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Tumor–host interactions through the lens of Drosophila

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Abstract

There is a large gap between the deep understanding of mechanisms driving tumor growth and the reasons why patients ultimately die of cancer. It is now appreciated that interactions between the tumor and surrounding non-tumor (sometimes referred to as host) cells play critical roles in mortality as well as tumor progression, but much remains unknown about the underlying molecular mechanisms, especially those that act beyond the tumor microenvironment. Drosophila has a track record of high-impact discoveries about cell-autonomous growth regulation, and is well-suited to now probe mysteries of tumor–host interactions. Here we review current knowledge about how fly tumors interact with microenvironmental stroma, circulating innate immune cells, and distant organs to influence disease progression. We also discuss reciprocal regulation between tumors and host physiology, with a particular focus on paraneoplasias. The fly's simplicity along with the ability to study lethality directly provide an opportunity to shed new light on how cancer actually kills.

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This Review discusses how fly tumors interact with the microenvironment and distant organs to influence disease progression and host physiology. The authors argue that the simplicity of flies and the ability to study lethality in this model provide an opportunity to address why patients ultimately die of cancer.

Introduction

A signature triumph of modern biology is deciphering the causes of cancer. From the isolation of tumor viruses to the discovery of oncogenes and tumor suppressor genes to contemporary cancer genomics, we now have a detailed understanding of the genetic, epigenetic, and signaling changes that drive the malignant growth of mutant cells. While these insights have strongly improved diagnosis and enabled development of rational therapies, their impact on patient mortality has been less than could be wished. Difficulties in drugging major oncogenic drivers, along with tumor heterogeneity and the development

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of chemotherapeutic resistance all impede treatment of patients through strategies that seek to prevent or reverse tumor growth.

Progress has also been limited by a historical focus on cell-autonomous characteristics of tumors. It is now clear that cancer, as a disease, emerges as a cellular and molecular dialog between genetically altered tumor cells and genetically wild-type surrounding cells in a patient's body. For example, four of the ten features highlighted in the 2011 update to the seminal review 'Hallmarks of Cancer' explicitly concern interactions between tumor and normal tissue[1]. These hallmarks encompass activation of invasion and metastasis, induction of angiogenesis, tumor-promoting inflammation, and avoidance of immune destruction. They reflect critical mechanisms through which local interactions in the tumor microenvironment (TME) promote or restrain growth and dissemination.

A hallmark still awaiting recognition is cancer's ability to induce systemic shifts of patient physiology. Such effects, in which the tumor can cause strong alterations in the activity of distant organs, can be grouped together as 'paraneoplastic syndromes'[2]. Though underappreciated, paraneoplasias have severe pathological consequences and are important drivers of mortality for a number of cancers. The best-known paraneoplasia is cancer cachexia, the wasting of muscle and adipose tissue that accompanies most advanced cancer cases, which is thought to drive more than 20% of patient deaths[3–5]. The mode of action of cachexia and many other paraneoplastic syndromes remains opaque, so there is much to be learned about the mechanisms through which tumors compromise the organ systems of patients to induce lethality.

The challenges of studying interactions between the tumor and the many tissues of a patient are exponentially more complex than those of studying tumor-autonomous growth regulation alone. Yet the payoff in terms of therapeutic strategies that could avoid obstacles faced by tumor-targeted approaches may be immense. To achieve this goal, simple model organisms have great potential to reveal underlying conceptual principles as well as specific mediators. Here we review current knowledge of tumor–host interactions in Drosophila, discuss how the fly system can uncover conserved molecular mechanisms, and consider what these reveal about how animal bodies cope with the presence of malignant growth.

We begin by laying out the case for using the fly to investigate this important question. In the following sections, for key tumor–host interactions seen in human patients, we describe what is known about the comparable interaction in Drosophila. We adopt vertebrate terminology for fly genes, proteins, and cell types where fair analogies can be made. We first discuss the tumor's juxtracrine and paracrine interactions with its immediate neighbors. We then consider fly models of metastasis, before moving on to systemic changes induced by long-distance tumor–host signaling and reflecting on the mechanisms by which tumors kill hosts. We close with a perspective on how the tumor–host studies possible in the fly can complement and enhance knowledge from traditional mammalian systems, particularly by highlighting the breadth of host responses to cancer.

Investigating fly tumor–host interactions

To probe mysteries about how tumors interact with normal tissues to promote disease progression and kill patients, we will need to study animal models. Vertebrates, with nearly identical tissue repertoire, endocrine signaling, and metabolic networks have obvious advantages for these studies. But there are also disadvantages. For mice, high animal care costs create a barrier to large-scale population cohorts as well as genetic screens. A ~28-month average lifespan discourages longitudinal experiments that reflect the course of human disease. Additionally, ethical and regulatory considerations usually prevent analysis of mortality as a study endpoint; instead, tumor size is taken as a proxy, resulting in a focus on tumor growth rather than lethality. Zebrafish share regulatory constraints around vertebrate animal suffering with mice, and have a comparably long lifespan. However, fish often tolerate tumors better than mice, and form an appealing substitute for several other reasons; some work has begun to leverage these advantages[6,7].

In this review we consider a simple alternative for investigating how tumors kill animal hosts: using the fruit fly Drosophila. Although it lacks central features of mammalian anatomy relevant to cancer, such as a closed circulatory system and an adaptive immune system, the fly shows remarkable conservation with humans not just of genes and molecular mechanisms, but also of functional organs and organismal physiology (Fig. 1)[8,9]. With an average lifespan of ~7 weeks and no concerns that necessitate premortem sacrifice, Drosophila is well-suited for explicit studies of mortality itself. Drosophila also has an unparalleled genetic toolkit that can be utilized in rapid experiments with high numbers of animals for low cost. This includes techniques to simultaneously and independently manipulate both tumor and host tissue genetics, leveraging publicly available reagents that allow analysis of both loss- and gain-of-function on a genome-wide scale. Moreover, the wealth of knowledge about normal Drosophila physiology, metabolism, immunity and inter-organ communication provides a solid basis for investigations of pathological conditions. The fly is thus poised to face the challenge of studying the entire suite of local and systemic interactions that a tumor can induce throughout the body.

