SJSU SAN JOSÉ STATE UNIVERSITY

SJSU Undergraduate Research Grants

Probing Autophagy in *Drosophila* Eye Development

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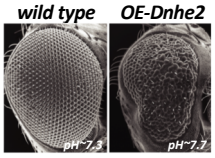
Abstract

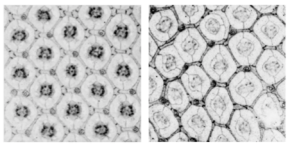
Cancer cells share common characteristics regardless of tissue type of genetic mutation, including increased cell proliferation, and deceased cell death. Many cancers also have increased intercellular pH (pHi), which is an understudied characteristic of cancer. We have preliminary data from other work in our lab that suggests that autophagic cell death is increased with higher pHi. This presents an intriguing paradox, as cancer cells are generally thought to be resistant to cell death.

The goal of this project is to resolve whether autophagy is increased in established tumor models in the developing Drosophila wing. We will perform genetic crosses to make flies with tumors that express green fluorescent protein (GFP) to visualize tumors. I will dissect these tissues, fix them and immunolabel to determine tumor size and levels of autophagy (Atg) regulatory proteins.

Rationale

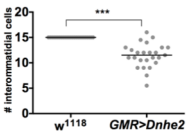


Grillo-Hill *et al.* eLife 2015;4e03270

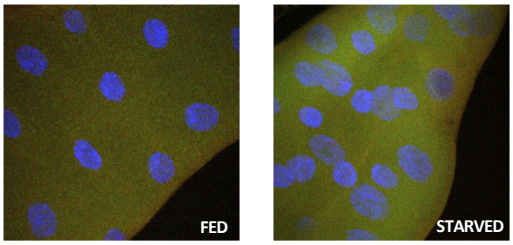


w1118, pHi 7.2 GMR>DNhe2, pHi 7.6

Over-expression of *DNhe2* in the fly eye increases pHi and causes a smaller eye. The wild-type adult eye has pHi ~7.3 and displays precisely patterned hexagonal ommatidia. Over-expression of *DNhe2* increased pHi to ~7.7 and significantly disrupted patterning. This is due to missing cells, as shown in the image of a pupal eye and quantification of cell number.

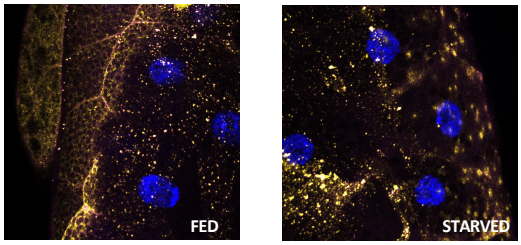


Genetic tools to detect autophagy- ATG8a::mCherry::GFP



In controls, we grew *Drosophila* larvae under fed or starved conditions. Previous reports showed that starvation induces increased expression of ATG8a, so we expected to see more red and green in our starved conditions. However, the signal appeared identical in both conditions.

Tools to detect autophagy- ATG5 antibody labelling



In controls, we grew *Drosophila* larvae under fed or starved conditions. Previous reports showed that starvation induces increased expression of ATG5, so we expected to see more yellow in our starved conditions. However, the signal appeared similar in both conditions.

Conclusions and Future Directions

Can we use published tools to detect autophagy in Drosophila?
We obtained published tools, however they do not show increased expression when we induce autophagy in controls.

Are there other tools we can use?
Yes, we are ordering other tools, and we will test them to be sure that they detect autophagy in our control experiments.

Ultimately, our goal is to combine autophagy reporters with tumor models in *Drosophila* to determine whether autophagy is increased in tumors, and if this is dependent on increased pHi.

Citations and Funding

1. Grillo-Hill BK, Choi C, Jimenez-vidal M, and Barber DL (2015). Increased H+ efflux is sufficient to induce dysplasia and necessary for viability with oncogene expression. *eLife*. 4:1-40.
2. White KA, Grillo-Hill BK, and Barber DL. (2017). Cancer cell behaviors mediated by dysregulated pH dynamics at a glance. *J Cell Sci*. 130:663-669.

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