How *Drosophila* Can Inform the Emerging Paradigm of the Role of Antioxidants in Cancer

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ABSTRACT

Drosophila melanogaster has proven to be an effective model system in uncovering both genetic and cellular contributions to human cancer. Many elusive genes and signaling pathways that control oncogenic growth were first identified using flies. In many cases, these discoveries were not driven by a direct search for novel genes involved in cancer but rather stemmed from research programs to uncover mechanisms that control growth and development. However, the bounty of genetic tools and the shared evolution of multicellular organisms places *Drosophila* in a powerful position to purposefully elucidate observations seen in human cancers. In the past decade, the role of antioxidants in cancer progression has shifted dramatically. This review highlights major findings driving this change in perspective and underscores an array of existing work and resources in laboratories using *Drosophila* that can make significant contributions to how the redox environment affects cancer progression.

Recent studies have revealed that several cancers appear to benefit from increased levels of antioxidants. Multiple studies in mice show that increased antioxidants accelerate cancer (1–3). In addition, some clinical trials have demonstrated that antioxidant supplements led to higher incidence of lung cancer in smokers (4, 5). As a consequence, arguments to decrease antioxidants have been offered as a therapeutic approach to cancer treatment (4, 5). But antioxidants are required to modulate protein function and stress responses in healthy cells; therefore, global suppression of antioxidants in patients with cancer will likely lead to unwanted secondary effects. Research in model organisms like *Drosophila* has the advantage of precisely controlling the location and timing of gene expression that alters redox signaling and the subsequent posttranslational modification of proteins. This ability, in turn, can help identify specific targets and add another important layer to the personalized medicine approach to cancer treatment.

Antioxidants have long been viewed as protective against cancer because they are able to inactivate reactive oxygen species (ROS) that damage DNA and accelerate genetic alterations that activate oncogenes or inactivate tumor suppressors. ROS are primarily produced by mitochondria during aerobic respiration. However, the direct link between ROS levels and DNA damage has become suspect, as studies in mice showed that increased ROS levels did not increase the mutational load of mitochondria DNA (6). This work is supported by earlier findings that the 5-fold increase in point mutations in mitochondrial DNA between young and elderly humans failed to reflect the mutational signature of oxidative damage (7). Finally, similar work in *Drosophila* has found that few mutations in mitochondrial DNA reflect the signature of oxidative damage (8). Therefore, while ROS species certainly have the potential to contribute to genetic mutations, high-throughput analysis *in vivo* indicates that the DNA proximate to peak production of ROS in the mitochondria is not markedly affected. Compartmental separation from nuclear DNA, likely further reduces the capability of ROS-mediated mutation in most cells.

While the contribution of ROS to DNA damage may be less than once ascribed, it seems intuitive that keeping levels of these volatile molecules low is beneficial to cell function. However, recent work from several labs has found that antioxidant enzymes can function to increase the incidence of some cancers. A principle regulator of the redox environment is the transcription factor NRF2 (nuclear factor erythroid2-related factor2), which induces expression of several protective antioxidant responses. As anticipated, loss of *NRF2* function results in higher cancer incidence in both humans and animal models (9). However, studies using lung cancer models in mice have made the distinction that while NRF2 impedes tumor initiation, it can accelerate tumor progression (10, 11). Several other studies corroborate NRF2 as having oncogenic properties with elevated levels of NRF2 reported in many human cancers and to serve as an indicator of poor prognosis (9). Interestingly, a mouse model for breast cancer demonstrated that two downstream targets of NRF2, the glutathione and thioredoxin antioxidant pathways, were needed for both initiation and progression of cancer (1). While this result seems to contradict the findings of increased cancer incidence in the *NRF2* knockout mice, it is important to note that NRF2 targets many stress responses in addition to ROS. For example, NRF2 targets stimulated xenobiotic and drug transport and their metabolism (**Fig. 1**); many of these studies involved exposure to carcinogens (12, 13). Loss of *NRF2* may have extended the potency of the treatments. The function and regulation of NRF2 appears to be well conserved in invertebrates, with *cncC* (cap "n" collar) serving as the *Drosophila* homolog of *NRF2* (14). To date, only a handful of studies have examined CncC in the context of cancer (15, 16), yet those findings and several other suggest *Drosophila* could serve as a powerful addition to unravel the role(s) of antioxidants in cancer.