Drosophila has a long track record of seminal contributions to cancer biology, including elucidating the mechanisms of oncogenic signaling pathways such as epidermal growth factor receptor (EGFR), WNT, Hedgehog (HH), Notch and Hippo. These pathways are among those dysregulated in fly tumors, about which more information is provided in Box 1. A bioinformatic search reveals that 90% of genes identified by The Cancer Genome Atlas to drive human cancer have an ortholog in the fly[10,11] (Supplementary Table 1). Though the precise genetic constituency of fly tumors generally differ from those of humans, the transformed phenotype – unrestrained and invasive growth of disorganized, immortalized cells – can be quite similar[12–15]. Using genetically engineered fly models (GEFMs), tumors can be generated *in vivo* in epithelial tissues of the larva (imaginal discs) or adult stage. Tumors can also be easily transplanted into the abdomen of adult hosts[16,17]; we will refer to this as the allograft model (Box 2). Most studies of fly tumor–host interactions analyze ‘neoplastic’ tumors that arise in epithelial organs, which are roughly analogous to mammalian malignant carcinomas. A popular variation coexpresses oncogenic Ras with mutation of a fly neoplastic tumor suppressor gene such as *scribble (scrib)* to enhance

malignancy[18], which can be termed a ‘cooperative neoplastic’ model. For simplicity, we will refer to neoplastic and cooperative neoplastic models as ‘tumors’, acknowledging that there are many different tumor models in the fly (e.g. Supplementary Table 2) and that results with one may not generalize to all.

Local interactions regulating fly tumors

As the transformed mutant cells that form a tumor overproliferate, they engage in a bevy of signaling exchanges with surrounding normal cells, altering the functions of the normal cells in ways that fuel tumor growth and progression. These local interactions take place in the TME, a specialized area containing a plethora of genotypically wildtype (WT) cell types from the tissue in which the cancer arose. The physical and signaling characteristics of the TME differ substantially from that seen in a healthy tissue; it is now appreciated that the TME is not a passive bystander but instead an active contributor to malignancy through intercommunication with tumor cells and more distant organs[19,20].

In human cancer patients, beyond a variety of infiltrating adaptive immune cells, several TME cell types are prominent in supporting tumor growth[21,22]. One type is the normal epithelial cells that neighbor the nascent tumor. A second type is cancer-associated fibroblasts (CAFs), which can enhance the proliferative properties of transformed cells and create pro-metastatic or immunosuppressive environments as well. A third is endothelial cells, which are recruited to transformed cells in the process of neovascularization to induce a tumor-directed blood supply that also provides a route to tumor dissemination. Each of these heterotypic interactions has an analogy in Drosophila tumor progression (Fig. 2). The influence of the non-cellular TME — the extracellular matrix — on fly tumor progression has yet to be investigated, and interactions with innate immune cells in the stroma are treated separately below.

Neighboring epithelial cells

The most immediate interaction that transformed cells have in their microenvironment is with the surrounding non-transformed epithelium, which can profoundly influence tumor progression. In fly cancer models where adult intestinal stem cells (ISCs) are transformed, these interactions induce mitogenic paracrine signaling loops akin to those that occur between tumor and stroma[23,24]. As an initially hyperplastic ISC-derived tumor grows, it physically displaces surrounding differentiated gut cells from the basement membrane, triggering a damage response from WT tissue. This response invokes signaling through interleukin 6 (IL-6)-like cytokines (the Unpaired (Upd) family in fly) to replace the displaced cells, which would normally be a self-limited process that ceases when homeostasis is restored. However, transformed ISCs make this process chronic, initiating a feed-forward loop that accelerates tumor growth.

Tumors can also signal one-way to accelerate growth of neighboring WT cells. In one class of neoplastic disc tumors, defects in endocytosis trigger inappropriate Notch cleavage; this ligand-independent Notch activation then drives production of Upd/IL-6 to stimulate proliferation of surrounding host cells[25,26] (Fig. 2A). A similar circuit is triggered by tumorigenic loss of epigenetic silencing mediated by the Polycomb repressive complex[27–29].

In all these cases, heterotypic signaling between genetically normal and transformed epithelia promote proliferative accumulation of tissue mass.

Importantly, local interactions with WT neighbors can prevent tumor growth instead of stimulating it. Fly imaginal epithelia display a remarkable ability to detect and then eliminate small groups of cells with malignant genotypes, particularly those bearing mutations in *scrib*-class genes (i.e. genes with functions similar to *scrib* such as *lethal giant larvae (lgl)* and *discs-large (dlg*). This tumor-suppressive ability is part of the larger phenomenon of cell competition through which cells with one genotype influence the proliferation of their immediate neighbors. This fascinating aspect of tumor–host interactions has been amply reviewed recently[30–33], and is recognized to play an important role in mammalian epithelial homeostasis and tumor development.

The mechanisms by which neoplastic *scrib* mutant cells are eliminated are molecularly distinct from those underlying competitive removal of cells with altered levels of growth regulators such as Myc or ribosomal proteins. The cue for the former is the disruption of cell polarity within the epithelium, which triggers JUN N-terminal kinase (JNK) signaling and a resultant apoptotic program[32]. Host cell factors required for elimination of neoplastic neighbors include the tumor necrosis factor (TNF) homolog (Eiger (Egr) in the fly) and a juxtracrine signaling molecule called Sas; both are latent in normal epithelia but appear to be activated by the architectural changes of mutant cells alone[34–37] (Fig. 2B). TNF signaling through JNK is part of a normal process that can restore epithelial homeostasis after physical damage and is also used to eliminate potentially malignant cells upon their onset. If this process fails, or if additional mutations in the mutant cells (e.g. activated *Ras* in the cooperative models) allow them to evade elimination, sustained tumor growth can ensue.

Mesenchymal stroma

Flies do not have fibroblasts or connective tissue, but they do contain mesenchymal cells that develop in close association with epithelia. An example is the larval myoblasts, muscle precursors that lie over regions of the wing imaginal disc. In a cooperative tumor model that generates neoplastic wing tissue through EGFR overexpression and depletion of the chromatin regulator Pipsqueak, a significant portion of the growing mass was composed of genetically normal myoblasts[38]. The transformed epithelial cells induced proliferation of adjacent myoblasts via transforming growth factor β (TGFβ) and Notch signaling[38,39] (Fig. 2C). Interestingly, the overproliferating mesenchymal cells were reciprocally required to drive epithelial growth and tumor progression, in a paracrine signaling loop reminiscent of that seen with mammalian CAFs[19]. However, myoblasts are not required for epithelial growth in several other neoplastic fly models[40], so CAF-like interactions may not be a general feature of fly tumors.

Oxygen-supplying tubules

FIies are small creatures without thick tissues, and their cellular need for oxygen is met by diffusion within an open circulatory system rather than blood cells carrying oxygen through vasculature. Internal oxygenation in flies is passively supplied through epithelial tubes called trachea (Fig. 1). Trachea form an elaborate network whose branches can ramify

directly on cells but also transfer oxygen into the circulatory fluid called hemolymph[41]. The normal pattern of tracheal branching is strongly influenced by fibroblast growth factor (FGF) homolog signaling via a process equivalent to mammalian angiogenesis[42].

