Effects of Tyrosine Kinase Inhibitor Drugs on Glioma

Kaitlyn Alleman[3], Molly Buchanan[3], Laura Bute[1], Nathan Holthaus[1], Kathleen McCaslin[3], Katie Parker[1], Karishma Gangwani[1], Kirti Snigdha[1], Madhuri Kango-Singh[1,2,3]
1) Department of Biology 2) Center for Tissue Regeneration and Engineering at Dayton (TREND)
3) Premedical Programs, University of Dayton, Dayton OH 45469

Abstract

- Glioma are brain tumors with very poor prognosis. The standard of care is surgery followed by radio- and chemo/immuno-therapy, or combinations thereof, however, healthy cells are affected as well as tumorous cells and all patients eventually die. Thus, there is a need to test if recently approved drugs can inhibit the growth and progression of this tumor. We have developed a Drosophila glioma model based on the two genetic/ oncogenic pathways known to be most frequently activated in patients viz., the Ras/MAPK pathway and the PI3K pathway. We designed a chemical screen involving drugs targeting Tyrosine kinases (Selleck Biochem Chemical library) – key enzymes that are activated by oncogenic pathways. The chemical screen involves feeding glioma containing larvae 10uM and 300uM drugs from the library at early third instar stage, then allow these larvae to grow and mature to the third instar stage (120h of development), and then dissect the brain to study effects on glioma growth and track survival on days 5-7 when other glioma positive larvae die. Here, we present data from our screen on promising drugs from this academic year's testing focusing on drugs H10 and H11. Once we identify potential glioma inhibitors in the primary screens, we will validate them in secondary screens.

Introduction

- This project is a chemical drug screen that has a goal to determine if newly approved Tyrosine Kinase Inhibitors are capable of limiting growth and progression of glioblastoma
- Two of the most frequent oncogenic pathways, Ras/MAPK and Pi3K, were used to model human glioma in *Drosophila*
- *Pten* is a tumor suppressor and the down regulation of Pi3K was deactivated through RNA interference
- *Pten* mutations are found in many patient biopsies of glioma
- Ras^{V12} is the most frequent oncogenic mutation found in many human tumors
- The expression of both $Pten^{i}$; Ras^{V12} in unison more accurately models human glioblastoma in *Drosophila*
- In this chemical screen and this specific poster presentation, the efficacy of chemical P1H10 and P1H11 are examined

Figure 1: Tumor Development

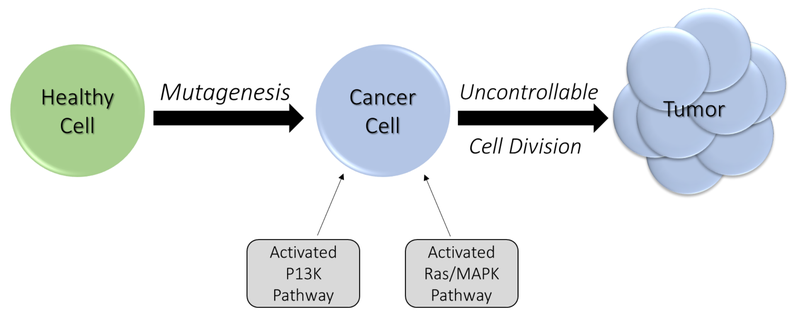
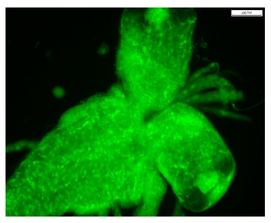


Figure 2: Drug Screen Process

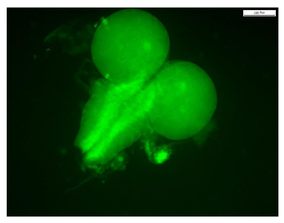
1. *Pten*^{i} and *Repo* Stocks are prepared
2. Female virgins are collected from the $Pten^{i}$;Ras^{V12} stock
3. Collected virgins are added to *Repo* males (2:1 ratio)
4. After Day 3, non-TM6B larvae are collected
5. The collected larvae are added to the drug vials
6. On Days 5 and 6, larvae are dissected, mounted and imaged
7. Day 7 pupated larvae are collected and imaged
8. Data is recorded and analyzed

Figure 3: Means for Comparison

A) Repo Control



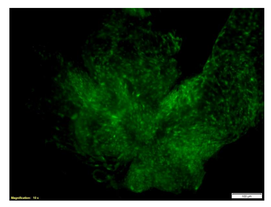
B) Enhanced Glioma



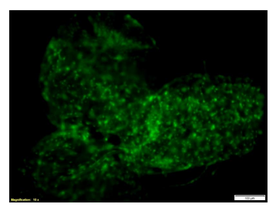
C) Suppressed Glioma

Figure 4: P1H10 and P1H11

P1H10 Potential Suppression

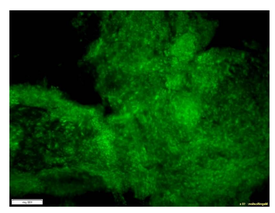


$Pten^{i}$; Ras^{V12} *X Repo; GFP*

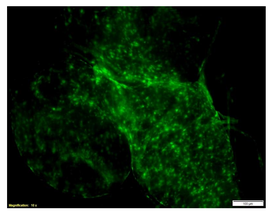


Repo; GFP Control

P1H11 No Effect



$Pten^{i}$; Ras^{V12} *X Repo; GFP*



Repo; GFP Control

Table 1: Drug Data Summary

Drug	Concentration	Dissection	Results
P1E7	300um	Day 5	Suppressed
P1E9	10um	Day 5	No Effect
P1E11	10um	Day 5	Suppressed
P1H10	300um	Day 5	Suppressed
P1H11	300um	Day 5	No Effect

Conclusions

- Our data has provided multiple candidates for the secondary screen, especially E7, E11 and H10.
- These drugs that are promising candidates for the second screen due to their potential suppression effects.
- The method of comparison between larval brain images continues to provide sufficient data to either pursue or eliminate a drug from further experimentation.

Future Directions

- We plan to continue the chemical screen using additional drugs from the Selleck Biochem Chemical library.
- We will begin to validate the potential glioma reducers to find the specificity and efficacy of these potential modifiers of glioma growth.

Acknowledgments

We would like to thank Dr. M Kango-Singh for her support and guidance throughout this experience. A special thanks to Kirti Snigdha and Karishma Gangwani for their help in teaching the techniques necessary for the experimental setup. We thank the Singh lab for the use of the fluorescence Stereomacroscope Zeiss Stemi-V8, and the Nakano Lab (University of Alabama at Birmingham) for the chemical library. Funding from GSSF to KS and KG, University Start-up support and subaward from NIH to MKS, and Stander Fellowship to Katie Parker, The Summer Collaborative Research Projects (CoRPs) program award to Nathan Holthaus and Timothy Cook are acknowledged.