Work on CncC in *Drosophila* has investigated its role in stem cell responses and tissue regeneration (17). In intestinal stem cells, suppression of CncC is required to enable this cell type to proliferate in response to stress (18). Work done in adult tissues identified *cncC* as an essential gene for regeneration of wing tissue following ablation during development (19). This result supports findings in mouse models highlighting that loss of *NRF2* impairs liver regeneration (20). Together, these reports suggest that CncC serves as a gatekeeper of tissue

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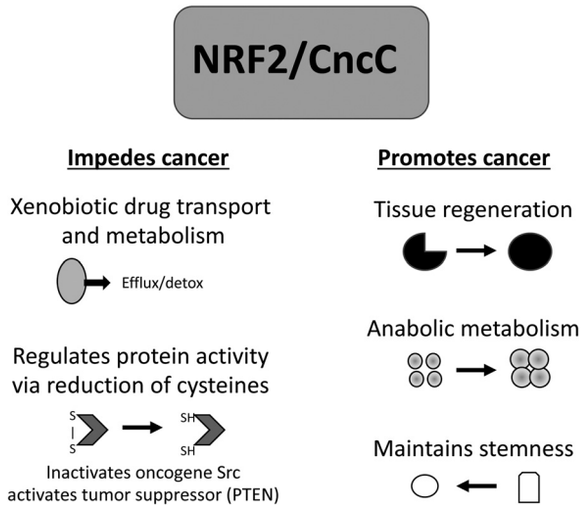

Figure 1.

Antioxidant activity can both impede and promote cancer. Summary of mechanisms that work downstream of mammalian NRF2, a master regulator of endogenous antioxidant activity, and its *Drosophila* homolog, CncC.

homeostasis, placing it in a key position to contribute to cancer (**Fig. 1**). The studies in *Drosophila* focused their attention on rising ROS levels in response to reduced CncC and found that outcomes could be phenocopied by increasing ROS levels through the diet or disabling the mitochondria. However, other means of modulating mitogenic signaling were not discounted (19), warranting more studies to explore the consequences of impaired CncC function in *Drosophila*, including conditions that do not activate a stress response.

Importantly, work in flies can easily examine genetic manipulations not only at the organismal and tissue levels, but at the single cell. This more realistically reflects cancer progression, which requires a founder, oncogenic cell to evade inhibitory signals and often coopt assistance from its noncancerous, neighboring cells. This experimental approach enabled *Drosophila* researchers to first characterize the process of cell competition; where the relative contribution of genetically mosaic cells to a developing tissue became tractable. The well-established oncogene *myc* was among the first genes to confer "winner" status to the cell expressing a higher level than neighbor cells via inducing apoptosis of the "loser" cells (21). This activity of Myc was subsequently demonstrated to apply to mammals, using heterogeneous mouse embryos (22). Intriguingly, in flies, overgrowth of cells with elevated Myc levels is dependent on CncC signaling (16). But elevated CncC has also been demonstrated to confer "loser" status relative to wildtype cells (15). The reciprocal experiment of determining whether reduced levels of CncC improves a cell's ability to compete has not yet been reported but would be important in determining whether CncC's role downstream of Myc-driven overgrowth is independent, and even in spite of, its effects on competition. Cell competition studies have the potential to explain the common clinical observation of "field cancerization"; a term used to describe tissue in which an unusually large area of normal tissue is replaced by premalignant cells. This single-cell approach could be employed more generally to quantify how CncC function interfaces with manipulation of various oncogenes and tumor suppressors, or in environmental conditions common to cancer, such as nutritional insufficiency. Notable, methods to fluorescently track CncC transcriptional activity and ROS levels are already established in flies (17), allowing visualization of both in living tissues.