Like growing human tumors, large neoplastic cell masses in the fly can show signatures of hypoxia and its associated response, stabilizing hypoxia-inducible factor 1α (HIF1α) homologs that induce metabolic reprogramming (discussed below). Downstream of HIF1α signaling, the fly FGF ligand Branchless (Bnl) is upregulated within tumors, inducing three changes in the host that echo vertebrate neovascularization[43–47]. First, endogenous trachea show ectopic branching towards tumor cells (Fig. 2D). Second, some tumor cells themselves develop tube-like architecture and tracheal gene expression, associating with normal tracheal cells in a phenomenon reminiscent of vascular mimicry[48]. Third, tumor cells in several models have been visualized migrating along trachea, perhaps using them as a route to dissemination. Though the requirement of neo-tracheation in tumor growth remains to be tested, these phenotypes are suggestive of thematic interactions between the fly tumor and the host oxygen supply that parallel those seen in mammals[49].

Fly tumors and the immune system

In addition to sessile cells in the stroma, tumors engage in extensive interactions with circulating immune cells. We will highlight here the role of macrophages, innate immune cells that play critical roles in tissue repair as well as the initial response to pathogens. In human patients, some tumor-associated macrophages (TAMs) can have tumor-promoting activity, including facilitating invasion and angiogenesis, while others are tumor-suppressive, assisting the immune recognition and subsequent execution of transformed cells[50]. The revolution in tumor immunology and recognition of the immune system's importance in shaping cancer progression raises the interesting question of whether these phenomena have a deep evolutionary origin. Flies do not have an adaptive immune system, but have a well-developed innate immune system that has been central to understanding conserved mechanisms of pathogen detection[51]. Exciting recent studies identify pro-oncogenic effects of fly innate immune cells that involve inflammatory signaling, as well as tumor-restricting activity by signaling to systemic immune effectors.

Cellular immune system

The cellular arm of the Drosophila immune system is composed of hemocytes. Hemocytes share functional properties with the vertebrate myeloid lineage, and may have a common evolutionary origin[52,53]. The bulk of hemocytes (95%) are a cell type called plasmatocytes, which are analogous to mammalian macrophages; we will refer to them hereafter as 'macrophages'. In the fly, macrophages are attracted to wounds, where they actively phagocytose dying cells and microbial invaders. In the immune response, they release anti-microbial peptides as well as cytokines including fly homologs of IL-6, TNF and ligands for the Toll innate immune pathway, which is homologous to mammalian TLR signaling.

Hematopoiesis takes place in the embryo and the larvae, but is absent in the adult[54]. As in mammals, many macrophages are sessile and reside in specific tissues, while others circulate, pumped through the hemolymph by the fly heart. Macrophages actively migrate

to or are passively captured by sites of tissue damage, regulated by cues that include H2O2 production or basement membrane destruction. There, in addition to phagocytosis, macrophages secrete factors that form an initial ‘soft’ clot; the clotting cascade also generates reactive oxygen species (ROS)[55]. Together, cytokines and ROS signal to remote tissues such as the fat body and trachea, activating the humoral arm of the immune system to secrete anti-microbial peptides in a systemic defense response. Thus, as in mammalian innate immunity, fly macrophages survey and defend the body from microbial threats and tissue damage. As we now describe, they can also detect and respond to the ‘altered self’ of malignantly transformed cells.

Interactions between fly tumors and macrophages (Fig. 2F) were first documented in 2008. In a pioneering paper, Pastor-Pareja et al[56]. demonstrated that macrophages are recruited to neoplastic imaginal discs in larvae. The presence of fly TAMs is due to their association with sites of basement membrane degradation, as well as systemic stimulation of macrophage proliferation by Upd/IL-6 signals from the tumor and perhaps other tissues. Strikingly, either elimination of all macrophages or blocking Upd/IL-6 signaling within them resulted in a significant increase in tumor burden within the animal. This provided the first evidence for immune restraint of tumors in invertebrates akin to that known in vertebrates.

Building on this study, Cordero et al. found that TAMs in disc tumors upregulated expression of the fly TNF, Egr[35]. Egr/TNF is a potent activator of JNK signaling in recipient cells, and when released from TAMs it binds directly to transformed but not WT disc tissue. In simple neoplastic tumors, it induces apoptosis, limiting tumor burden. On the other hand, when neoplastic cells coexpress oncogenic Ras^{V12}, cell death is blocked and Egr/TNF instead promotes phenotypes such as upregulation of matrix metalloproteases (MMPs) that drive invasion. A further consequence of TNF signaling in both tumor types is the upregulation of Pvf1, homologous to vascular endothelial growth factor (VEGF), which increase macrophage numbers in circulation. Whether due to mobilization of sessile cells or stimulated proliferation, this increase of Egr/TNF-expressing TAMs creates a feed-forward loop that enhances the tumor’s response. Together, these data show that fly TAMs, like their mammalian counterparts, can suppress tumors in some contexts but in others promote tumor growth. It should be noted that a second study did not find a role for either TAMs or TNF signaling in growth of a comparable tumor type[40]; the reasons for this discrepancy remain to be determined.

Humoral immune system

The number of macrophages that associate with fly tumors is not large, suggesting that they might restrict tumor growth by calling on additional partners. Interestingly, TAMs not only upregulate Egr/TNF, but also Spaetzle (Spz), the major ligand for the fly Toll signaling pathway[57]. Spz secretion into circulation then triggers activation of the humoral arm of the immune system. Both Toll and the IMD pathway, a second innate immune signaling pathway driven by nuclear factor κB (NF-κB) homologs, are activated in peripheral tissues of tumor-bearing larvae, and manipulations that block Toll or IMD signaling prevent the tumor-suppressive activity of macrophages.

A further paper revealed that this fly immune response kills tumor cells by an unexpected mechanism[58] (Fig. 3A). Death is not due to direct induction of apoptotic signaling by TAM-derived Egr/TNF; instead TNF signaling promotes tumor cell exposure of the lipid phosphatidylserine. Phosphatidylserine generally marks cells for death in several ways, including serving as a recognition signal for macrophages. In this case, it enhances binding of a circulating anti-microbial peptide called Defensin. Defensin is normally secreted during the Toll-dependent humoral response to microbial infection. In tumor-bearing larvae, TAM stimulation of immune-responsive organs produces Defensin and other anti-microbial peptides, although only Defensin seems to bind specifically to tumor cells. Interestingly, the authors provide evidence that Defensin exhibits direct killing activity on tumor cells. This coordinated link between the cellular and humoral arms of the innate immune system allows a microbial defense effector to eliminate the ‘altered self’ of transformed cells. Interestingly, humans produce Defensin orthologs that also bind phosphatidylserine-enriched tumor cells and exhibit oncolytic properties on several cancer cell lines[59].

