutations in the Gq-alpha protein (NIH,2019). The development of this disease is attributed to the activation of YAP, a transcription factor that induces cell growth and suppresses apoptosis, from this mutation (Yu, 2014). Attempts to directly target that protein have been too toxic for prolonged use in patients. To combat this, researchers have now been looking to find less toxic targets in the that still have the desired impact of cancer cell death (Yu, 2014) . Before new targets can be acquired, it is imperative to locate to what extent the Gq-alpha mutation effects uveal melanoma growth and development. Studying the impact of the inhibition of Gq-alpha mutation on uveal melanoma growth can be done by determining changes in programmed death mechanisms by measuring caspase3 and tumor metastatic potential through PRAME (Preferentially Expressed Antigen in Melanoma) expression. From this the extent to which the mutation and YAP (yes-associated protein) drive uveal melanoma progression and the effectiveness of targeting factors in the mutated pathway can be determined.

b.**Question/problem:** How does the inhibition of the Gq-alpha mutation effect Uveal Melanoma growth?

Goal: To determine the impact of Gq-alpha inhibitors on uveal melanoma growth.

Hypothesis: The higher the concentration of Gq alpha inhibitor present, the lower number cells that will be alive and/or expressing high levels of a proliferative factors.

Role of Mentor

The student and mentor will work alongside each other in the passaging of cells, the transfection process, as well as administration of the Gxq mutation drug. The student will perform all calculations using imagej and cell profiler while the mentor will perform the statistical analysis.

c. **Procedures:**

1. **Providing Cells Fresh Media/Seeding the Cells:**

- The student will be involved in the cell culture activities of passaging and
- Confluent cell culture OMM1.3 will be grown within a proper container with a matrigel base to replicate the 3D environment that the tumor would naturally

2. **Apply Gxq Inhibitor**

- The inhibitor of the Gxq mutation will be administered to the cells line with predetermined quantities of 100um, 200um, and 300um of drug. The student will not be involved in this part of the study and this will be performed solely by the mentor.

3**. Cell Imagining**

- Using a confocal microscope the cells will be stained to identify the quantity of NR2F1 gene expression
- DAPI (4′,6-diamidino-2-phenylindole) will be used to confirm the presence of an intact cell.
- NR2F1 will be noted to determine the impact of the drug on cell dormancy.

4**. Analyzing PRAME in the cells**

- Select channels 0-2 and open them 00-DAPI (blue) 01-NR2F1(green) 02-PRAME (red). Put in the condition and picture name if the data (ex:C1.1-1)
- Next click Image then color. Next click merge color while selecting channels 00 and 01 so that the NR2F1 and DAPI will merge, allowing the cells that are NR2F1 positive to be seen..
- Use the color tool to mark the nuclei that were previously counted, counting NR2F1 positive cells first, which makes the overall calculation of total cells easier.Record data and determine general outline to use for analysis. (What cells

are being counted in the measurement). Then the student will click Analyze and set measurements, checking only the mean gray value background.

- Analyze with free hand tool and draw around previously decided path and Record that data. Then, divide average PRAME intensity by the number of nuclei to get expression per cell.

Analyzing Caspase in the cells

- Create a new folder for the experimental condition (control or drug treated), then add in the three channels for the condition into the pipeline.
- Go to export spreadsheet and that all measurements are being done recorded
- Check the image and see how many cells are present and if the image has any disturbances. Then, under identify primary object adjust the specificity of the object between 10-50// 300. The lower the number the more specific the computer will be, so with a few cells it will be better to be more general so disturbances aren't highlighted by the computer as well.
- When individual circled cells appear, some adjusts may need to be made. Right click on cells to highlight them
    - If two cells are highlighted together use S- split drag line from one side of the two cells to the other to split them
    - If one cell is broken up into two, select the areas then click J-join to merge the cells back together
    - If the cell is not highlighted at all, click f to freedraw around it.
    - If areas that are not cells are highlighted

        - Select them using a right click and then use x to create a box over the selected area you want deleted (it will only delete selected stuff)
        - Adjust the specificity under identify primary object is there are two many to delete.
- After proper adjustments have been made, click done and let the pipeline get the measurements.
- After results have finished processing, save all tables 11-16 and image 10 (cell number labels) to the correct folder using the save table feature for the tables to turn them into excel sheets and the pdf save function for image 10.
    - Make sure the texts are in the folder. If they are not then you did not export correctly.
    - Never click override in the beginning without making sure that the texts are being sent to the right folder.

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 face masks will be used when handling materials with a BioSafety Level greater than 1.

Data Analysis: Data analysis was performed using the software imagej by Java and cell profiler version 3.1.5 by the Broad's Institute to measure the respective quantities of PRAME and Caspase3 in uveal melanoma cells. Statistical analysis will be performed using Graph pad Prism8.

d. Vertebrate Animal Research

- Live animals were not used for the purposes of my experiment. I did not come into contact with any vertebrate animals during the time of my research. All of my experimentation was performed with cell lines obtained from the American Type Culture Company (ATCC).

e. Potentially Hazardous Biological Agents Research

a. The cell line will be obtained from the American Type Culture Collection (ATTC) under the catalog number MP46 CRL-3298. The BSL-1 is determined by the (ATTC)
b. For safety precautions the cells will only be handled under an adequate cell tissue hood while personal protective equipment is on. All waste will be disposed of in the designated biological waste containers.

f. Hazardous Chemicals, Activities, and Devices

a. All chemicals used in the experiment were determined by Mount Sinai to be BSL-1. To ensure further safety gloves and a hood will be used when handling any chemicals. Mentor will also be present with the student when any chemicals are in use.

d. **Bibliography**

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