

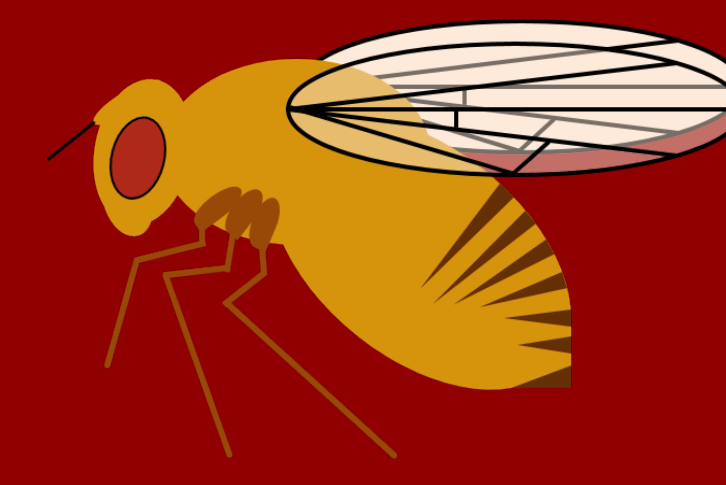


Effectiveness of Metformin in Cancer Combination Chemotherapy

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Introduction

Background

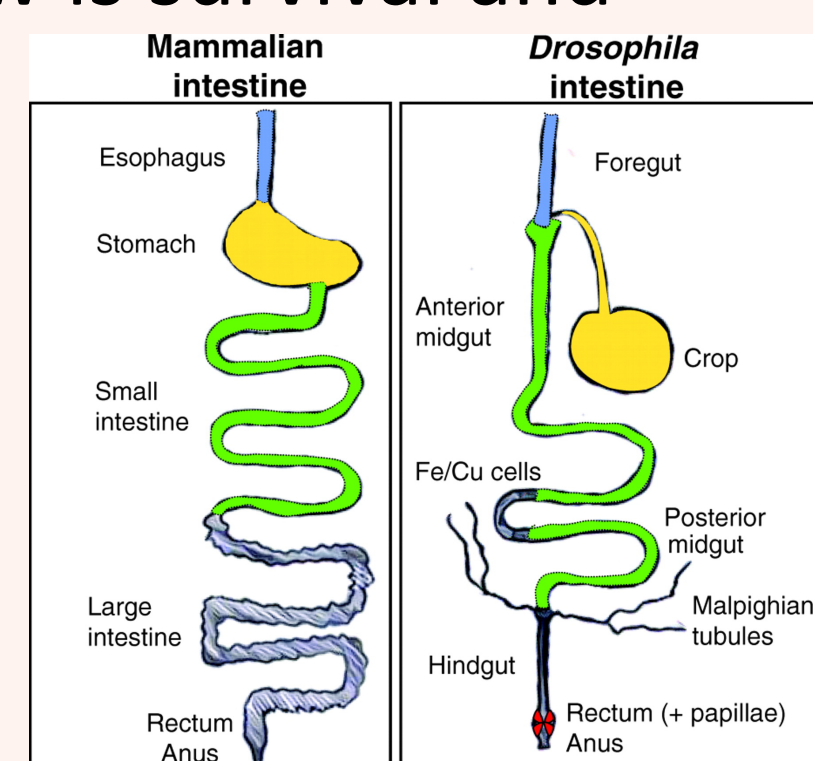
- Combination chemotherapy has been shown to be effective
- ABC transporters were recently discovered to pump out drugs, causing cancer cells to be drug resistant

Goals

- Can metformin, an inexpensive diabetic drug, amplify the effects of rapamycin and lower the toxicity of vincristine in cancer chemotherapy?
- If MRP, an ABC transporter, is removed, how is survival and cancer resistance affected?

