Modulating the Cancerous Effects of the Oncogene Src

Katherine Segar
ksegar@pugetsound.edu
Cancer is fast becoming the largest global disease burden, affecting millions of people across the world. The oncogene Src has been implicated in breast and colon cancers as well as melanomas. Src is related to cell-to-cell adhesion and is regulated by reactive oxygen species (ROS). ROS are highly reactive compounds produced via cellular metabolism and are used for cellular signaling can cause cellular damage at high levels. High levels of Src activation and ROS leads to a the formation of a tumor in the Drosophila melanogaster but it is unclear what mechanisms allow this to happen.

How does the combination of Src and ROS promote tumorigenesis?

**Question**

**Methods**

- Allows for tissue specificity via UAS (we are using UAS that are only expressed in wings and thorax)
- Temperature sensitive; higher temperature leads to higher expression
- Overexpression mimics oncogene inducing mutations, creating an artificial oncogene
- Using Immunofluorescence to track proteins indicative of characteristics of cancer cells; (i.e. DCP-1 stain for apoptosis and E-cadherin stain for migration)

**Background**

- Cancer cells are characterized by six hallmarks: evading apoptosis, resistant to anti-growth signaling, sustaining proliferative signaling, inducing angiogenesis, divisional immortality, and tissue invasion
- Cancer cells have higher levels of ROS due to an increased metabolic rate
- The role of Src in cancer has long been unclear as overexpression of Src has been shown to lead to apoptosis in addition to proliferative effects
- Increased levels of Src activation with high levels of ROS could be changing proliferation rate, evading apoptosis as a form of organellar compensation for increased proliferation, or inhibiting cellular migration as a form of organellar compensation

**Results**

- Overgrowth phenotypes seen in adult ↑Src ↑ROS wings do not appear to reflect less apoptosis or organellar compensation as apoptosis does not vary between ↑Src and ↑Src ↑ROS wing discs (Figure 4) and the addition of DIAP does not increase tissue size (Figure 5).
- E-cadherin staining did not demonstrate differences in localization or intensity between ↑Src and ↑Src ↑ROS and controls (data not shown), indicating that migration does not play a significant role in tumorigenesis. Instead, the data suggests that ↑Src ↑ROS combination is impacting proliferation rates.

**Figure 1.** ROS changes phenotype of Src overexpression. ↑Src by itself does not produce a notable phenotype, with either increased levels of proliferation or migration leading to organellar compensation which prevents a notable phenotype. ↑Src + ↑ROS however could change either Src expression or organellar compensation.

- ↑Src + ↑ROS

**Figure 2.** Formation and functions of Reactive Oxygen Species. Under normal conditions, high levels of ROS leads to apoptosis whereas at lower levels, ROS are regulated by antioxidants and function in cellular signaling that can promote cell survival and proliferation. By increasing antioxidants, cancer cells can balance higher levels of ROS, to induce higher levels of cell signaling and proliferation, but avoid apoptosis (red arrow).

**Figure 3.** UAS-GAL4 system. The UAS line has the gene of interest under the control of the UAS while the Gal4 line generates the promoter to increase expression of genes under the control of the UAS. When the two parental lines are crossed, one with UAS and the other with Gal4, Gal4 will interact with the UAS and activate Src (UASsrc) expression and/or increase ROS levels (UASsrcCRNAi).

**Figure 4.** Increased size and apoptotic activity at 25°C and 18°C in Larval Drosophila wing disc. Larval wing discs were dissected and stained to investigate general wing disc size (DAPI) and apoptosis activity (DCP-1) (Figure 4A). Both ↑Src and ↑Src, ↑ROS were found to be larger than either control (p<0.05) at both 25°C and 18°C. There was no statistical difference between ↑Src and ↑Src, ↑ROS due to wing disc size. Both ↑Src and ↑Src, ↑ROS were also found to have a higher proportion of caspase activity than either control (p<0.05). There was no statistical difference between ↑Src and ↑Src, ↑ROS due to apoptosis activity (Figure 4B).

**Figure 5.** Posterior wing compartment size differences in adult Drosophila. Src and ROS were expressed in posterior adult wing compartments (Figure 5A). Adult Drosophila wing anterior and posterior compartments were compared to identify size differences (Figure 5B). DIAP indicated inhibition of apoptosis. ↑Src ↑ROS was larger than the control (engal) (p<0.05) but was not significantly larger than ↑Src. 73% of ↑Src and 78% of ↑Src ↑ROS demonstrated cross vein overgrowth as compared to the 0% of the ↑Src +DIAP and ↑Src +ROS +DIAP wings.

**Conclusion**

Overgrowth phenotypes seen in adult ↑Src ↑ROS wings do not appear to reflect less apoptosis or organellar compensation as apoptosis does not vary between ↑Src and ↑Src ↑ROS wing discs (Figure 4) and the addition of DIAP does not increase tissue size (Figure 5). E-cadherin staining did not demonstrate differences in localization or intensity between ↑Src and ↑Src ↑ROS and controls (data not shown), indicating that migration does not play a significant role in tumorigenesis. Instead, the data suggests that ↑Src ↑ROS combination is impacting proliferation rates.

**References**


**Acknowledgements**

I would like to thank the Sherman Fairchild foundation and the University of Puget Sound for financial support of this project. I would also like to thank my mentor Dr. Saucedo and my lab mates, Ali and Chris.