Key to all of these responses is the ability of TAMs to be recruited to a tumor. Evidence for fly macrophage adhesive receptor roles comparable to mammalian innate immune recognition of tumors[60] does not yet exist, but several recruitment mechanisms lie downstream of JNK signaling. JNK transcriptionally upregulates *Mmp1* in all neoplastic fly tumor models[61], and basement membrane damage by MMPs may be the proximate cue detected by hemocytes, as it is sufficient to recruit them to imaginal discs[56,62]. JNK in tumor cells also stimulates ROS production through the plasma membrane-localized NADPH oxidase; this process is dependent on activation of initiator caspases, and is enhanced if activated Ras is expressed in tumors to block the execution of the apoptotic program[63]. ROS is required alongside JNK for hemocyte recruitment, perhaps by stimulating MMP enzymatic activity. Finally, JNK activation may also trigger cells to produce other secreted signals that attract hemocytes. Once attracted to the tumor, the transformation into TAMs also seems to involve ROS, which induces acquisition of a distinct morphology and upregulation of Egr/TNF. Such fly studies may shed light on the important roles that ROS plays in mammalian anti-tumor immunity[64].

The extensive interactions described above raise the question of whether paraneoplastic impacts on other immune cell functions exist. Macrophages and a second cell type called crystal cells play critical roles in the clotting cascade[55], but whether fly tumors affect hemostasis, as in human paraneoplastic coagulopathies such as Trousseau’s syndrome[65], has not been investigated. Similarly, macrophages are important mediators of the immune response, but it is unknown if tumor- and microbial-initiated activation of these pathways synergize or interfere with each other. Both of these questions are ripe for future study.

Metastasis in Drosophila

In humans, the tumor–host interaction most strongly associated with lethality is metastasis. Metastasis in vertebrates is a multistep process that involves not only exit from the primary tumor but both entry into and exit from the vasculature, as well as survival and proliferation at a secondary site. The open circulatory system of Drosophila is not optimal for studying vascular intravasation and extravasation, although fly immune cells do

traverse other vessel-like structures[66]. Nevertheless, fly tumor cells show clear invasive behaviors[67,68]. Tumor cells lose polarized architecture, expand their actin cytoskeletal network and acquire mesenchymal morphology with pro-migratory characteristics. As mentioned above, concomitant upregulation of MMPs degrades the basement membrane (Fig. 2E) to allow exit from the tissue of origin. For the purposes of this review, we refer to movement into neighboring tissues as invasion, and reserve metastasis for the presence of tumor-derived cells at a site that is not contiguous with the tissue of origin.

Invasive behavior of fly tumors was evident in early allograft studies, where neural-derived tumors were noted to envelop and penetrate adult host organs[69]. Epithelial-derived tumors, by contrast, were less invasive and predominantly formed compact independent growths. Nevertheless, in GEFM larvae containing cooperative neoplastic eye discs, tumor cells consistently move into the neighboring ventral nerve cord[18]. The dependence of this phenotype on MMP activity[70,71] is consistent with it being true invasion.

Histological tracing clearly documents cell dispersal beyond the primary tumor. In neural tumors transplanted into adult abdomens, genetically labeled cells can be later found in distant organs, including sites that required crossing a host basement membrane[72]. Dispersal required MMP activity and involved a handful of cells[73]; these ‘micrometastases’ of 2–100 cells are typical of several types of neural tumor[17]. The small size of such secondary tumors compared to the robust growth of the primary may suggest tumor dormancy. Long-distance dissemination can also be seen in larvae bearing cooperative neoplastic eye discs[18]. Although this phenotype can be confounded by ‘leaky’ labelling[74], a more recent study indicated that bona fide cellular migration from the primary tumor occurs and shows organotropic characteristics, as tumor cells travel to certain tissues while avoiding others in a manner dependent on a host-derived signal[45]. However, allografts of cooperative neoplastic discs into adults result only in very rare metastases (JK, DB, and A. Figueroa-Clarevega, unpublished data).

A robust system to study invasion and dispersal is emerging in GEFMs that manipulate the adult gut. When targeted to the differentiating epithelial cells of the hindgut, transgenic models that mimic multi-hit genetic constituencies of human colorectal cancers yield cells that frequently disseminate into the body cavity, driven by oncogenic *Ras*[46]. In a model using midgut ISCs, Ras activation alone is sufficient to drive invasion and dissemination[75]. A recent paper shows that ISC fly tumors carrying three genetic changes — activation of *Ras*, mutation of the colorectal tumor suppressor gene ortholog *Apc* and overexpression of the epithelial-mesenchymal transition regulator *snail* — can form large metastases at secondary sites[76]. Although rare (~1%), these metastases are amenable to analysis; the animals also frequently generate circulating tumor cells in the hemolymph. Advances in both short-term and long-term *in vivo* live imaging[77,78] promise to shed further light on metastasis in these systems.

To summarize, invasion into the local microenvironment is a prominent feature of fly neoplastic tumors. Dispersal is more variable and depends on tissue origin and context; it is high in neural tumors allografts, intermediate in cooperative neoplastic larvae, and low but consistent in adult gut GEFMs. In allografted disc tumors, metastasis is sufficiently rare that

effects remote from the primary tumor are much more likely to be mediated by mobile cells or secreted bioactive molecules. We now turn to these long-distance tumor-host interactions.

Physiological changes induced by fly tumors

Beyond the TME, cancers can induce profound systemic shifts that manifest in distant tissues throughout the organism. These changes are collectively called paraneoplastic syndromes, and although different tumors have propensities for particular effects, there is a set that is commonly induced irrespective of tissue-of-origin or genetic constituency. Paraneoplastic syndromes are not thought to contribute to oncogenic growth or metastasis per se, and are thus not specifically selected for during tumor evolution[2,79]. Instead, they are regarded as epiphenomena that are caused by tumor secretion of diffusible molecules with the ability to impact remote organs. Such secreted factors have profound effects in flies as well as humans[79], and we coin the term ‘oncokine’ to refer to these as well as signaling molecules that act locally in the TME. Table 1 lists currently known fly oncokines and their influence on host physiology; in the following sections we describe several examples of systemic oncokine signaling.

Many paraneoplastic syndromes have been documented in human cancer patients. Some of these, such as hypercalcemia, the syndrome of inappropriate antidiuretic hormone secretion (SIADH), Cushing’s syndrome (involving excess cortisol production) **[**and paraneoplastic autoimmune reactions involve signaling axes or organ systems that are specific to mammals, and will not be considered here. Several others that impact tissues with fly analogs, such as paraneoplastic coagulopathies and remote immunosuppressive effects, have yet to be investigated in Drosophila. Fig. 3 provides a summary of fly paraneoplasias. We discuss first fly studies of cachexia, anorexia, and related research linking tumor growth to host metabolic changes. We then consider other fly paraneoplastic syndromes whose relation to human morbidity is not yet clear.

Tissue wasting

Recent years have seen the development of several fly models of cancer cachexia. Cachexia is a complex phenomenon, but a defining criterion is that it involves tumor-induced systemic tissue wasting that is not due to insufficient nutritional intake. Despite its high prevalence and lethal impact, cachexia is a poorly understood syndrome with little available therapeutic amelioration[3–5]. A wealth of experimental studies in rodents have identified secreted factors that have cachectogenic properties, but there is often poor correlation with factors detectable in cachectic patients. This gap increases the appeal of novel models for study.

