Drosophila as a Model for Tumor-Induced Organ Wasting

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Abstract

In humans, cancer-associated cachexia is a complex syndrome that reduces the overall quality of life and survival of cancer patients, particularly for those undergoing chemotherapy. The most easily observable sign of cachexia is organ wasting, the dramatic loss of skeletal muscle and adipose tissue mass. Estimates suggest that 80% of patients in advanced stages of cancer show signs of the syndrome and about 20% of cancer patients die directly of cachexia. Because there is no treatment or drug available to ameliorate organ wasting induced by cancer, cachexia is a relevant clinical problem. However, it is unclear how cachexia is mediated, what factors drive interactions between tumors and host tissues, and which markers of cachexia might be used to allow early detection before the observable signs of organ wasting. In this chapter, we review the current mammalian models of cachexia and the need to use new models of study. We also explain recent developments in *Drosophila* as a model for studying organ wasting induced by tumors and how fly studies can help unravel important mechanisms that drive cachexia. In particular, we discuss what lessons have been learned from tumor models recently reported to induce systemic organ wasting in *Drosophila*.

Keywords

Drosophila · Cachexia · Muscle · Fat body · Organ wasting

11.1 Cancer-Induced Cachexia

Cachexia induced by cancer is characterized by increased systemic inflammation, general metabolic dysfunction, and elevated resting energy expenditure; it can be accompanied by anorexia and loss of appetite but it is not usually reversed by increasing nutritional intake [55]. All of these symptoms lead to a progressive loss of body weight due to organ wasting, particularly of the skeletal muscle and, in many cases, of adipose tissue and fat reserves [6, 56, 113, 133, 147]. Even though cachexia is often observed in a high proportion of cancer patients and correlates with poor life expectancy and reduced quality of life, the mechanisms driving this syndrome are poorly understood and efficient treatment therapies are needed [56, 91].

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Most of our knowledge of cachexia induced by cancer has been acquired from studies using rodent models [13, 17]. The induction of cachexia has no clear correlation with either tumor mass or tumor type [49, 71, 98], yet it is widely accepted that circulating factors secreted directly by tumor cells or from normal host cells cause wasting [56, 147]. Lysates of Krebs2 carcinoma samples, when injected in mice, reduce fat levels and cause weight loss, arguing for the presence of a wasting-inducing factor [42]. In a study using extracts of mouse thymic lymphoma, a soluble lipid-mobilizing factor (LMF) derived from the cancer cells also induced lipolysis [83]. A distinct LMF, isolated from human melanoma A375 cells [143], and later found also in MAC16 mouse adenocarcinoma cells and urine samples of cancer patients, was further identified as zinc-a2-glycoprotein [150]. Interestingly, MAC13 cells, derived from the same tumor type as MAC16, are histologically similar and have a similar growth rate, but do not produce zinc-a2-glycoprotein and do not provoke weight loss, arguing that similar types of tumor can have different potential to induce cachexia [98]. Further, a secreted proteolysis-inducing factor (PIF) isolated from both MAC16 cells and human tumor samples was shown to cause muscle-specific proteolysis [148, 149]. Taken together, these examples shed light on the heterogeneity of tumor-secreted factors that can induce cachexia independently of the type of tissue that originates the tumor.

Cachexia involves systemic inflammation, and several pro-inflammatory cytokines, either derived from host tissues or from tumors, have been shown to have a relevant role in cachexia [7]. Muscle samples incubated with interleukin-1 (IL-1), a cytokine usually produced by human leukocytes in the context of sepsis, exhibit increased proteolysis and signs of wasting [15]. Tumor necrosis factor alpha (TNF-α), initially called "cachectin" [19], was also shown to induce organ wasting in mouse models [103], as was IL-6 [25, 141]. Although rodent models have been crucial to understanding cachexia, data from human patients has not always correlated with mouse models, particularly since there is no clear link between circulating levels of TNF-α in the serum of cancer patients and their respective weight loss [93]. Further, the observation that antibodies against TNF-α in cancer patients do not improve prognosis puts in question the role of TNF-α in cachexia [77]. Regarding IL-6, higher circulating levels of this cytokine in patients with lung cancer correlates with lower survival rates [99, 134, 142]. In mouse models, higher levels of IL-6 are linked to higher tumor burden and decreased survival in the presence of certain tumor types [141]. However, overexpression of IL-6 in tumor-free mice does not cause organ wasting, indicating a tumor-dependent role of IL-6 in organ wasting [14]. Moreover, circulating cytokines can directly promote tumor growth [31] or stimulate production of tumor-derived factors [24] and, consequentially, influence the development of cachexia. These synergistic interactions emphasize the difficulty in understanding the role of cytokines and other secreted factors in cancer-induced cachexia when relying only on data from rodent models [13, 108]. It also highlights that using other model organisms to study organ wasting might help to expand our understanding of cachexia.

