Genetic Manipulation of the Cellular Redox Environment Alters Oncogenic Phenotypes in *Drosophila*.

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ABSTRACT

The paradigm of antioxidants serving a protective role in cancer has shifted dramatically in recent years. Cancers often aberrantly express enzymes that control the redox environment and administration of antioxidants has been shown to accelerate the progression of some cancers. Research in model organisms like *Drosophila* have the advantage of precisely controlling the location and timing of gene expression that alters redox signaling. We genetically manipulated the levels of NRF2 and Keap1; two conserved master regulators of the cellular redox environment, while simultaneously overexpressing the oncogenes Src or Ras. We found that increasing antioxidant activity counters Ras-induced proliferation in stem cells and MAPK signaling in epithelial cells. We also found that decreased antioxidant activity promotes a Src-induced tumorigenic phenotype in adult flies. However, because the tumors formed in conjunction with reduced apoptosis rather than increased proliferation, this suggests that Src activity was likely mitigated with a lessening of antioxidant activity. Inhibiting antioxidants as a therapeutic approach to cancer is gaining momentum, therefore we hope that work in *Drosophila* could serve as a powerful system to isolate discrete mechanisms regulated by redox signaling and subsequently, better inform treatment.

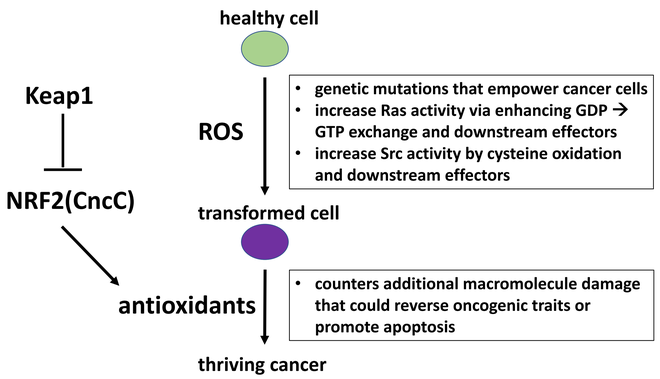

Fig 1. ROS and antioxidants can both contribute to cancer. Increased ROS activity can contribute to cancer formation through genetic damage and upregulation of Ras and Src function. Once established, cancers can prevent excessive damage by countering ROS with increased antioxidant activity. We use genetic manipulation of two master regulators of cellular redox environments, Keap1 and NRF2 (fly homologue = CncC), to examine effects on Ras and Src-induced phenotypes.