Cachectic phenotypes in the fly were first demonstrated in two adult tumor models: in allografts of neoplastic discs and in a GEFM when ISCs overexpress an active form of the Hippo pathway transcription factor Yorkie (Yki), orthologous to mammalian YAP and TAZ[80,81]. As the tumors grow, several host tissues display clear wasting (Fig. 3B, C). These include the fat body, in which triglyceride levels are reduced, and muscle, in which mitochondria degenerate with associated reductions in energy production and motor activity. Such phenotypes are also seen when animals are starved, but careful analysis showed that – even in the presence of gut tumors—the host flies feed normally and do not display markers

of starvation. Thus, the wasting is not due to anorexia (reduced nutrient consumption) but instead cachexia (failure of ingested nutrients to support metabolic homeostasis).

How do fly tumors induce wasting of distant organs? Remarkably, although the models involve different driver genes that transform different cell types, wasting in both depends on a shared target gene that is strongly upregulated in the tumor tissue. This target is *ImpL2*, which encodes a secreted antagonist of Insulin signaling with functions analogous to the insulin growth factor binding protein (IGFBP) family in vertebrates[82]. Insulin signaling is a major endocrine regulator of biosynthesis, metabolism, and cell survival in all animals, and is activated by ligands that in flies are called insulin-like peptides (ILPs). ImpL2/IGFBP is released into the hemolymph where it binds to and sequesters circulating ILPs; expression of ImpL2/IGFBP alone in the absence of a tumor is sufficient to cause peripheral wasting. Accordingly, tissues that waste when a tumor is present show reduced insulin signaling, with a hyperglycemic state throughout the animal. Most importantly, depletion of ImpL2/IGFBP in the tumor itself leads to a rescue of wasting, with associated improvements in peripheral organ function. This rescue is not complete, and a recent study identified Pvf1, a VEGF family ligand produced in the ISC model as an additional, independent cachetogenic factor[83]. Another group using a larval cooperative tumor model enhanced by a high-sugar diet (see below) identified Bnl/FGF, rather than ImpL2/IGFBP or Pvf1/VEGF, that promotes muscle wasting in this context[84]. As with human patients, fly cancer cachexia can be driven by several different endocrine signaling axes.

How do the mechanisms that drive tumor-induced wasting in flies compare to human cancer cachexia? Cachexia is a heterogeneous condition, likely induced in different cancer types by multiple distinct pathways. One mechanism frequently implicated in mammals is upregulation of E3 ubiquitin ligases to drive protein degradation[3]; fly models do not appear to show this. Nevertheless, systemic insulin resistance, akin to that induced by ImpL2/IGFBP in the fly models, is seen in some cancer patients, and evidence supports a role for reduced insulin signaling in several rodent cancer cachexia models [85,86]. Moreover, certain human tumors induce IGFBP3, and IGFBP3 can directly induce wasting of cultured muscle cells[87]. Finally, in a mouse cancer cachexia model, muscle-specific ERK signaling akin to that demonstrated in tumor-bearing flies[83] was shown to drive atrophy associated with increased proteolysis[88]. Thus, discovery-based approaches in the fly can provide new leads for effectors of cachexia and other paraneoplasias.

Anorexia

Weight loss in cancer patients is often driven not only by cachexia, but also by accompanying anorexia[89]. Although clinically intertwined, anorexia is distinct from cachexia as it results from reduced food intake due to behavioral changes in appetite. Our understanding of human cancer-associated anorexia is complicated by the impacts of concomitant therapies, although rodent models implicate imbalances of appetite-regulating neuropeptides as well as changes in central neural regions such as the hypothalamus[90]. A recent paper has used Drosophila to identify an oncokine that acts directly on neurons to reduce feeding behavior[91]. In the fly model, hyperactivation of Yki in adult eye cells induces a secreted protein called insulin-like peptide 8 (Ilp8), previously discovered as the

oncokine responsible for developmental delay of tumor-bearing larvae (see below). Despite its name, Ilp8 resembles relaxins more closely than insulin-like growth factors, and signals through Lgr3, a homolog of LGR7 and LGR8 receptors (also known as relaxin receptors 1 and 2, respectively) found in a small set of central brain neurons[92–95]. Ilp8 binding to Lgr3 suppresses the fly's food intake by cell-autonomously increasing production of an anorexigenic hormone homologous to mammalian NUCB2 (also known as Nesfatin) while decreasing production of the orexigenic neuropeptide Y (NPY) hormone. The authors show that the same signaling axis exists in mammalian hypothalamic cells: relaxin-like peptide INSL3 binds to LGR8 to upregulate NUCB2 and downregulate NPY. Moreover, they provide strong evidence that several implanted mouse tumors induce anorexia using this INSL3-dependent circuit, and show a correlation between anorectic severity and serum INSL3 serum levels in a small cohort of human pancreatic cancer patients. Thus, by leveraging simple experiments in the fly, this work revealed a mechanism for paraneoplastic anorexia through altering appetite-regulating brain hormones.

Autophagy and Amino acid import

All tumors require substantial anabolic input to enable their inappropriate growth, arousing debate around whether transformed cells actively solicit nutrients from normal tissues[3,5,96]. Fly tumor models have revealed that indeed, some metabolic building blocks come not from ingestion but instead from catabolic processes in the host. Prominent among these is autophagy. Tumor cells in cooperative neoplastic larvae induce strong autophagic processes in WT neighboring epithelial cells as well as distant tissues (Fig. 3D), and blocking autophagy in these cells suppresses growth of the tumor itself[97]. The signaling pathway responsible for non-autonomous autophagic induction is not yet defined, but requires autocrine Upd/IL-6 signaling in tumor cells and perhaps local generation of ROS. Pharmacological blockade of autophagy, as well as depletion of a specific amino acid transporter in tumor cells, is sufficient to block tumor growth, although neither manipulation impacts the growth of WT cells. These results are consistent with a model in which tumor cells signal to drive autophagy in the TME, importing the liberated amino acids to fuel their own growth. Thus, autophagy in near or distant host cells, alongside the well-documented role for tumor-intrinsic autophagy[98], can generate nutrients used for tumor growth.