Model: *Drosophila melanogaster*

- MRP: transporter in ABC family
- 59% similar to ABC transporter coded by ABCB3 gene in humans



Materials and Methods

Confirm MRP expression in the gut

- Set up genetic cross such that GFP is expressed where MRP is expressed

MRP-T2A-gal4	✗	UAS-GFP
Male		Female

- Dissect offspring gut and image with fluorescence microscopy

Determine fly survival without MRP

- Set up genetic cross such that MRP is not expressed in offspring (marked with straight wings) and count amount of alive straight-winged offspring

UAS-RNAi-MRP	✗	Act-gal4
Male		CyO (curly)
		Female

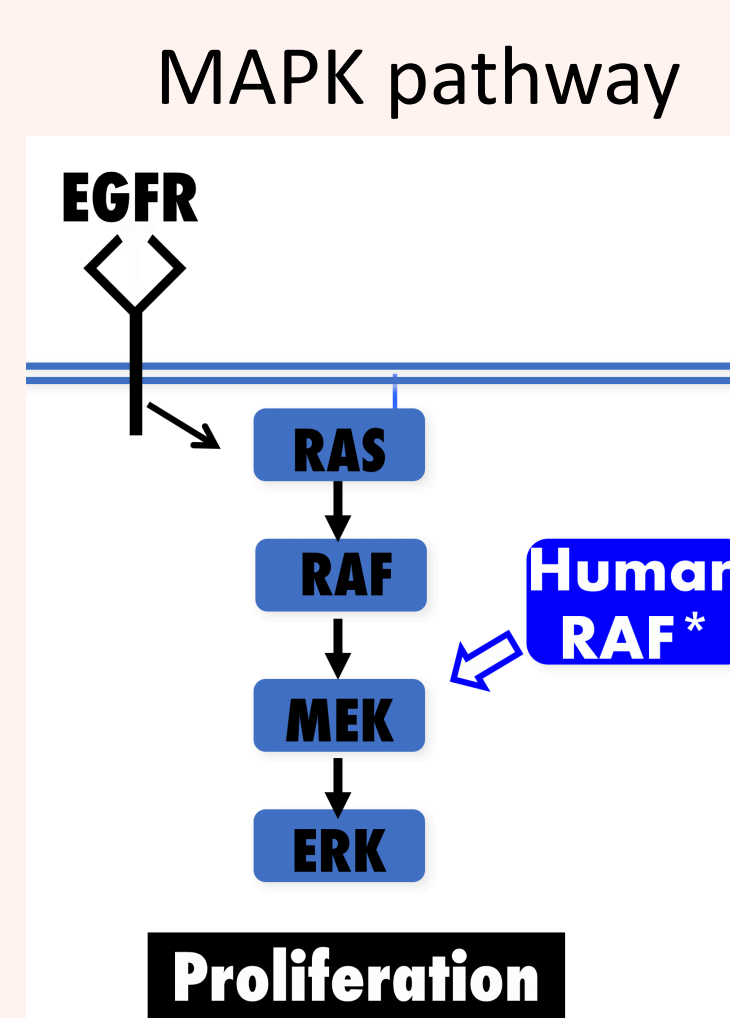
Create tumors

- Engineer flies with mutated proto-oncogene, which activates MAPK pathway leading to proliferation

UAS-RAF:EGT

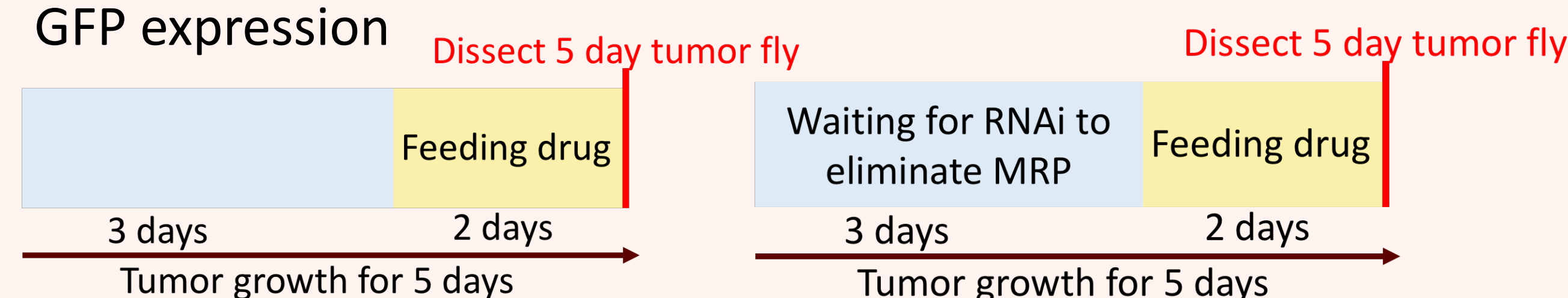
- To get tumor flies without MRP, set up genetic cross with MRP RNAi