11.2 Muscle Wasting

Loss of skeletal muscle mass is a hallmark manifestation of cancer-induced cachexia and results from an imbalance in the coordination between protein synthesis and protein degradation [52, 63, 79, 132]. In skeletal muscle, protein synthesis is mainly regulated by the insulin-like growth factor 1 (IGF1) [39, 99]. IGF1 activates the PI3K/AKT pathway, which stimulates expression of downstream target genes involved in protein synthesis and hypertrophy of muscle fibers [27, 120]. Conversely, proteolysis can be caused by different catabolic inputs such as starvation, denervation, or cachexia, which typically lead to increased activity of the ubiquitin-proteasome system and the autophagy/lysosome pathway [10, 88, 94, 125].

The proteasome system acts in the muscle by two muscle-specific E3 ubiquitin ligases, the muscle RING finger-containing protein 1, MuRF1, and the muscle atrophy F-box protein, MAFbx [38]. Both are upregulated under several catabolic states and are extensively used as mark-

ers of muscle wasting; deletion of either of these two ubiquitin ligases in mice ameliorates skeletal muscle atrophy [26, 64]. In addition, both are transcriptionally controlled by Forkhead box O (FoxO) transcription factors, which are negatively regulated by the insulin/AKT pathway, putting both MuRF1 and MAFbx under direct control of the IGF-1 pathway [96, 130, 140]. MuRF1 and MAFbx act in the muscle by ubiquitinating specific proteins and targeting them for degradation by the proteasome system: MuRF1 mediates ubiquitination of myosin heavy chain (MyHC) and other thick filaments that compose the muscle fibers [35, 37]; MAFbx targets both eIF3-f, a translation initiation factor, and MyoD, a key regulator of myoblast identity and differentiation [87, 145]. Notably, the latter finding suggests that MAFbx acts by suppressing protein synthesis rather than by increasing proteolysis of muscle fiber components [11].

The autophagy/lysosome pathway can also be elevated during muscle wasting [129]. Muscle denervation or starvation induces FoxO3-mediated expression of autophagy-related genes in mouse skeletal muscle [94, 96] and in *in vitro* models of C2C12 myoblast cells [163]. Importantly, the autophagy/lysosome pathway has also been shown to be upregulated in muscles of mice with cachexia [10, 110]. Bnip3, a member of the Bcl-2 family of apoptosis regulators, is a mediator of autophagy [67, 94, 151], and is upregulated during wasting [10], making it a relevant gene involved in muscle wasting.

11.3 Adipose Tissue Wasting

In many cases of cancer-induced cachexia there is loss of adipose tissue [50, 124, 152]. Fat accumulates in the form of triglycerides in lipid droplets (LDs) located in the cytoplasm of adipocytes. Brown adipocytes form the brown adipose tissue, whereas white and beige adipocytes constitute white adipose tissue (WAT) [36]. Brown and beige adipocytes have higher mitochondria content than white adipocytes, and brown adipocytes express higher levels of uncoupling protein 1 (UCP-1) to generate heat in response to cold stress through a process called thermogenesis [107, 155]. In rodent models of cachexia, browning of WAT by activation of UCP-1 in beige cells increases energy expenditure [112], and is induced by the parathyroid-related peptide (PTHrP), a tumor-secreted factor [82].

WAT is the storage tissue in mammals for energy reserves, and works as an endocrine organ that controls general metabolic homeostasis [60]. In cancer patients with cachexia, loss of adipose tissue seems to be due to excessive lipolysis in LDs, rather than reduction of lipid synthesis [44, 123]. Lipolysis is driven by a cascade of three lipases, adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase, which sequentially process triglycerides into diacylglycerol, monoacylglycerol, and finally glycerol and fatty acid that are further released into circulation [159]. The specific removal in adipose tissue of ATGL or HSL prevents excessive lipolysis and wasting of adipose tissue in a mouse model of cachexia [45]. Furthermore, in cancer patients with cachexia, some tumors may lead to increased activity of ATGL and HSL [45], whereas other tumors only induce activity of HSL [3], arguing for a role of both lipases in induction of adipose tissue wasting by stimulating lipolysis [44, 123].