A requirement for host tissue-derived nutrients has been further demonstrated in a second fly tumor model, in which modest benign growth of larval imaginal disc cells expressing activated *Ras* and *Src* oncogenes is enhanced by a high-sugar diet, promoting full cooperative neoplastic transformation[44]. As mentioned above, these tumors induce wasting of host muscles in an Bnl/FGF-dependent manner, and the authors find that free circulating amino acids are elevated coordinately with muscle breakdown[84]. Intriguingly, in the high-sugar diet, tumor cells upregulate several amino acid transporters, one of which increases import of proline. Blocking this transporter activity either genetically or pharmacologically reduced tumor growth, while feeding the larva extra dietary proline was sufficient to increase tumor size and malignancy in the absence of the high-sugar diet. Together, these studies support the idea that in some cases tumor-induced host catabolic processes can be not just epiphenomena but instead active contributors to tumor growth. They also illustrate

how the fly system, with sophisticated genetic manipulation of different tissues, can resolve questions that are challenging to approach in vertebrate models.

Other fly paraneoplasias

Fly tumor models display several paraneoplasias that do not yet have obvious parallels in humans. Indeed, the first tumor suppressor mutant was identified because of its unusual systemic phenotype. Animals lacking the *lethal giant larvae (lgl)* gene are incapable of entering the pupal stage[99,100]. During the subsequently prolonged larval stage, the animal becomes strikingly enlarged and filled with fluid. *lgl* mutant larvae were later found to develop neoplastic imaginal discs and brains,[101] raising the question of whether developmental delay and fluid retention were due to cell-autonomous activities of the *lgl* gene product or instead to tumor–host interactions. Genetically mosaic larvae demonstrate that the latter is the case: a single pair of neoplastic discs in an otherwise WT animal is sufficient to induce both phenotypes[102]. Interestingly, the edema-like ‘bloating syndrome’ is also seen in allograft as well as GEFM adult tumor models, demonstrating a consistent and dramatic systemic perturbation in fluid balance (Fig. 3E)[16,81,103].

Developmental delay and bloating are separable paraneoplasias. One signaling molecule responsible for the former is Ilp8, discussed above as a regulator of cancer-associated anorexia. *Ilp8* is the most highly upregulated gene in neoplastic fly tumors[104,105], and tumor-derived Ilp8 binds to Lgr3 in neurons to regulate a circuit that releases hormones triggering the larva-to-pupa transition (Fig. 3F). When produced and secreted into the hemolymph, Ilp8 binds to Lgr3 in the brain and causes neuroendocrine changes that delays pupation until Ilp8 levels drop. Ilp8 expression in imaginal discs declines as the animal enters the L3 larval stage, but can be strongly upregulated by JNK and Hippo signaling, for instance in response to wounding or cell stress. This signaling axis thus normally couples tissue damage to the neuroendocrine system in order to allow time for tissue repair before metamorphosis. However, like an unhealing wound, tumors with chronic activation of JNK and Hippo signaling hijack this pathway and prevent normal maturation. Interestingly, Upd/IL-6 production in the imaginal disc shows similar JNK and Hippo-dependent regulation[106], and a recent paper suggests that tumor-derived Upd/IL-6 acts directly on neuroendocrine cells to promote developmental delay, enhancing the effect of Ilp8[107] (Fig. 3F).

In contrast to developmental delay, the mechanism by which tumors induce fluid retention is not yet understood. Bloating correlates with wasting phenotypes in ISC tumor models, and one proposal is that fluid retention results from osmotic compensation for elevated hemolymph sugar levels, induced by systemic insulin resistance (see below)[81,83]. Alternate possibilities also exist. Both solute balance and fluid secretion are ultimately regulated by the malpighian tubules, the Drosophila kidney analog[108]. In tumor-free adults, bloating phenotypes can result from developmental defects of the tubules[109,110], from defective signaling of a diuretic hormone that regulates tubule function[111], or from defects in ion channels in the tubule cells themselves[112]. Whether tumors interrupt any of these endogenous fluid-balancing circuits merits investigation.

Host impacts on tumor progression

So far, we have discussed how the tumor impacts host tissues. Conversely, systemic physiology is a critical regulator of disease progression in cancer. Indeed, amongst the many challenges of studying human cancer patients, the complex interactions between individual environment, comorbid conditions and tumor genotype looms large. Obesity in particular is a major risk factor for cancer, through mechanisms that include inflammation; diet may also have more direct interfaces with tumor metabolism[113]. We now describe what is known in the fly about the impact of these host environmental parameters on tumor progression.

The influence of diet on Drosophila tumors differs widely with the model used. For example, the mild overgrowth of mitotic clones mutant for the tumor suppressors *Pten, Tsc1 or Tsc2* in imaginal discs is strongly enhanced in nutrient-deprived conditions[114]. However, in *Pten* mutants this is due to increased cell proliferation, while with *Tsc1* or *Tsc2* mutants it is due to cell hypertrophy. Diet can also affect the stability of the fly homeodomain interacting protein kinase (Hipk), which has oncogenic properties[115]. In a study mentioned earlier, diet had a dramatic impact in a cooperative oncogenesis model combining *Ras* and *Src* hyperactivation[44]. Under standard conditions, most eye disc cells with these genetic changes die. When the host larvae are fed a high sugar diet, mutant cells undergo full neoplastic transformation including dispersal to secondary sites. In WT host tissue, a high-sugar diet induces hyperglycemia, hyperinsulinemia and insulin resistance. In the transformed cells, it creates a feed-forward loop involving Wnt signaling that results in upregulation of the Insulin receptor[116], allowing the cells to evade insulin resistance, and express the full malignant phenotype.

Evasion of insulin resistance may be a frequent feature of Drosophila tumors. For instance, neoplastic clones can escape competitive elimination and form tumors if systemic insulin levels are elevated[117]. When reviewing fly cachexia above, we described how allografted and GEFM flies upregulate ImpL2/IGFBP, which blunts insulin signaling throughout the animal[80,81]. Tumors in these models nevertheless proliferate aggressively, although they remain dependent on PI3K activity[118]. How such cells decrease reliance on insulin for growth is not known, but fly tumors have a distinct metabolism, with aspects of the glycolytic Warburg effect seen in many human tumors[119–121]. Many fly tumors upregulate lactate dehydrogenase (LDH), perhaps through oncogenic ERK and PI3K signaling pathways as well as hypoxia. Moreover, in one model, LDH expression has been shown to drive the transition from hyperplasia to neoplasia[121]. Thus diet and particularly metabolic dysfunction can enhance tumor progression in the fly.

To date, few studies have looked deeply at the effects of obesity on fly cancer models[122]. Our group has found that tumor-bearing flies on a high-fat diet show accelerated death (JK and DB, unpublished data). Flies fed a high-fat diet are known to upregulate Upd/IL-6, a central inflammatory player implicated in many fly tumor phenotypes, suggesting one possible mechanism[123]. It is known that dysbiosis of commensal bacteria can fuel gut tumor production in flies[124,125], but there are few studies on the interface between infection and fly tumor progression[126,127], and none on the impact of the changing physiology of aged

animals. All of these are feasible in the Drosophila system, where approaches described in sections above can untangle the role of tumor-autonomous, local, and paraneoplastic effects.