UAS-RNAi-MRP	✗	UAS-RAF:EGT
Male		Female



Feed drugs and observe tumor response

- After tumor grows for 3 days, mix in drug with fly food
- After 2 days of drugs, dissect and image guts with fluorescence and confocal microscopy
- Visually inspect images to qualitatively determine amount of GFP expression

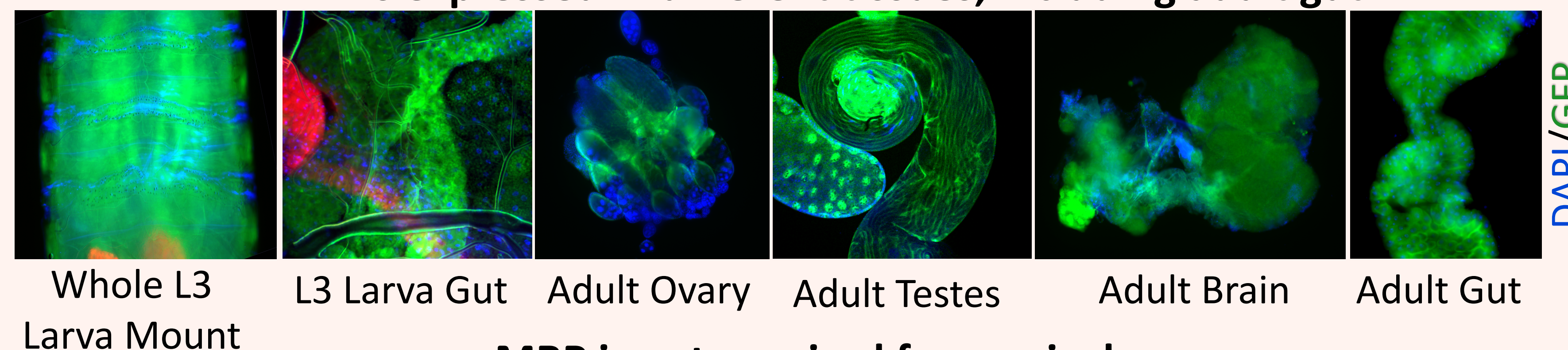


Drugs Used

- Rapamycin:** immunosuppressant, modest antiproliferative medicine
- Vincristine:** strong, toxic chemotherapy drug with side effects, such as hyponatremia and neuropathy
- Metformin:** Type 2 diabetic drug, lowers blood sugar levels

Results

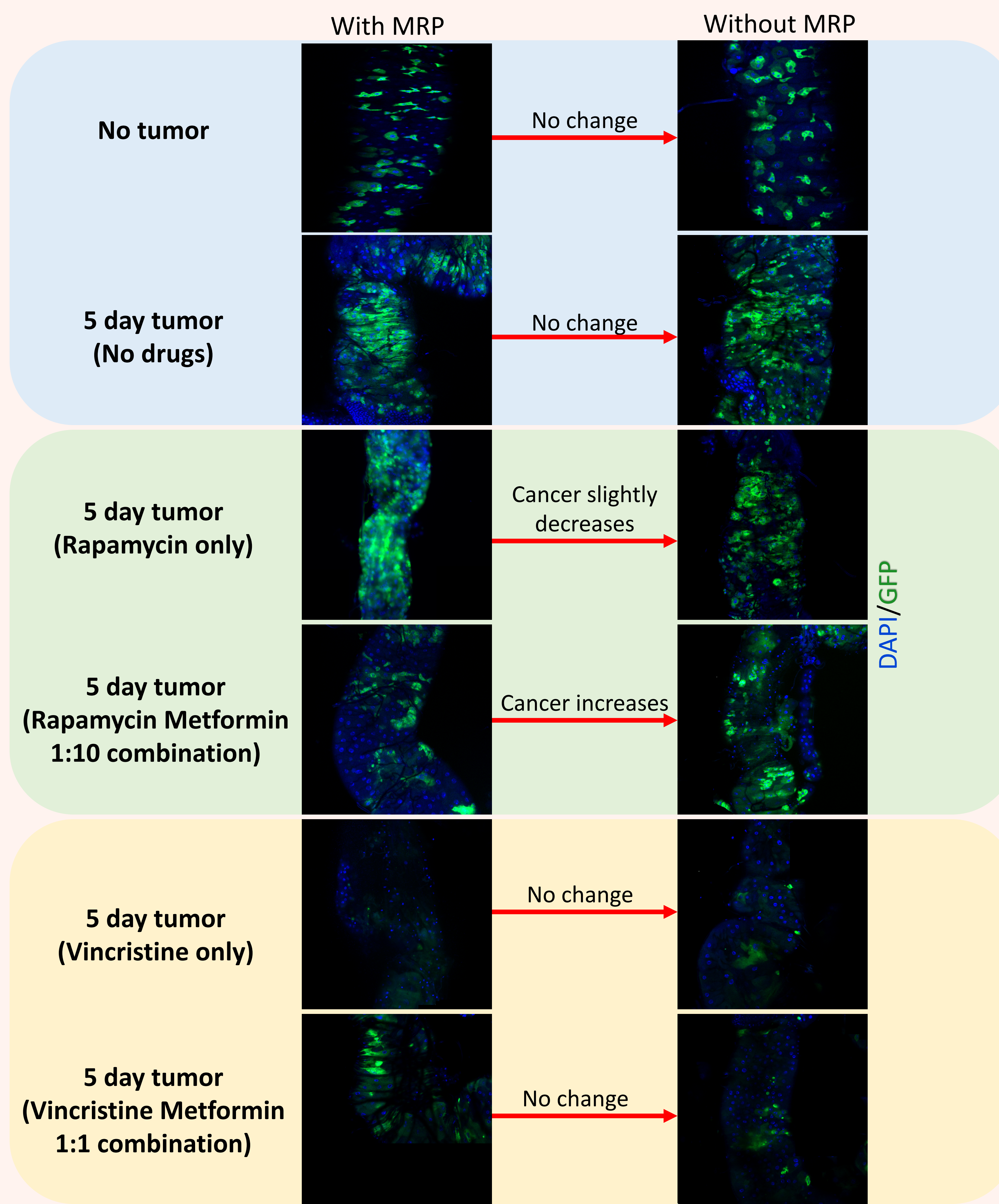
MRP is expressed in different tissues, including adult gut