ROS and NRF2 are intricately linked to metabolism and likely contribute to the metabolic signatures of both stem and cancer cells, which display reduced aerobic respiration. In stem cells, a switch to oxidative phosphorylation and subsequent increase in ROS pushes stem cells toward proliferation and differentiation (23). Similarly, converting differentiated cells into pluripotent stem cells is accompanied by a reduction in oxidative phosphorylation. Commonly described as the "Warburg effect" in cancer cells, a benefit to reducing aerobic respiration is that the subsequent increase in glycolysis feeds anabolic pathways, needed to provide cellular growth to match cellular proliferation. NRF2 also activates expression of several enzymes for anabolic metabolism of nucleic acids, amino acids, and phospholipids (24). *Drosophila* has nicely served as an informative model for how growth and division are not always linked following overexpression of oncogenes (25). In addition, a model for the Warburg effect has been genetically developed in flies (26). Importantly, even though enzymes involved in mitochondrial function were reduced, an increase in ROS was detected, which further upregulated glycolytic enzymes. Future work in *Drosophila* could combine epistasis tests to ascertain CncC's contribution to macromolecule production and growth as well as whether it affects the positive feedback loop mediated by ROS. NRF2 has been implicated in other anabolic

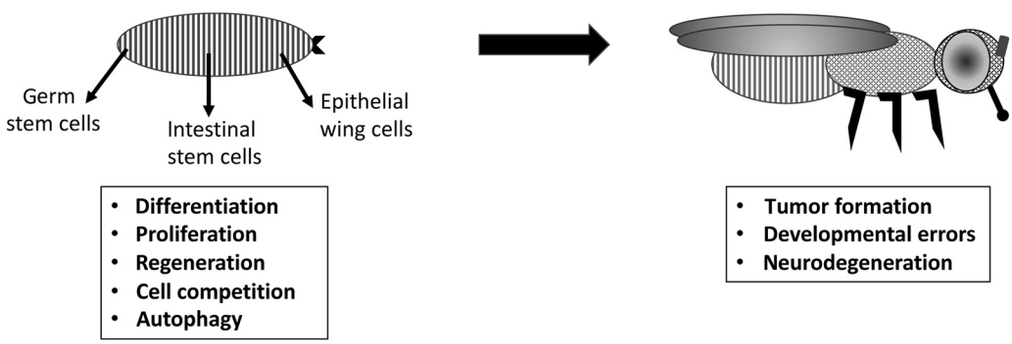

Figure 2.

Using *Drosophila* as model to measure outcomes of manipulating antioxidant activity. Flies are a powerful system to manipulate the genetics of antioxidant and cancer genes. Many hallmarks of cancer and possible side effects (e.g., neurodegeneration) can be quantified at the cellular level in larvae (left) or the tissue level in adults (right).