Perspectives

We feel that the general field of tumor–host interactions is well-poised to benefit from work in the reductionist system of Drosophila. Beyond molecules and mechanisms, fly studies are providing general insight into how an animal reacts to the growth of a pathological ‘neo-organ’. For instance, the anti-neoplastic activities of neighboring WT epithelial cells, circulating macrophages, humoral immune-induced factors and systemic cytokines make it clear that even in this short-lived invertebrate multiple potent mechanisms to prevent tumor formation have been selected for. Moreover, fly data support the idea that tumor defense was an early evolved role for the innate immune system, rather than a later cooption of adaptive pathogen-fighting cells. Finally, similarities between the fly responses to injury and to cancer demonstrate that Dvorak’s formulation of a tumor as a ‘wound that does not heal’, first used to describe the composition of tumor stroma[128], can also serve as an insightful guide to the evolutionary origin of the host response.

The emphasis in this review on paraneoplasia reflects our belief that the growing interest in tumor–host interactions should expand well beyond the TME, and embrace a view of cancer as a disease not just of mutant cells, but of interacting physiological systems throughout the body. Approaching cancer as a multi-organ disease brings with it an intimidating complexity. Yet Drosophila research has a long and impactful track record in the ‘discovery’ phase of complex biological questions, when organizational principles have yet to emerge. Flies’ superb experimental approaches, including forward genetic screens, have untangled phenomena that appeared unapproachably intricate, such as the molecular bases of development and pathways mediating intracellular communication[129–131]. Analogous genetic screens are conceivable for the tumor–host interactions described above, and high-throughput therapeutic screening as well as follow-up mechanism-of-action studies are also possible[132,133]. While the example of cachexia shows the value of the fly for deciphering mechanisms of recognized paraneoplasias, this simple system also can be used to identify new and potentially conserved tumor-host interactions.

We have discussed mechanisms of short- and long-range tumor–host interactions, yet the impacts of these interactions on host survival *per se* are seldom explicitly studied. Tumor growth and lethality are often treated as synonymous, but patients can also die with a relatively small tumor burden that does not impede an essential organ. Moreover, the ultimate cause of cancer death can often be unclear. In these situations, paraneoplastic alterations might play an important role, highlighting the value of a better understanding of how they actually promote lethality. It is not currently known why flies bearing tumors die (Fig. 3G). Aging flies, like other model organisms, often present with defects in intestinal permeability shortly before death[134]. However, comparable defects are not regularly seen in flies bearing tumor allografts (JK and DB, unpublished data). Flies suffering from infection may die not only from pathogenic effects of the microbe itself but also from the deleterious impact of the host immune response, such as prolonged inflammation[135]. The organ(s) whose failure is responsible for death of such infected flies, like tumor-bearing flies, remain

unknown. Answering ‘how cancer kills flies’ appears achievable using existing tools and knowledge, and should shed light on what is truly the ultimate tumor–host interaction.

Just as fundamental cancer research focuses on mechanisms of tumor growth, most current therapies focus on limiting or reversing this growth. Radiotherapy, chemotherapy and immunotherapy all place a selective pressure directly on cancer cells. Given the genetic instability of human tumors, this pressure can lead to the emergence of resistant clones, which account for the high frequency of cancer recurrence after initial treatment success. Appreciating the scope of paraneoplastic syndromes and their fatal impacts suggests an alternative tactic. Mechanistic understanding of these syndromes could permit targeted therapeutics that interfere with the host side of the tumor–host dialog. These are less likely to select for resistant variants; they would also — given the fact that widely different cancers elicit a common handful of paraneoplastic syndromes — reduce the need to customize therapy to an exact tumor genotype.

An important paradigm in microbial pathogenesis makes the distinction between resistance and tolerance: in the former, the patient fights disease by actively reducing microbial burden, whereas in the latter, the patient instead endures by ameliorating the pathological effects of the infection[136]. This paradigm has recently been ported to cancer, and explored in Drosophila[137,138]. Given the success of fly models in revealing functionally relevant host responses to tumors, it is appealing to consider that the lessons learned could inspire host-directed therapeutic strategies for human patients. With molecular knowledge of paraneoplastic morbidity mechanisms, one can envision developing cancer therapies that focus on ‘tolerance’, promoting longer health and life even in the presence of a tumor. Such an approach would provide a distinct and appealing complement to the standard tumoricidal strategies being pursued today.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1:

Drosophila tumor genetics

As in mammals, tumors in Drosophila are defined as groups of cells that overproliferate due to a loss of normal growth-restraining mechanisms. Though relatively short-lived, Drosophila and other insects can develop tumors spontaneously, for instance in the gut[139–141]. In the laboratory, tumors can be readily induced through genetic manipulation of a variety of epithelial, neural, hematopoietic and germline tissues. Homologs of some fly tumor suppressor genes and oncogenes (e.g. RAS family genes, *TSC1, TSC2, NF2,* and the Hippo pathway are frequently mutated in human cancer; others (e.g. *SCRIB*) are not but have nevertheless been experimentally implicated in mammalian oncogenesis[142,143]. A striking feature of Drosophila tumors is that they can be induced by altered activity of single genes, and do not require multiple genetic lesions nor loss of p53. The resultant tumors are grossly genetically stable and therefore relatively homogenous[144], simplifying analyses.

Drosophila tumors are divided into two classes, called hyperplastic and neoplastic[12,142]. These are roughly comparable to benign and malignant mammalian tumors respectively. Hyperplastic tumors preserve the fate as well as cell architecture of their tissue of origin, and undergo accelerated mitoses. Neoplastic tumors, by contrast, often grow more slowly than WT cells, but never stop proliferating. Neoplastic cells show signs of dedifferentiation, lose cell polarity and show invasive behaviors including degradation of basement membranes; they also become functionally immortal. While flies can often tolerate extensively overgrown hyperplastic tissue, the presence of a neoplastic tumor causes more rapid death (Fig. 2). Comprehensive reviews on the biology of fly tumors are available for readers seeking more detail[7,12–15,142,143,145].

Box 2:

Fly models for tumor – host interaction studies

While transformation can occur in other cell types, epithelial tumors have been best characterized for tumor-host interactions (Supplementary Table 2). Neoplastic carcinomas are most readily induced by mutation of *scrib*-class genes [12,143,146]. A popular model combines *scrib* mutants with overexpression of oncogenic *Ras*[18]. This cooperativity creates a more aggressive and rapidly malignant tumor.

Neoplastic transformation can also occur through other means, involving e.g. activation of Notch and Src or chromosomal instability[145]. Some models mimic the genetic constituency and tissue of origin of human cancers, with a focus toward therapeutic screening[133,147]. Despite differing driver genes, many neoplasms act through a common set of signaling pathways --JNK, Hippo, STAT and ERK-- that regulate the shared phenotypic hallmarks. In this review we treat these tumors collectively, as providing a new and malignant source of growth that host physiology must react to, while also acknowledging that there may be significant differences in the details of the host response.