Adipose tissue loss has been shown to precede skeletal muscle wasting in patients with cachexia [59]. In addition, the removal of either ATGL or HSL from adipose tissue also protects skeletal muscle from wasting [45], suggesting a link between excessive lipolysis in adipocytes and subsequent induction of muscle wasting. An excessive rate of lipolysis increases the cellular levels of lipids in the muscle, leading to insulin resistance and glucose metabolism impairment [127, 158]. Although the mechanisms of insulin resistance induced by lipid accumulation are unclear, it has been hypothesized that intracellular accumulation of diacylglycerol in muscles activates a subgroup of protein kinase C (PKCs) that inhibit the insulin receptor, causing skeletal muscle insulin resistance [33, 77, 159]. Interestingly, insulin resistance is present in both human patients and mouse models of cachexia [73, 158], although in some clinical cases of cachexia there is a lack of correlation between tumor-secreted factors and insulin resistance [2]. Nevertheless,

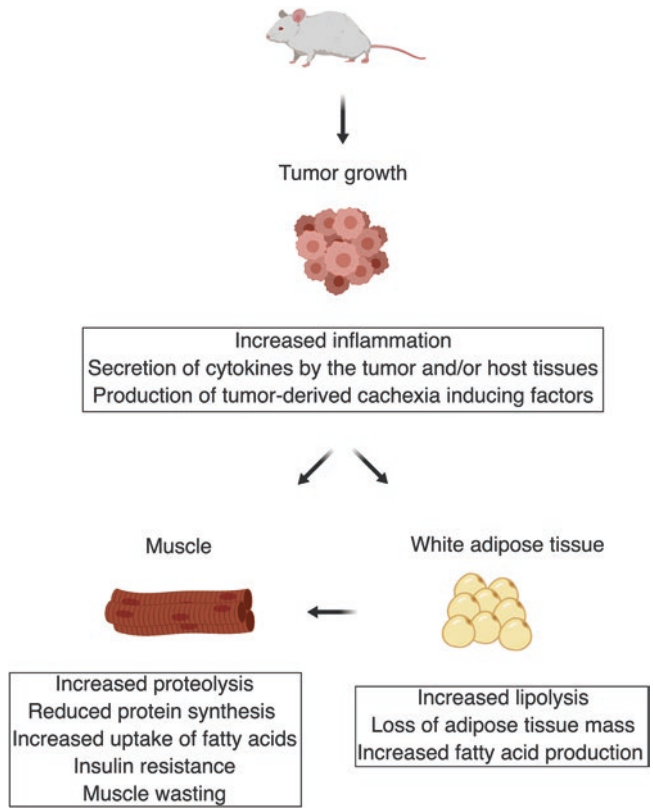

Fig. 11.1 Cancer-induced cachexia in mammals. Tumors promote a systemic inflammatory response and pro-inflammatory cytokines produced by either tumor or host tissues induce organ wasting. Tumors can also produce other cachexia-inducing factors that promote muscle and white adipose tissue wasting. In the muscle, wasting is caused by excessive proteolysis and reduction of protein synthesis. Wasting of the white adipose tissue consists of increased lipolysis, loss of fat, and production of circulating fatty acids. The excessive uptake of fatty acids by muscles leads to tissue-specific insulin resistance that can also contribute to muscle wasting

changes in glucose metabolism in skeletal muscle and adipose tissue correlate with organ wasting [73]. Therefore, excessive lipolysis causing insulin resistance and impaired glucose metabolism in the muscles may lead to a synergistic effect that, when combined with the activity of circulating factors derived from tumors or host tissues, induces muscle wasting [6] (Fig. 11.1).

In summary, cachexia is a syndrome with several layers of complexity. Tumors and host tissues can produce different types of circulating factors that cause systemic metabolic impairment and organ wasting. These factors induce catabolism, lipolysis, or proteolysis directly in muscles and adipose tissues, and/or work synergistically to promote tumor growth. Excessive lipolysis in adipose tissues can lead to insulin resistance in the muscle and decreased anabolism, which may contribute to the wasting process. Moreover, data from rodent models of cachexia does not always reflect what is observed in human patients. As such, as well as due to the extreme heterogeneity of cancer and cachexia, it has been very difficult to pinpoint if there is a general mechanism of systemic organ wasting, and therefore other models of research are necessary.