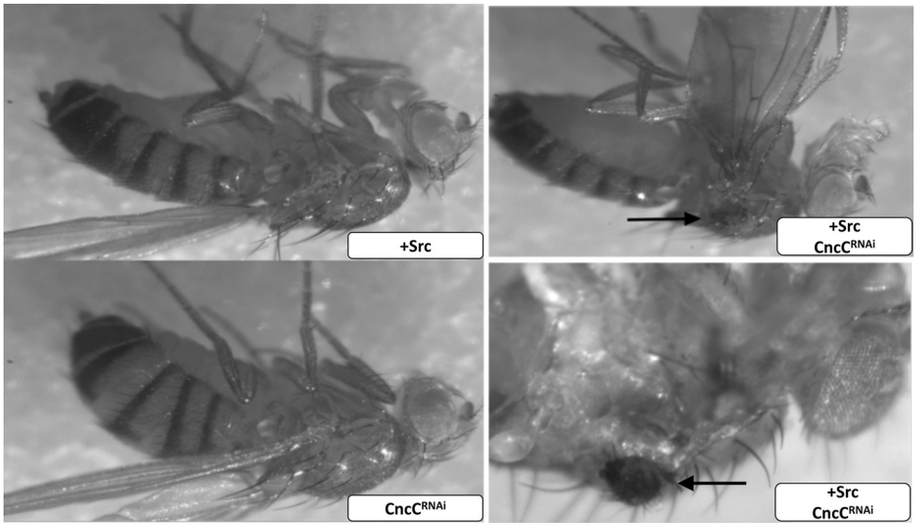

Figure 3.
Reducing antioxidant activity enables the Src oncogene to form tumors in adult *Drosophila*. Driving overexpression of Src (+Src) or reducing levels of endogenous antioxidant activity ($CncC^{RNAi}$) in developing flies produces no phenotype, while the combination (+Src, $CncC^{RNAi}$) results in tumors.

processes that contribute to cancer, such as autophagy (9). The conservation of mechanisms and numerous genetic tools and markers available in flies to modify and observe autophagy (27) suggests that assessment of a relationship between CncC and autophagy would be constructive (**Fig 2**).

While the redox environment broadly affects cellular physiology, it also serves to directly alter the function of many proteins, including several oncogenes and tumor suppressors. One common mechanism is the oxidation/reduction of cysteines to control disulfide bond formation **Fig 1**). This process has been most fully examined for protein tyrosine phosphatases, and appears to modulate their role in signaling downstream of growth factors. For example, the tumor suppressor, PTEN, is temporarily inhibited via an intramolecular disulfide bond following exposure of cells to growth factors. This, in turn, allows PI3K to increase levels of PIP3 sufficiently to propagate intracellular signaling pathways (28). The function of the oncogene Src also appears to be sensitive to oxidation of cysteines, with the majority of findings reporting that oxidation promotes Src activity (29). These studies were performed *in vitro* and in mammalian cell culture, inviting continuing work in whole organisms. Indeed, our own lab has determined that genetic reduction of *cncC* stimulates tumor formation by Src in adult *Drosophila* (**Fig 3**). Importantly, these examples of posttranslational regulation involve proteins that localize at the cell membrane. At this subcellular location, ROS are generated in subdomains via NADPH oxygenases and degraded by peroxiredoxins (4). Inactivation of peroxiredoxin appears to allow growth receptors to propagate local intracellular signaling (30) but paradoxically, elevated levels of peroxiredoxins appear to promote numerous cancers (31). Currently, no reports on the fly homologs of human peroxiredoxins have directly assessed whether they have oncogenic properties. However, one family member, Jafrac, affects the stability of the tumor suppressor E-cadherin (32). This cell–cell adhesion molecule is frequently compromised in invasive cancer cells and is similarly studied for its role in cell migration in embryonic flies (32). Importantly, oxidation/reduction of oncogenes and tumor suppressors can allow them to influence cancer progression without being mutated and so their contribution will be missed using genome/transcriptome approaches. Therefore, tools that reveal protein activity are essential. For example, a fusion protein has been genetically engineered in *Drosophila* that nicely serves as an indicator of PI3K activity in living cells. This protein contains the plectrin homology (PH) domain joined to GFP; the PH domain binds PIP3 and membrane aggregation is then visible by fluorescence microscopy (33). The ability of redox regulators to promote or inhibit PI3K activity at localized membrane regions could thus be ascertained.

Uncovering cellular redox mechanisms that are subverted in cancer cells is very exciting because it represents posttranslational alteration of oncogene and tumor suppressor functions that may be "reversible" via environmental changes. It has been proposed that a modest increase of ROS in cancer cells would meet the threshold of cell death while remaining tolerable in healthy cells (4, 5). Importantly, potential side effects should be proactively investigated. Neurons seem especially sensitive to alterations in cellular redox conditions, with NRF2 generally serving a protective role in neurodegenerative diseases (34). Strikingly, a *Drosophila* model for Parkinson disease demonstrated that increased function of CncC rescued neuron loss and locomotor activity (35), indicating that studies in flies may also disclose undesirable outcomes of redox-based treatments (**Fig 2**).

