Fly as Cancer Model? Genomic Analysis of Drosophila's Hypoxia Tolerance

Project description: Mammalian organs and tissues are highly oxygen-dependent and have adapted to operate under a perfectly equilibrated O_2 supply-and-demand balance. These systems usually answer to challenges of falling oxygen partial pressure (pO_2) and any inadequate (hypoxia) or lacking (anoxia) cellular O_2 supply through the attempt to restore pre-existing energy- and oxygenation-levels with defenses that encompass strong glycolytic, angiogenic or erythropoietic components. However, while these energy compensating defenses are capable of preventing massive cell death in the O_2/glucose deprived brain or embryo during short-term or milder insults, they will quickly become ineffective or even harmful (e.g. glycogen depletion and lactic acid overload due to excessive glycolysis) should the organism be faced with more extreme stresses. In contrast to the situation in healthy organs, most solid tumors are able to cope with otherwise lethal perturbations in the supply of O_2 and glucose and the resulting pH- or redox-based insults on cellular homeostasis. This resilience of mammalian malignancies and a few healthy cell types (e.g. astrocytes) towards hypoxic or ischemic stresses is paralleled by many hypoxia tolerant species, which are known to mainly utilize energy conserving survival strategies.

Induced by severe and persistent stresses (e.g. hypoxia, ischemia, dehydration), and maintained throughout it, numerous invertebrates and some ectothermic vertebrates will quickly, yet reversibly, reduce their metabolic rate just enough to enter a new energy supply=demand steady state called hypometabolism. This new energetic equilibrium prevents lethal falls in cellular ATP levels and is the single most protective and unifying feature of non-transformed hypoxia tolerant tissues. Both, *Drosophila* flies and S2 cells from the especially resilient late *Drosophila* embryos express with the hypoxia-inducible factor (HIF) the key regulator protein for survival and adaptation during oxygen deprivation. We recently reported on the critical *in vivo* function of fly HIF whereby the factor is necessary, albeit not sufficient, to coordinate, angiogenesis-like, both plasticity and sprouting of tracheolar end cells via fibroblast growth factor receptor (FGFR)-signaling during moderate hypoxia. When flies or fly embryos are exposed to a more severe O_2 shortage, however, they will switch from a tracheal (angiogenic) to a hypometabolic strategy, which, at first glance, seems to mimic the behavior of cancer cells during progressive deoxygenation.

To understand, genome-wide, the remarkable ability of *Drosophila* to survive severe and prolonged deprivations of oxygen, transcriptome profiles of S2 cells were recorded through microarray and Northern blot surveys as a function of graded hypoxia. Increasing severity of the hypoxic stress was reflected in rising numbers and magnitudes of hypoxia-responsive gene regulations. Entrance of S2 cells into the ultimate hypometabolic defense mode saw marked transcriptional regulations of 'hypometabolic factor (HMF)' candidate genes, in correlation with a progressive reduction of ATP-costly biochemical functions such as protein or DNA synthesis and cell cycle progression. The biological role of several of these protein synthesis inhibiting and cell cycle arresting molecules will be studied by collaborative *in vitro* and transgenic approaches. Together with our collaborators (i.e. group of Prof. Pablo Wappner, Instituto Leloir, Buenos Aires), we are currently focusing this line of investigation on the highly hypoxia inducible *Drosophila* gene THOR, a single-copy homolog of the mammalian 4E-binding protein family that acts, once hypo-phosphorylated, as repressor of the rate limiting mRNA translation initiation factor 4E. Various cancer cell lines also activate 4EBP1 during hypoxia, but in contrast to flies, this activation proceeds via the hypo-phosphorylation of the protein rather than the induction of its RNA.

Interestingly, a 4EBP1 loss-of-function considerably sensitized both fly null mutants and shRNA knockdown variants of different cancer cell lines to low oxygen levels (work done in Wappner group). Whereas mutant *Drosophila* could no longer adapt to hypoxia, or needed significantly more time to recover from it, cultures of 4EBP1 knockdown cancer cells lost almost their entire capacity to survive extreme oxygen deprivation. Furthermore, our published collaboration with molecular oncologists

and radiologists revealed that tumor xenografts generated from 4EBP1 knockdown cells developed a greater susceptibility to irradiation-triggered cell kill when compared with control tumors. Thus, down-regulating the rates of protein synthesis via the control of cap-dependent translation repressors (i.e. 4EBP1/THOR) is required, from fly to cancer cells, to facilitate energy conservation and to gain hypoxia tolerance. For tumors, this tolerance acquisition is associated with radioresistance. The results of this collaborative effort, thus, represent the necessary proof-of-principle to show that targeting translational controls could well be an effective new way for the sensitization of cells to hypoxia and of solid cancers to radiotherapy.

Key words:
Drosophila, tumors, HIF, hypoxia tolerance, hypometabolism, 4EBP1, radioresistance

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