11.4 *Drosophila* as a Model to Study Cancer

The *Drosophila* genome encodes orthologs of many human genes associated with diseases, and several fly organs have analogous functions to human organs [119, 122, 153]. The repertoire of genetic tools available in *Drosophila* includes the GAL4/UAS system for tissue specific modulation of gene expression and the FLP/FRT MARCM system to induce and label mosaics of genetically distinct cells in a specific tissue [28, 46, 157]. In addition, an extensive collection of fly strains for RNAi and overexpression, covering most fly genes, allows for spatially-controlled knockdown or ectopic expression of any gene of interest. Moreover, some of the most important signaling pathways involved in cancer were first discovered in flies [111], making *Drosophila* an invaluable model for the study of human diseases, including cancer [34, 153].

Despite being short-lived animals, flies can spontaneously develop tumors [139]. Tumors can also be readily induced by ectopic expression of oncogenes or disruption of tumor-suppressor genes in target tissues [23, 62]. *Drosophila* tumors display the typical hallmarks of cancer, namely resistance to apoptosis, chronic mitogenic signaling, evasion of tumor suppressor action, genomic instability, metabolic alterations, and invasion of tissues [68]. Given this, the use of *Drosophila* as a model to study cancer has revealed new genes involved in tumorigenesis and contributed to our understanding of the mechanisms of tumor growth and metastasis [54, 97, 137, 146].

Both fly larvae and adult stages have been used to study tumor development [65, 146]. In larvae, tumor models have been established in various tissues: lung cancer in the trachea [90], gliobastoma in the brain [118, 154], rhabdomyosarcoma in muscle [61], and leukemia in hemocytes [43]. However, the imaginal discs have been the most-used tissues for study of tumorigenesis [69]. Imaginal discs are epithelial tissues composed of highly proliferative diploid cells, making them an easy tissue in which to induce gene knockout or overexpression mosaics of cells [157]. Several signaling pathways that can drive cancer in mammals have been manipulated in the imaginal discs to induce tumors, including EGFR-Ras-Raf, Hippo-Salvador-Warts, TGFbeta, Notch, JAK/STAT, and have been extensively studied in the context of tumorigenesis [69].

Most genetic manipulations used to model tumors in larvae lead to hyperplastic tumors but some become metastatic and invade other tissues [22, 62, 97]. Expression of an activated form of Ras ($Ras^{[V12]}$) or activated Notch ($Notch^{[ACT]}$) in clones in imaginal discs, for example, induces overproliferation and hyperplastic growth [30, 80]. Similarly, ablation of the cell polarity genes *scribble (scrib), discs-large-1 (dlg1)* or *lethal (2) giant larvae (lgl)* drives loss of apico-basal polarity and induces hyperplasia [23]. However, when a mutation for a polarity gene is combined with overexpression of either $Ras^{[V12]}$ or $Notch^{[ACT]}$, cells become severely malignant, invade other tissues, and induce secondary tumor growths [30, 104].

The study of tumor progression in adult flies has mainly consisted of dissecting and transplanting larval tumors into adult flies [121]. Unlike malignant tumors, benign tumors transplanted into adult flies do not display metastatic behavior, such that the transplantation method provides a way to distinguish between neoplastic and hyperplastic tumors [62]. An alternative to the transplantation method is to generate tumors directly in adult fly tissues [65]. One example of this strategy is the overexpression of an activated form of Yorkie (Yki), a transcriptional co-activator of the Hippo pathway, in adult stem cells of the intestine, which generates gut tumors [81, 135]. Other adult tissues used as sites for induction of tumor formation include the Malpighian tubules [161], germline [126], brain [16, 92], and hemocytes [5], arguing that induction of tumors in adult flies is a valid alternative to the transplantation method of larval-induced tumors.

