Investigating the Role of a *p53* Mutation in Glioma Progression and Therapy Resistance in *Drosophila*

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Abstract

Gliomas, which are brain tumors that arise from glial cells, are some of the most aggressive and lethal types of tumors. These brain tumors are difficult to treat because not enough information regarding the mutations present in these tumors exists. This project studies effects of a *p53* mutation on *Drosophila* glioma progression and then will test to see if this results in resistance to current chemotherapy. *Drosophila* are used as model organisms to mimic these processes. The current genetic crosses that have been created will be studied, and an effective *p53* knockdown will be made. In essence, this will effectively mimic a human brain tumor so the treatments tested and the data collected from this model can be applied to the current understanding of human gliomas. In addition to studying just the *p53* mutation, PI3K and oncogenic Ras signaling will be coactivated. This will lead to an even more accurate glioma model because multiple mutations, such as the ones added are present in human tumors as well. These genetic crosses will be treated with Tyrosine Kinase Inhibitors, which are currently used to treat brain cancer patients in order to find out whether or not this mutation plays a role in resistance to current therapy. The main goal of this endeavor is to investigate the numerous defects occurring at the cellular and biochemical level in gliomas, which will give insight into why these types of tumors are so difficult to treat. Data gathered from this project will lead to further inquiry into the role of *p53* mutations in gliomas and hopefully, to better outcomes for those affected by this type of cancer. Here, we present the data gathered from this project thus far.

Fig. 1: Loss of p53 causes reduction in glia

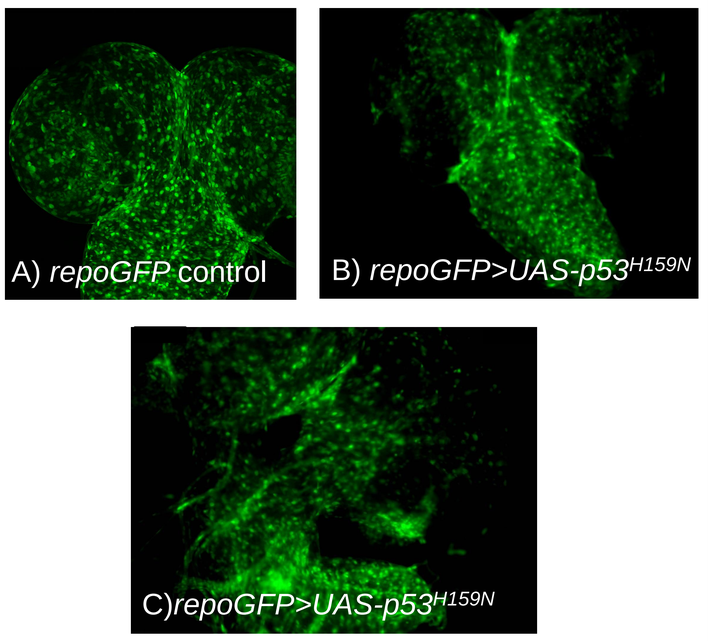
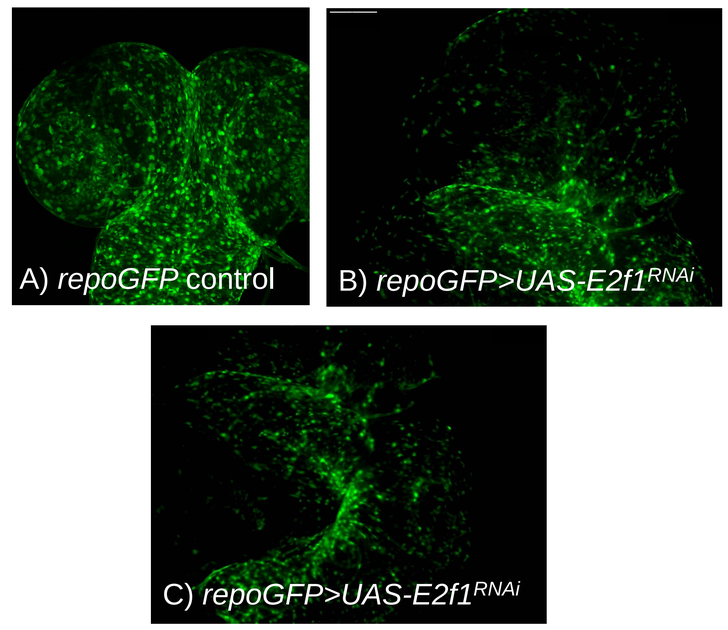


