Tumor transplantation assays of fluorescently-labeled uveal melanoma cell lines in zebrafish

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Celebration of Scholars 2019: Exposition of Student & Faculty Research, Scholarship & Creativity

Background

Uveal Melanoma (UM)

- Cancer that presents in the melanocytes causing abnormalities in cell growth.
- Represents 3-5% of all melanoma cases
- Develops in the pigmented part of the eye in the uvea, which consists of the iris, ciliary body, and choroid.
- Develops asymptomatically and typically metastasizes within 15 years to the liver.

Cell Lines

- Mel290: Melanocytes from a primary tumor of a UM patient
- OMM2.5: Melanocytes from a secondary (metastatic) tumor of a UM patient

Objective

To use *in vitro* methods to develop fluorescently labeled uveal melanoma cell lines. The cells will then be injected *in vivo* into zebrafish to better visualize and track the behavior of cancer in a living organism.

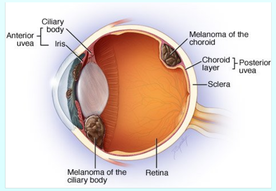

Figure 1. Uveal melanoma arises in the uvea of the eye, which includes the iris, ciliary body, and choroid.

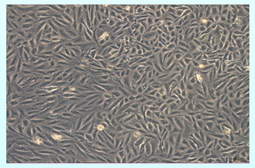

Figure 2. Morphology of live UM cells. Cells cultured *in vitro* have an oblong, epithelial shape and are adherent.

Methods

- ***Goal**:* fluorescently labeled UM cell lines
- ***Step 1:*** **Dosage curve** to determine most efficient antibiotic concentration for selection of transfected cells. What is the concentration of antibiotic that will kill 80% of cells at 48 hours?

  The antibiotic geneticin (G418) is used as a selective agent

  Designed a kill curve with geneticin concentrations ranging from 0 ug/ml to 1500 ug/ml
- ***Step 2:*** **Transfection** of cell lines with a fluorescent plasmid.
- ***Step 3:*** **Selection** of the cells that have been efficiently transfected with the plasmid.
  - Continue to add antibiotic to select the cells that successfully took up the plasmid.
  - Determine the percent of the total cell population that is green or red fluorescent via flow cytometry.
  - Confirm fluorescent expression via microscopy.
- ***Step 4:*** **Optimizing** the injection technique of cells into perivitelline space of zebrafish embryos.
  - Day 1: Breed adult zebrafish for embryos.
  - Day 2: Collect eggs and put in E2 media with PTU.
  - Day 3: Grow cells to 80% confluency and wash in Dulbecco's Phosphate Buffered Saline (DPBS), then replate.
  - Day 4: Trypsinize cells and wash in DPBS. Resuspend cells at a concentration of 30 cells per nl in media. Inject 5 nl of cell suspension containing 150 cells into the perivitelline space.

Figure 3. Injection of cells into the perivitelline space of a zebrafish embryo. At 48 hours post fertilization, embryos are injected with 150 GFP or RFP fluorescent cells. After another 48 hours, the embryos are imaged using a confocal fluorescent scope to show fluorescent cells dividing within the space.

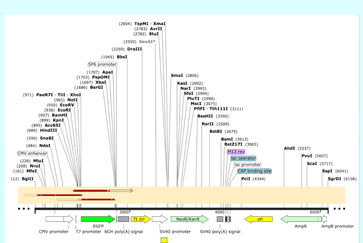

Figure 4. Green fluorescent protein plasmid used for transfection. With this plasmid, we can visually see the fluorescence expression in cancer cells and select the cancer cells that stably incorporated the plasmid DNA into their genome. Those cells are resistant to geneticin because they express the neomycin resistant cassette. A red fluorescent protein plasmid was also used in our research.

Results

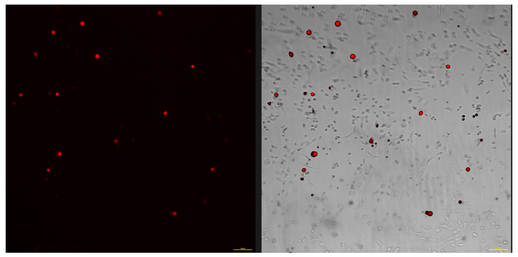

Figure 5. Epifluorescent microscopy of OMM2.5 RFP-expressing cells. (*Left*) Red designates cells that successfully express the RFP plasmid. (*Right*) Overlay of red demonstrates the cells in the population that took up plasmid.

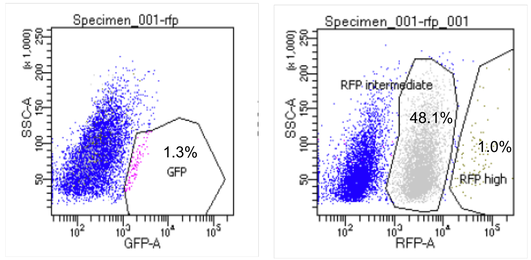

Figure 6. Flow cytometry of OMM2.5 from a fluorescence activated cell sorter. Blue represents cells that are not fluorescent. Pink represents GFP+ cells and grey represents RFP+ intermediate and high fluorescence. After sorting, cells were replated for maintenance of pure fluorescent populations.

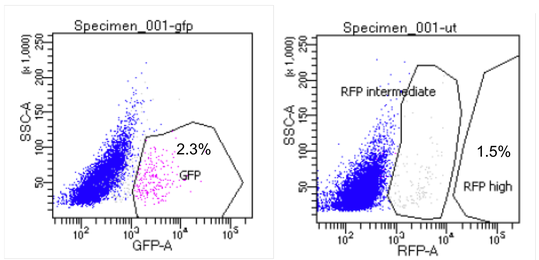

Figure 7. Flow cytometry of Mel290 from a fluorescence activated cell sorter. Blue represents cells that are not fluorescent. Pink represents GFP+ cells and grey represents RFP+ intermediate fluorescence. After sorting, cells were replated for maintenance of pure fluorescent populations.

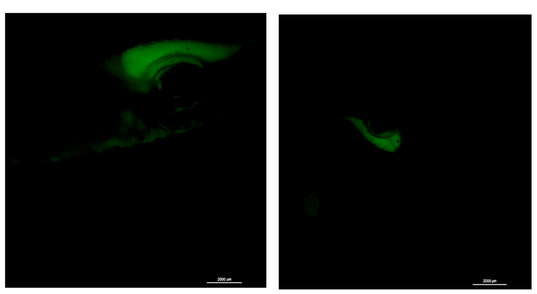

Figure 8. Injection site of OMM2.5 GFP cells. 150 cells were injected into the perivitelline space of larvae. 2 days post fertilization (dpf). To allow time for cell division, images were taken 9 dpf, which was 7 days after injection.

Conclusions and Future Directions

- Continue to inject the fluorescent cells into zebrafish embryos for *in vivo* studies of tumor development.
- Monitor growth of fluorescent tumors in hypoxic environments.
- Use the fluorescent cells to test effective cancer treatments.

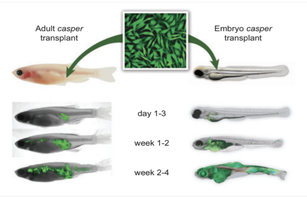

Figure 9. Tumor cell injections were started for the OMM2.5 GFP cell line to mimic previous *in vivo* studies that have been done to observe tumor development. This allows for easier visualization of tumor growth through development of the zebrafish which can correlate to how the cancer behaves in humans (Heilmann et al, 2015).

References

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Acknowledgements

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