11.5 *Drosophila* as a Model of Cachexia

Developmental biology studies in *Drosophila* have unraveled important signaling pathways that are implicated in cancer [111]. Moreover, *Drosophila* has been used as model to study tumorigenesis, but it has only recently been considered as a model of organ wasting [54, 69, 137]. Two independent studies have described how tumor progression in adult flies induces phenotypes consistent with organ wasting, in a manner similar to what is observed for rodent and human models [57, 86]. In one study, the authors induced tumors in the adult midgut by specifically expressing Yki in intestine stem cells (gut yki-tumors) [86]. In the other study, the authors generated neoplastic tumors in the eye disc by inducing clones of cells mutant for *scribble* while ectopically expressing $Ras^{[V12]}$ ($Ras^{[V12]}$/*scribble*) and transplanted the tumors into adult flies [57].

In both tumor models, flies display a loss of muscle function and severe wasting of the ovaries and fat body. Interestingly, both tumors secrete high levels of *Imp-L2*, an insulin-like binding peptide, and flies show reduction of systemic insulin signaling, while being hyperglycemic, suggesting that the peripheral tissues become insulin resistant [57, 86]. Ectopic expression of *Imp-L2* is sufficient to induce wasting, whereas suppressing expression of *Imp-L2* specifically in the tumors significantly ameliorates the wasting phenotype [57, 86]. Transplanted discs with yki-induced tumors have lower production of Imp-L2 and do not cause wasting, even though their tumor burden is larger than that of $Ras^{[V12]}$/*scribble* tumors [57]. Therefore, high levels of Imp-L2 induce organ wasting independently of the tumor burden, making Imp-L2 a novel tumor-secreted factor that can cause organ wasting. Notably, IGF-binding protein-3 (IGFBP-3) was found to be upregulated in pancreatic cancer samples of human patients, supporting the possibility of a role for insulin binding peptides in induction of organ wasting [74].

Imp-L2 is a circulating peptide that forms a ternary protein complex with the acid-labile subunit of the IGF1-binding protein, dALS, and with the *Drosophila* insulin-like peptides (Dilps) [9]. Increased levels of circulating Imp-L2 correlate with systemic insulin signaling reduction, although it is unclear how binding of Imp-L2 to circulating Dilps modulates insulin signaling [4, 58]. Under starvation, the fat body, the fly counterpart organ of WAT in mammals, produces Imp-L2 to protect flies from starvation by reducing systemic insulin signaling [72]. The fact that Imp-L2 is induced by starvation raises the question of whether yki-tumors drive organ wasting by simply disrupting the basic gut functioning of food intake, inducing general starvation [86]. However, the feeding behavior of flies with gut yki-tumors and the expression of *pepck,* which is upregulated during starvation conditions, are not severely affected, suggesting that Imp-L2 is not simply increased due to starvation caused by the impairment of gut function [86]. More importantly, both yki-tumors in the gut and imaginal disc tumors produce Imp-L2, suggesting that tumor-driven wasting by Imp-L2 might be a general mechanism to "starve" the peripheral tissues by reducing insulin signaling (Fig. 11.2).

Interestingly, the insulin pathway, and in particular the rate-limiting enzymes involved in the glycolytic pathway, are highly active in yki-tumors in the gut, despite the fact that the peripheral organs show reduced insulin signaling [86]. This evidence highlights the importance of glucose metabolism in supporting growth of yki-tumors, and is in accordance with other tumor models in which high-sugar diets promote malignant growth in imaginal discs [53, 70].

Another study reported that yki-induced tumors in the intestine of adult flies also secrete a PDGF- and VEGF-related factor 1 (Pvf1) ligand that leads to a pathological activation of ERK/MAPK signaling non-autonomously in host tissues to induce wasting of muscles and the fat body [136]. Similarly, in a mouse model of cachexia, ERK signaling was increased in skeletal muscle, leading to upregulation of MAFbx and increased proteolysis [109]. In both studies, pharmacological inhibition of the ERK pathway ameliorated the wasting phenotype caused by tumors, independently of affecting tumor growth [109, 136]. As such, these results support a role for ERK signaling in promoting catabolism in peripheral tissues like the muscle [109, 136].