Fig. 2: Loss of E2F1 causes reduction in glia



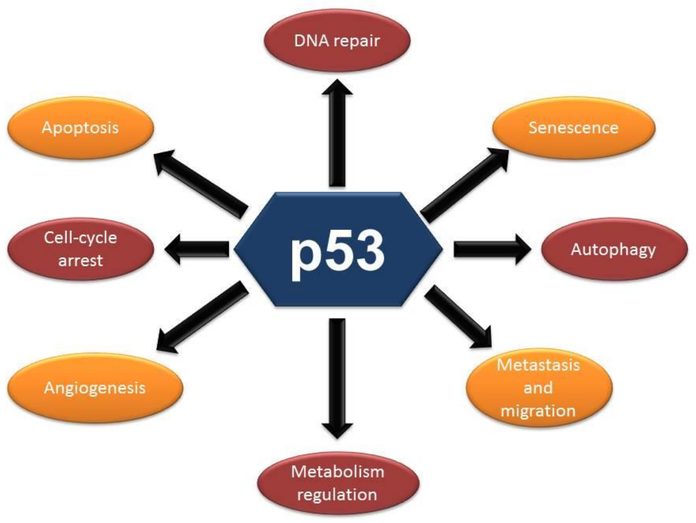
Introduction

The goal of this project is to investigate why some gliomas do not respond well to chemotherapy. One approach to this is to compare the response of two genetically different glioma to a sleuth of drugs known to partially affect or weakly inhibit tumor growth. The *p53* mutation is thought to play a role in this resistance. This specific mutation can be modeled in *Drosophila melanogaster*, both independently and in conjunction with other commonly occurring mutations in glioma.

The *p53* gene acts as a tumor suppressor in addition to its numerous other functions concerning the cell cycle. The E2F family of genes, which will also be tested along with *p53*, help control the cell cycle. Mutations in both of these genes are known to occur in patients with gliomas, but their role in glioma progression and response to therapies is not well understood.

If more information regarding the exact mutations present in gliomas existed, more targeted therapies could be used to act on the pathways known to contribute to tumor development and chemotherapy resistance. This project aims to explore these mutations in an effort to bridge this gap in information, so therapies can efficiently destroy the tumor without causing damage to healthy tissues. We present the preliminary data necessary to create the various glioma model here.

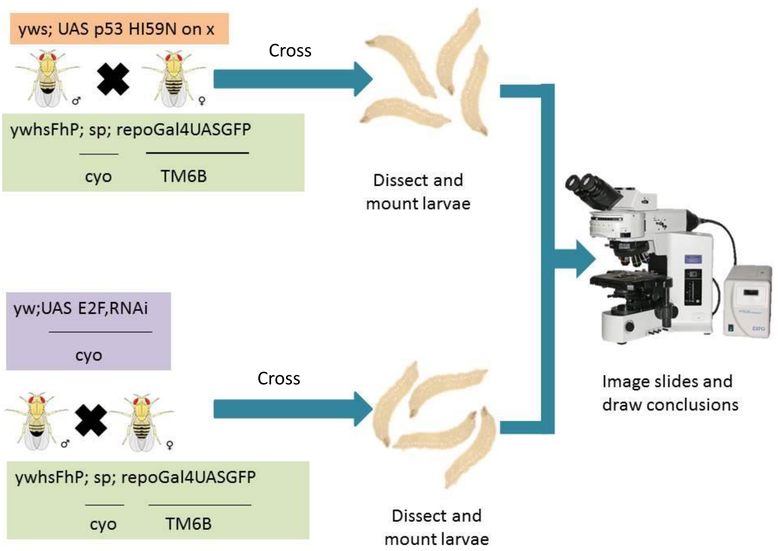
Functions of *p53*



Conclusions

- The larvae produced from the *UASp53H159N; repoGal4* cross had decreased glia in the optic lobes and they were clustered in the ventral nerve cord.
- The larvae produced from the *UASE2FRNAi; repoGal4* cross had glia clustered in the ventral nerve cord as well.
- Some of the larvae from the UASE2FRNAi; repoGal4 were deformed and had their ventral nerve cord curved upward, attached to an optic lobe.
- A majority of the larvae hatched. This viability shows that further crosses made with this offspring are possible.
- This data shows promise for continued genetic crosses to eventually create a *p53* knockdown which exhibits glioma.

Experiment Design



Role of *p53*

This specific gene is known to be a tumor suppressor but it also has many other vital functions, such as modulating the cell cycle, repairing DNA and triggering apoptosis. Certain p53 mutations are thought to be early events in the process of tumorigenesis. Loss of *p53* can cause an increased risk for gliomas to develop when they occur as germline mutations and often occur with the deletion of the chromosome 17p. *p53* mutations are most often seen in patients with high-grade gliomas rather than those with low-grade tumors, which suggests its possible usefulness in predicting prognoses. We will use a dominant negative p53 mutation [UAS-p53H159N] for our studies.

Role of E2F

E2F genes encode transcription factors that are responsible for modulating the cell cycle. The transcription factors are known to play a role during the transition from the G1 to the S phase, however, they are thought to modulate other phases of the cycle as well. These E2F genes can also work as tumor suppressors and when mutations occur, this function no longer works correctly. Furthermore, E2F makes proteins that mediate p53-independent/dependent apoptosis. We will knock-down dE2F1 using a RNA interference approach [UAS-E2F1RNAi].

Future Directions

1. Create an effective glioma model expressing a *p53* mutation
2. Combine the *p53* mutation with coactivation of Ras PI3 Kinase along with additional mutations that are present in human gliomas.
3. Treat the *p53*, Ras PI3 Kinase gliomas with Tyrosine Kinase Inhibitors in order to test for therapy resistance.
4. Use Western blotting to determine the mechanism of the mode of action.

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