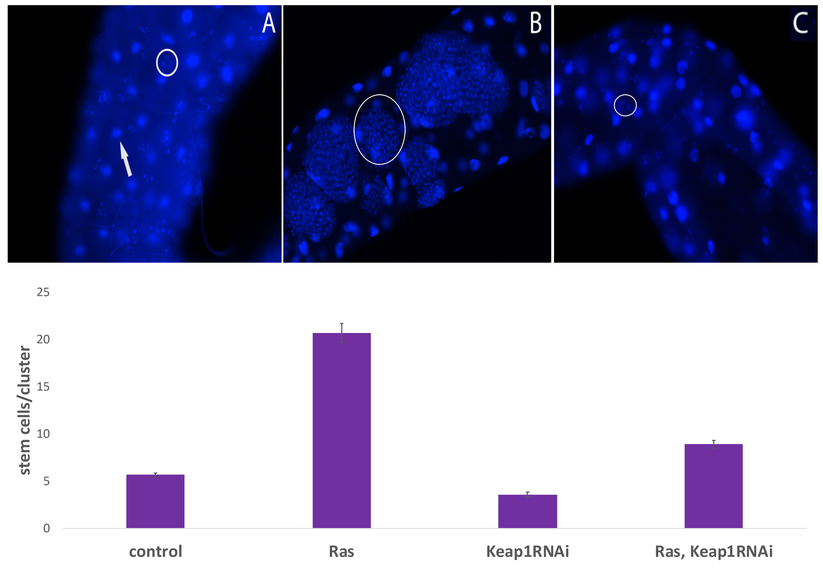

Fig. 2. Ras-induced overproliferation of larval gut stem cells is suppressed by modulating an antioxidant pathway. Using the UAS/GAL4 system to direct expression of oncogenic Ras^{V12} to intestinal stem cells (ISCs) in the larval gut leads to a 4-fold increase in ISCs/cluster (top panel, B). Simultaneous overexpression of an RNAi construct to Keap1 significantly counters Ras-induced overproliferation. Control guts express only GAL4 (top panel, A) with a stem cell cluster indicated by the open circle and a differentiated cell indicated by an arrow. Tissues stained with DAPI. Medians +/- std error shown in bottom panel.

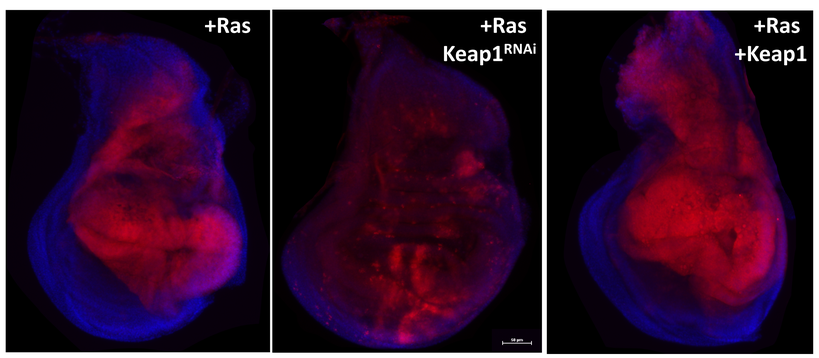

Fig 3. Increased antioxidant activity reduces Ras-mediated activation of MAPK. Expressing Ras^{V12} in the hinge and dorsal compartment of the developing wing leads to adult lethality, which is fully rescued by co-expression of an RNAi construct to Keap1 (data not shown). This rescue is accompanied by reduced levels of activated MAPK (middle panel) seen in response to Ras overexpression (left panel). Antibody to phosphorylated MAPK in red and DNA stained with DAPI (blue).

Figure 4. Decreasing antioxidant pathways enables tumorigenesis by Src. Expressing Src in combination with an RNAi construct to CncC in the hinge and dorsal compartment of the developing wing leads to tumor formation on the thorax near the wing (right panels, arrows), not seen with expression of either alone (left panels).

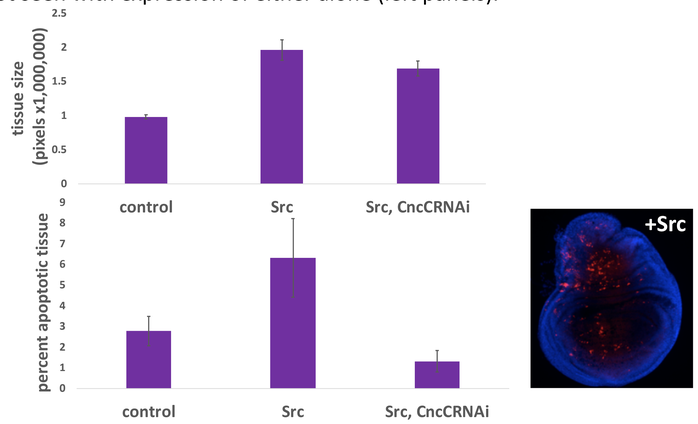

Figure 5. Examining effects of decreased antioxidant pathways on Src-induced growth and apoptosis. Src expression in developing wings increases their size by almost 2-fold (top). However, Src also induces high levels of apoptosis (bottom, DCP-1 staining in red). Reduced levels of CncC did not alter tissue size but significantly reduced Src-induced apoptosis ($p<0.05$).

CONCLUSIONS

Our findings demonstrate that using *Drosophila* as a model for redox-regulation of oncogenic phenotypes is promising. Future studies will further investigate the mechanisms responsible. Uncovering cellular redox mechanisms that are subverted in cancer cells is very exciting because it represents posttranslational alterations of oncogenes that may be "reversible" via environmental changes.