While the larva is excellent for studying autonomous tumor growth, it presents disadvantages for studying tumor–host interactions. The accessible third larval instar lasts only 48 hours, terminating when metamorphosis creates a pupa. Such insect-specific events impair analysis of long-term tumor–host interactions familiar in mammals. Studying cancer in adult Drosophila avoids these limitations. The earliest model uses ‘allograft’ transplantation of imaginal discs or brains from the larva[16]. Flies have an open circulatory system and no transplant histoincompatibility, so simple injection of tissue allows growth in the permissive environment of the adult abdomen[17]. Transplanted WT tissue does not undergo excess growth, but tumorous tissue can increase ~200-fold in size over 1–2 weeks before the host dies. Whereas neural tissue disseminates throughout the body cavity, epithelial disc tissue remains intact, growing as a compact mass. Remarkably, tumors can be propagated to successive hosts apparently indefinitely, supporting an ‘immortal’ transformation of the original tissue.

In addition to transplants, autochtonous tumors can be induced in the adult via ‘genetically engineered fly models’ (GEFMs)[15,148]. The adult fly has only a few active zygotic stem cell populations, but transgenic manipulation of these can induce tumorous growth. Activation of Hippo or Wnt, or reduction of Notch in intestinal stem cells (ISCs) creates malignant tumors, as does Ras overexpression in normally quiescent renal stem cells. Adult blood and neural cells are post-mitotic, but oncogenic expression in neural or glial progenitors in the larva can yield viable adults with continuously proliferating tumors. Finally, our lab has achieved neoplastic transformation of adult ovarian epithelial cells (DB, TH, and JK unpublished data).

Fig. 1: Drosophila organ systems and their human analogs.

Many fly organs have straightforward structural and functional homologs in vertebrates: brain, muscle, digestive tract, and the dorsal vessel (the Drosophila heart). Neuroendocrine cells in the fly brain secrete glucagon-like and insulin-like peptides similarly to pancreatic alpha and beta cells, respectively. The fly fat body stores lipids and carbohydrates, akin to adipose tissue and the liver in humans; it is the metabolic hub as well as a major secretory organ. Fly oenocytes also play a hepatocyte-like role in lipid processing and mobilization. The fly malpighian tubule serves the excretory and diuretic (water and ion homeostasis) function of human kidneys, while nephrocytes serve the glomeruli and podocyte role in filtering circulatory fluid. Oxygenation takes place through passive transport through tracheal tubules, whose complete network of air sacs and extensively ramifying branches are not shown here. The hemocoel is the open body cavity of the fly; it is filled with circulatory fluid that transports oxygen, nutrients, waste and immune cells analogous to human blood. Figures 1, 2, and 3 were drawn by Nature Reviews Cancer art editor, not the authors of the paper. They can be found in the published version of the paper at DOI: 10.1038/s41568-021-00387-5.

Fig. 2: Interactions in the fly tumor microenvironment

Tumor-produced signals alter the surrounding stroma. **a)** Upregulation of Upd/IL-6 in tumor cells can stimulate proliferation of neighboring WT cells. **b)** Mispolarization of Sas and Ptp10D at the interface between a tumor clone and WT epithelium promotes death of the tumor cells, triggered by Egr/TNF. The source of Egr/TNF is not yet clear. **c)** Paracrine Dpp/TGFβ and juxtacrine Delta produced by tumor cells can promote proliferation of underlying mesenchymal myoblasts. **d)** Bnl/FGF production from hypoxic tumors can attract new tracheal branches. **e)** Tumor production of MMPs degrades basement membrane and promotes invasion. **f)** Tumors attract macrophages (dashed arrow) that detect tumor-produced Pvf/VEGF, extracellular ROS and basement membrane damage. Macrophages then upregulate Egr/TNF, which binds to tumor cells and promotes their death.

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Fig. 3: Paraneoplastic effects of fly tumors.

Endocrine signals from tumors cause pathologies in distant organs. Some effects have been demonstrated in larvae and others in the adult; see text for details. Tumor-associated macrophages secrete Spz, which activates Toll signaling in the fat body to trigger production of the antimicrobial peptide Defensin (**a**). Defensin, working along with macrophage-produced Egr/TNF, binds to and kills tumor cells. ImpL2/IGFBP induces systemic insulin resistance, leading to cachexia-like wasting and degeneration of fat body (**b**) and muscle (**c**). Reception of tumor-produced Pvf/VEGF in adult fat body and muscle, and Bnl/FGF in larval muscle, also induces wasting. Wasting muscles supply amino acids via autophagy (**d**) that are taken up by the tumor and promote tumor growth. Ilp8/INSL3 acts on brain neurons to inhibit the production and release of pupation-promoting hormones in the larvae; it also acts in the adult to stimulate brain production of anorexigenic peptides (**e**). Unknown tumor-dependent factors cause the host to retain excess fluids; this may be due to actions on the malpighian tubules (**f**). Some combination of these pathologies, along with other currently unrecognized effects, kills hosts prematurely (**g**).

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Table 1:

Drosophila oncokines that mediate tumor–host interactions.

Fly Oncokine	Human Orthologue	Host Effect	Target Tissue	Tumor Model	References
Endocrine action					
ImpL2	IGFBP family	Cachectic wasting	fat, muscle, ovaries	neoplastic and cooperative neoplastic disc, Activated *yki*-expressing ISCs	80, 81
Pvf1	VEGF and PDGF	Cachectic wasting	fat, muscle	Activated *yki*-expressing ISCs	83
		Immune cell proliferation	macrophages	neoplastic disc	56
Branchless	FGF	Cachetic wasting	muscle	*csk mutant* + *RasV12* + High sugar diet disc	83
		Neotracheogenesis (?)	trachea	neoplastic disc	43, 47
Ilp8	Relaxins or INSL3 (?)	Neuroendocrine regulation	neurons	neoplastic and cooperative neoplastic disc	104, 105
		Anorexia	neurons	cooperative neoplastic disc	91
Unpaired 1, 2 and 3	IL-6	Immune cell proliferation	macrophages	neoplastic and cooperative neoplastic disc	56
		Neuroendocrine regulation	neurons	cooperative neoplastic disc	107
Paracrine action					
Unpaired 1, 2 and 3	IL-6	Epithelial proliferation	WT epithelia	*ESCRT* and *PRC1* mutant disc clones	25–29
MMP1 and 2	MMPs	Invasion	basement membrane	neoplastic and cooperative neoplastic disc	70, 71, 73
Decapentaplegic	TGFβ	Stromal proliferation	myoblasts	*psq* mutant + *EGFR* disc	38
Delta	DLL family	Stromal proliferation	myoblasts	*psq mutant* + *EGFR* disc	39

DLL, delta-like ligand; ISCs, intestinal stem cells