Previous work performed in flies has identified novel genes and signaling pathways that were later demonstrated to contribute to human cancers (36). This perspective offers an inverse approach: to clarify findings from mammalian studies via the powerful genetic tools and reduced complexity of *Drosophila*. If *Drosophila* can serve as a model for the role of antioxidants in cancer, then flies can be additionally exploited for drug screening and discovery. Promisingly, flies have already been shown to upregulate CncC in response to chemopreventative treatments as had been reported in rodents (17). This affordable, whole-animal approach is gaining momentum thanks to some early successes and the demonstration that at least 60 drugs share the same target between flies and man (37).

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

L.J. Saucedo: Conceptualization, investigation, methodology, writing-original draft, writing-review and editing. **R.E. Triolo:** Conceptualization, investigation. **K.E. Segar:** Conceptualization, investigation.

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References

1. Harris IS, Treloar AE, Inoue S, Sasaki M, Gorrini C, Lee KC, et al. Glutathione and thioredoxin antioxidant pathways synergize to drive cancer initiation and progression. Cancer Cell 2015;27:211–22.
2. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, Bergo MO. Antioxidants accelerate lung cancer progression in mice. Sci Transl Med 2014;6:221ra15.
3. Le Gal K, Ibrahim MX, Wiel C, Sayin VI, Akula MK, Karlsson C, et al. Antioxidants can increase melanoma metastasis in mice. Sci Transl Med 2015;7:308re8.
4. Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy. Biochem Pharmacol 2014;92:90–101.
5. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 2013;12:931–47.
6. Kauppila JHK, Bonekamp NA, Mourier A, Isokallio MA, Just A, Kauppila TES, et al. Base-excision repair deficiency alone or combined with increased oxidative stress does not increase mtDNA point mutations in mice. Nucleic Acids Res 2018;46:6642–69.
7. Kennedy SR, Salk JJ, Schmitt MW, Loeb LA. Ultra-sensitive sequencing reveals an age-related increase in somatic mitochondrial mutations that are inconsistent with oxidative damage. PLoS Genet 2013;9:e1003794.
8. Itsara LS, Kennedy SR, Fox EJ, Yu S, Hewitt JJ, Sanchez-Contreras M, et al. Oxidative stress is not a major contributor to somatic mitochondrial DNA mutations. PLoS Genet 2014;10:e1003974.
9. Basak P, Sadhukhan P, Sarkar P, Sil PC. Perspectives of the Nrf-2 signaling pathway in cancer progression and therapy. Toxicol Rep 2017;4:306–18.
10. Satoh H, Moriguchi T, Saigusa D, Baird L, Yu L, Rokutan H, et al. NRF2 intensifies host defense systems to prevent lung carcinogenesis, but after tumor initiation accelerates malignant cell growth. Cancer Res 2016;76:3088–96.
11. Tao S, Rojo de la Vega M, Chapman E, Ooi A, Zhang DD. The effects of NRF2 modulation on the initiation and progression of chemically and genetically induced lung cancer. Mol Carcinog 2018;57:182–92.
12. Becks L, Prince M, Burson H, Christophe C, Broadway M, Itoh K, et al. Aggressive mammary carcinoma progression in Nrf2 knockout mice treated with 7,12-dimethylbenz[a]anthracene. BMC Cancer 2010;10:540.
13. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc Natl Acad Sci USA 2001;98:3410–5.
14. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell Mol Life Sci 2016;73:3221–47.
