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Effect of Redox Environment on Number of Stem Cells in Drosophila Gut

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Introduction

Cancer originates from normal cells that undergo mutations in their DNA, allowing them to proliferate in an uncontrolled manner. The abnormal growth can be due to either overactive oncogenes, which promote growth, or loss of tumor suppressors which inhibit growth.

Drosophila melanogaster is a model organism used for studying cancer due to ease of genetic manipulation, having similar genetics to humans, and rapid generation turnover.

Cancerous cells can contain stem cells, allowing "self-renewal" to occur and thus having the ability to proliferate indefinitely [1].

PRL-1 has been seen to function as both a tumor suppressor and an oncogene. A possible mechanism is that redox conditions cause this switch [2-3].

Increased expression of enzymes that promote reduction, i.e. antioxidants, have been found in multiple cancers. In addition studies have shown that the activity of PRL1 is increased by an enzyme that serves as an antioxidant [4].

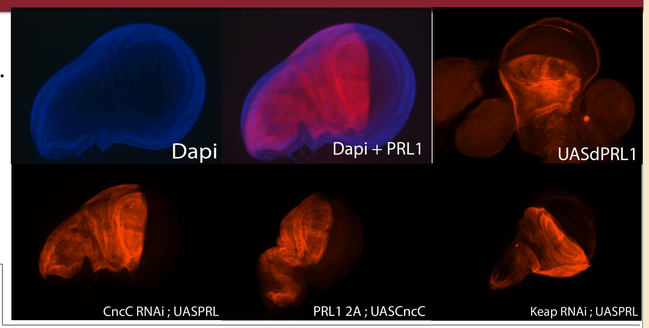
Objectives

- Determine baseline effect of altering redox condition in *Drosophila* Intestinal Stem Cells (ISCs).
- Determine if PRL-1 will switch from growth suppressing to growth promoting in reduced environments.
- Determine if the function of PRL1 is altered by increasing/decreasing antioxidant pathways.

Results

PRL was found to be in three of the four lines: PRL + CncC, PRL + CncC^{RNAi}, and PRL + Keap^{RNAi} (Figure 2).

Figure 2. Confirmed expression of PRL. *Wandering larvae wing discs were examined to confirm PRL was in the flystock, an apterous driver was used to express PRL + CncC, PRL + CncC^{RNAi}, PRL + Keap^{RNAi}, PRL + Keap in ½ of the wing. An entire wing disc is shown (Dapi), with the dorsal tissue expressing PRL (Dapi + PRL1). PRL was found to be in the dorsal tissue of PRL + CncC, PRL + CncC^{RNAi}, and PRL + Keap^{RNAi} , however, Keap + PRL did not express PRL (data not shown).*



Keap1^{RNAi}, reduced the size of stem cell niches by 43.5% (p value < 0.0001). Keap1^{RNAi} + PRL did not statistically differ from the positive control of PRL (p value = 0.2108) (Figure 3).

The addition of CncC III, which promotes reduction, resulted in a 35.9% overgrowth in the stem cells (p value = 0.006). PRL + CncC III resulted in a reduced environment that had 17.2% overgrowth (p value = 0.0117). While PRL + CncC^{RNAi} increases oxidation, shown by an 18% increase in stem cell count (p value = 0.0083) (Figure 4).

Ras was a positive control for oncogene phenotype (overgrowth), resulting in a 14.6-fold overgrowth in the stem cells (p value < 0.0001) (Figure 5).

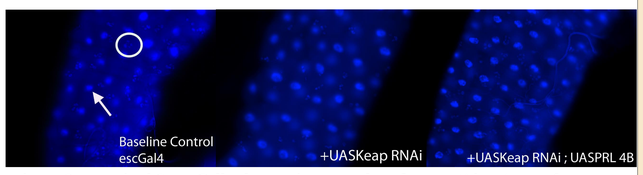


Figure 3. Intestinal Stem Cells clusters in Keap altered guts. *Midgut issue of wandering larvae was stained using Hoechst's Blue stain. An escargot driver (escGal4) was used to express Keap^{RNAi} and Keap^{RNAi} + PRL1^{4B}. The escGal4 control shows a stem cell cluster (circle), and a large gut cell nuclei (arrow). Keap^{RNAi} has fewer stem cells per cluster suggesting inhibits growth.*

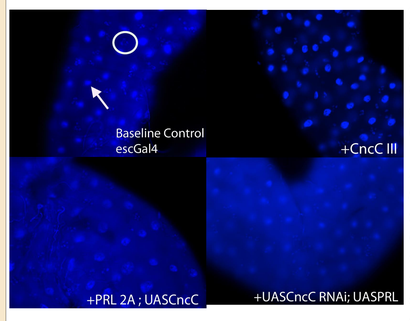


Figure 4. Intestinal Stem Cells clusters in CncC altered guts. *Midgut issue of wandering larvae was stained using Hoechst's Blue stain. An escargot driver (escGal4) was used to express CncCIII, PRL + CncCIII , CncC^{RNAi} + PRL1^{4B}. The escGal4 control shows a stem cell cluster (circle), and a large gut cell nuclei (arrow). The addition of CncC resulted in a 36% overgrowth, while the further addition of PRL, reduced the overgrowth to 17-18%.*