MRP is not required for survival

	Males	Females	Total
Straight-winged offspring (lack MRP)	62	61	123
Curly-winged offspring (contain MRP)	27	30	57

Combination chemotherapeutics were effective Evidence about role of MRP in response to drugs is not conclusive



Conclusions

Chemotherapeutic effectiveness in cancer suppression

- Rapamycin alone was not noticeably effective
- Rapamycin Metformin 1:10 combination was effective
- Vincristine alone was very effective but also was toxic, killing most of the normal stem cells as well
- Vincristine Metformin 1:1 combination was very effective and spared normal stem cells

Influence of MRP on tumor stem cells

- Fly survival was not impacted by lack of MRP
- Tumor stem cells was not affected by MRP presence/absence
- Effect of MRP in tumor reduction with drug combinations is not conclusive
- Rapamycin Metformin 1:10 combination with MRP and Vincristine Metformin 1:1 combination without MRP were most effective

Metformin amplified the effectiveness of Rapamycin and lowered the toxicity of Vincristine

Future Directions

- Determine statistical significance of results by conducting more experiments
- Quantify tumor using precise methods, such as luciferase assay
- Determine impact of varying drug ratios on cancer reduction
- Determine effectiveness of other drug combinations
- Determine rate of tumor progression with drug combinations

References

- FlyBase. (n.d.). Retrieved from <https://flybase.org/reports/FBgn0032456>
- Drosophila Workers Unite! (n.d.). Retrieved from <http://marksteinlab.org/dwu/>
- Chalfie, Tu, Y., Prasher, D., & WW Ward. (1994, February 11). Green fluorescent protein as a marker for gene expression. Retrieved from <https://science.sciencemag.org/content/263/5148/802>
- Markstein, M., Dettorre, S., Cho, J., Neumüller, R. A., Craig-Müller, S., & Perrimon, N. (2014, March 25). Systematic screen of chemotherapeutics in *Drosophila* stem cell tumors. Retrieved from <https://www.pnas.org/content/111/12/4530>
- Zhang, J.-W., Zhao, F., & Sun, Q. (2018, February). Metformin synergizes with rapamycin to inhibit the growth of pancreatic cancer *in vitro* and *in vivo*. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5774390/>
- Yi, Y., Gao, L., Wu, M., Ao, J., Zhang, C., Wang, X., ... Xiao, Z.-X. J. (2017, August 21). Metformin Sensitizes Leukemia Cells to Vincristine via Activation of AMP-activated Protein Kinase. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/28900501>

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