15. Kucinski I, Dinan M, Kolahgar G, Piddini E. Chronic activation of JNK JAK/STAT and oxidative stress signalling causes the loser cell status. Nat Commun 2017;8:136.
16. Nagy P, Varga A, Pircs K, Hegedűs K, Juhász G. Myc-driven overgrowth requires unfolded protein response-mediated induction of autophagy and antioxidant responses in Drosophila melanogaster. PLoS Genet 2013;9:e1003664.
17. Pitoniak A, Bohmann D. Mechanisms and functions of Nrf2 signaling in Drosophila. Free Radic Biol Med 2015;88:302–13.
18. Hochmuth CE, Biteau B, Bohmann D, Jasper H. Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in Drosophila. Cell Stem Cell 2011;8:188–99.
19. Brock AR, Seto M, Smith-Bolton RK. Cap-n-collar promotes tissue regeneration by regulating ROS and JNK signaling in the Drosophila melanogaster wing imaginal disc. Genetics 2017;206:1505–20.
20. Beyer TA, Xu W, Teupser D, auf dem Keller U, Bugnon P, Hildt E, et al. Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance. EMBO J 2008;27:212–23.
21. Gallant P. Myc, cell competition, and compensatory proliferation. Cancer Res 2005;65:6485–7.
22. Clavería C, Giovinazzo G, Sierra R, Torres M. Myc-driven endogenous cell competition in the early mammalian embryo. Nature 2013;500:39–44.
23. Ito K, Suda T. Metabolic requirements for the maintenance of self-renewing stem cells. Nat Rev Mol Cell Biol 2014;15:243–56.
24. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci 2014;39:199–218.
25. Saucedo LJ, Edgar BA. Why size matters: altering cell size. Curr Opin Genet Dev 2002;12:565–71.
26. Wang CW, Purkayastha A, Jones KT, Thaker SK, Banerjee U. In vivo genetic dissection of tumor growth and the Warburg effect. Elife 2016;5:e18126.
27. Maruzs T, Simon-Vecsei Z, Kiss V, Csizmadia T, Juhász G. On the fly: recent progress on autophagy and aging in Drosophila. Front Cell Dev Biol 2019;7:140.
28. Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, et al. Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. Proc Natl Acad Sci USA 2004;101:16419–24.
29. Giannoni E, Taddei ML, Chiarugi P. Src redox regulation: again in the front line. Free Radic Biol Med 2010;49:516–27.
30. Woo HA, Yim SH, Shin DH, Kang D, Yu DY, Rhee SG. Inactivation of peroxiredoxin I by phosphorylation allows localized H(2)O(2) accumulation for cell signaling. Cell 2010;140:517–28.
31. Nicolussi A, D'Inzeo S, Capalbo C, Giannini G, Coppa A. The role of peroxiredoxins in cancer. Mol Clin Oncol 2017;6:139–53.
32. DeGennaro M, Hurd TR, Siekhaus DE, Biteau B, Jasper H, Lehmann R. Peroxiredoxin stabilization of DE-cadherin promotes primordial germ cell adhesion. Dev Cell 2011;20:233–43.
33. Britton JS, Lockwood WK, Li L, Cohen SM, Edgar BA. Drosophila's insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. Dev Cell 2002;2:239–49.
34. Yamazaki H, Tanji K, Wakabayashi K, Matsuura S, Itoh K. Role of the Keap1/Nrf2 pathway in neurodegenerative diseases. Pathol Int 2015;65:210–9.
35. Barone MC, Sykiotis GP, Bohmann D. Genetic activation of Nrf2 signaling is sufficient to ameliorate neurodegenerative phenotypes in a Drosophila model of Parkinson's disease. Dis Model Mech 2011;4:701–7.
36. Mirzoyan Z, Sollazzo M, Allocca M, Valenza AM, Grifoni D, Bellosta P. Drosophila melanogaster: a model organism to study cancer. Front Genet 2019;10:51.
37. Su TT. Drug screening in Drosophila; why, when, and when not? Wiley Interdiscip Rev Dev Biol 2019;8:e346.