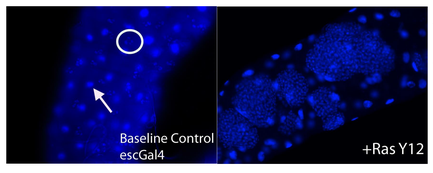


Figure 5. Intestinal Stem Cells clusters in Ras altered guts. *Midgut issue of wandering larvae was stained using Hoechst's Blue stain. An escargot driver (escGal4) was used to express Ras. Ras has a 14 fold increase in stem cells per cluster.*

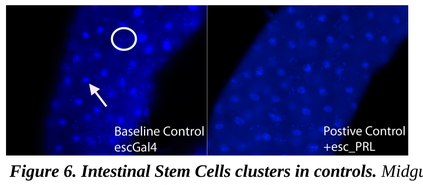


Figure 6. Intestinal Stem Cells clusters in controls. *Midgut issue of wandering larvae was stained using Hoechst's Blue stain. An escargot driver (escGal4) was used to express PRL1^{4A}. The escGal4 control shows a stem cell cluster (circle), and a large gut cell nuclei (arrow). The controls do not differ statistically significantly.*

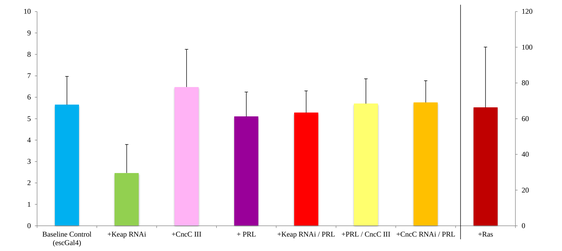


Figure 7. Overgrowth with the addition of PRL.
A comparison of the averages of all three trials for every cross. Ras, on a second axis scaled to the right, resulted in a significant increase in stem cell count, while Keap^{RNAi} resulted in a significant decrease in stem cell count. With PRL present, the number of stem cells increases, slightly.

Conclusions

Keap1^{RNAi} increases the amount of CncC and the antioxidant activity, however suppresses stem cell number.

CncC III increased the antioxidant activity and resulted in an increase of stem cell count.

Future Direction

- Continue analyzing data.
- Get data for Keap, using same experimental methods.
- Look at Keap^{RNAi} and how it suppressed growth. Run more trials and introduce other inhibitors.

Methods

The UAS/GAL4 driver system was used to genetically alter specific tissues, while leaving the rest of the animal unaltered. UAS enhances the transcription of the target gene only when until the gene is bound by GAL4.

1) Crosses were set up to confirm if previously established lines contain PRL1. Wings from larvae were fixed and antibodies were used to detect the presence of PRL1. Dapi for DNA
   apGal4 → dorsal wing tissues (wing discs in larvae)
2) Wandering larvae were dissected, fixed, and stained with the nuclear dye Hoechst's. Using epifluorescence, the guts were examined and stem cells in clusters were counted.
   escGal4 → gut stem cells
3) Redox levels were altered through crossing escGal4 with PRL, CncC, CncC^{RNAi}, Keap, Keap^{RNAi}. CncC promotes reduction (antioxidant), while Keap1 negatively regulates CncC (figure 1). Subsequently, the effect of redox alterations in combo with overexpression of PRL1 was examined.

Figure 1. Altering Redox Conditions. *CncC promotes reduction (antioxidant), while Keap1 inhibits CncC, regulating reduction. In the presence of Keap1 + CncC^{RNAi} there is a decrease in antioxidant. In the presence of Keap1^{RNAi} + CncC there is an increase in antioxidant.*

References

[1] Shackleton M (2010) Normal stem cells and cancer stem cells: similar and different. *Semin Cancer* Biol 20(2):85-92 doi: 10.1016/j.semcancer.2010.04.002

[2] Pagarigan KT, Bunn BW, Goodchild J, Rahe TK, Weis JF, Saucedo LJ (2013) Drosophila PRL-1 is a growth inhibitor that counteracts the function of the Src oncogene. *PLoS One* 8(4): e61084

[3] Basak S, Jacobs SB, Krieg AJ, Pathak N, Zeng Q, Kaldis P, Giaccia AJ, Attardi LD (2008) The metastasis-associated gene Prl-3 is a p53 target involved in cell-cycle regulation. *Mol. Cell*. 30(3):303-314 doi: 10.1016/j.molcel.2008.04.002.

[4] Biaglow JE, Miller RA (2005) The thioredoxin reductase/thioredoxin system: novel redox targets for cancer therapy. *Cancer Biol Ther* 4(1):6-13.

Acknowledgements

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