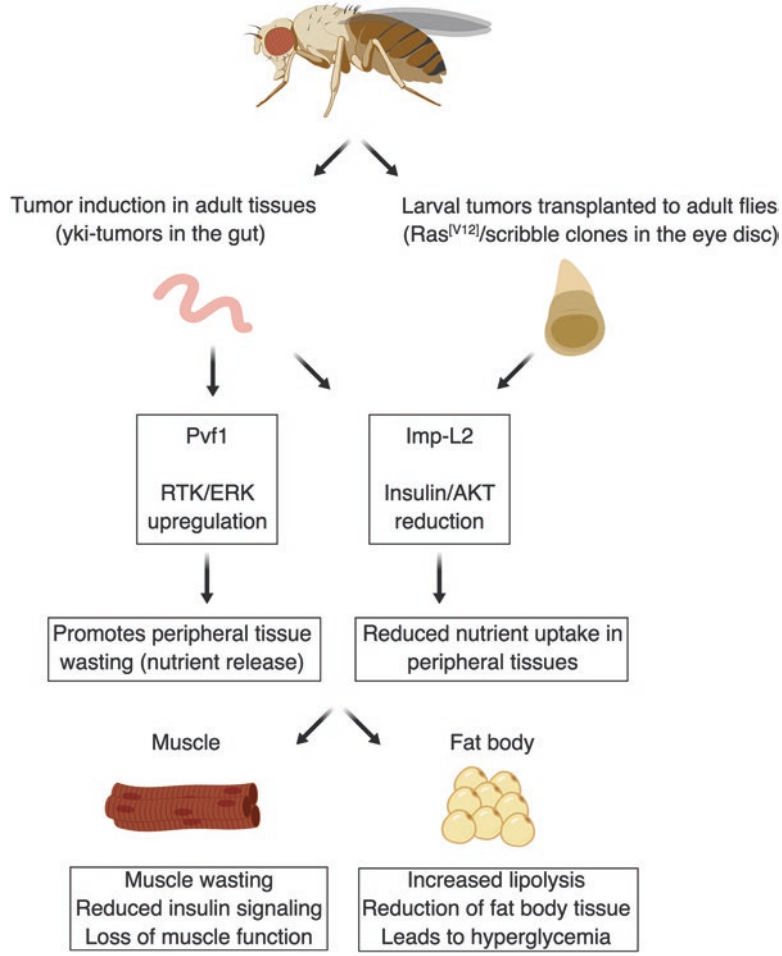

Fig. 11.2 Putative model of organ wasting induced by Pvf1 and Imp-L2 in *Drosophila*, using two different types of tumors. One tumor is generated by overexpressing Yki in the stem cells of the adult intestine. The other type of tumor is formed by transplantation to adult flies of larval imaginal discs that have clones of cells that express an activated form of Ras ($Ras^{[V12]}$) and are mutant for the polarity gene *scribble* ($Ras^{[V12]}$/*scribble*). Both tumor types produce Imp-L2, but yki-tumors in the adult gut also secrete Pvf1. $Ras^{[V12]}$/*scribble* transplanted discs induce organ wasting by overproducing Imp-L2. However, in gut yki-tumors, wasting is thought to be driven by a combination of Pvf and Imp-L2. Increased levels of circulating Imp-L2 reduce systemic insulin signaling, which leads to a reduction of nutrient uptake by muscle and adipose tissue, and further drives organ wasting. Pvf1 activates ERK signaling in the peripheral tissues and possibly reinforces the wasting of muscle and fat body already caused by Imp-L2

In summary, the studies describing organ wasting in the fly [57, 86, 136] suggest that *Drosophila* can be a useful model to study tumor-induced organ wasting (Fig. 11.2). Furthermore, the fly genetic tools, short generation time, and conservation of signaling pathways that induce tumors make *Drosophila* an important alternative to rodent models of cachexia.

11.6 *Drosophila* as a Model for Studying Muscle Wasting

Drosophila muscles are composed of actomyosin cables formed by repetitive contractile units – the sarcomeres – and share both functional and structural similarities with mammalian skeletal muscles [114, 116, 138]. Growth and atrophy of muscle are regulated by insulin signaling in a

manner similar to what is observed for vertebrate skeletal muscle [48]. Moreover, specific reduction of insulin signaling in muscles promotes FoxO-mediated expression of autophagy genes [12, 48]. However, although MAFbx and Bnip3 orthologs are present in the *Drosophila* genome (*CG11658* and *CG5059*, respectively), there is no apparent ortholog for MuRF1, arguing whether other ubiquitin ligases might be involved in muscle wasting in *Drosophila*.

In both Imp-L2-secreting tumors described above, flies show reduced climbing ability and defects in wing position [57, 86], indicating impaired muscle function. Also, AKT activity is reduced in muscles due to a decrease in insulin signaling [86]. Interestingly, whereas in mice models of cachexia, Bnip3 and MuRF1 are increased in muscle wasting [10], the expression levels of *CG11658* and *CG5059* are not significantly elevated during wasting [57], suggesting that either these genes are post-transcriptionally regulated during wasting or that other genes related to the proteasome system or the autophagy/lysosome pathway are involved. Furthermore, since FoxO transcription factors play a role in mammalian muscle wasting [129], and in *Drosophila* FoxO modulates expression of autophagy genes [12, 48], it remains to be addressed if FoxO activity is required in both fly tumor models to induce organ wasting.

11.7 *Drosophila* as a Model for Studying Adipose Tissue Wasting

The *Drosophila* fat body has an analogous function to the white adipocytes in mammals, while there is no apparent counterpart to brown adipose tissue. The fat body is the main organ where energy is stored in the form of fat and glycogen, with fat being stored in the form triglycerides in lipid droplets of the fat body [8]. *Brummer* (*bmm*), the fly ortholog of ATGL, and dHSL drive the hydrolysis of triglycerides in free fatty acids and glycerol that are further released into circulation [21, 66]. Mutations in any of these genes lead to accumulation of triglycerides in LD and obesity in flies [21, 66]. Conversely, feeding flies a high-sugar diet leads to hyperglycemia, insulin resistance, and obesity [101] – hallmarks of type 2 diabetes – suggesting that flies are a useful model to study lipid and glucose metabolism [20, 102, 144].

In the adult gut yki-tumor model, several rate-limiting enzymes of the glycolytic pathway are downregulated in muscles, and flies have lower levels of triglycerides stored in the fat body [86]. Since flies with yki-tumors are hyperglycemic, the systemic reduction of insulin signaling combined with an increase in lipolysis of triglycerides could indicate a dramatic accumulation of diacylglycerides in the muscle and induction of insulin resistance, similar to what occurs in mammalian skeletal muscle as discussed previously [33, 127]. However, it is unclear if *bmm* and dHSL mediate lipolysis in both fly models of organ wasting, and further studies are needed to understand the interactions between the fat body wasting and muscle wasting.

The fat body functions as an endocrine tissue that regulates systemic metabolism and organismal growth [40, 89]. In addition to Imp-L2, cytokines and hormones such as Unpaired-2 [117]; the ortholog of TNF-α, Eiger [1]; Stunted, the ligand of the Methuselah receptor [47]; growth-blocking peptides (GBP) [85, 95]; and the peptide hormone CCHamide-2 [131] are secreted from the fat body in response to nutritional cues to modulate systemic insulin signaling.

A link between cytokines, inflammatory responses, and tumorigenesis has been established in larvae [41, 76, 105, 106]. JNK signaling induction in $Ras^{[V12]}$/*scribble* disc tumors upregulates the cytokine-encoding *unpaired* genes *(upd1*, *upd2,* and *upd3)*, which further activate JAK/STAT signaling and promotes tumor growth and metastasis [106, 156]. Curiously, the release of Upd cytokines from the tumor also induces a systemic inflammatory response that limits tumor growth [106]. Circulating hemocytes produce Eiger, which activates JNK pathway in tumors to induce apoptosis and suppress growth, highlighting the complex interaction between cytokines and JNK signaling in tumorigenesis [41]. Nevertheless, it remains unknown if circulating cytokines induce organ wasting in *Drosophila*.

11.8 Considerations for Studies of Organ Wasting in *Drosophila*

Perturbation of different conserved signaling pathways in tissues, either in larvae or adult stage, generates different types of tumor in *Drosophila*. Though two types of tumor have already been identified to induce organ wasting, it remains unknown if more tumor types are capable of inducing wasting. While it is easy to generate tumors in imaginal discs with mosaic induction [157], the developmental time of the larva before reaching pupariation is short and, therefore, organ wasting might only be detected with very aggressive tumors. Thus, adult flies seem more suitable for studying organ wasting. However, transplantation of larval tumors into adults is technically demanding and often lethal, making it difficult to obtain a high number of surviving individuals to study [121]. In addition, it is difficult to control the amount of tumor sample transplanted, raising a concern that there could be considerable variability among individuals.

An alternative approach is to induce tumors directly in adult flies, as in the case of the gut yki-tumors [86]. In larvae the imaginal discs, in which tumors are induced, are formed of highly proliferative diploid cells [51]. Alternatively, the yki-tumors in adults are formed by stimulating overproliferation of intestinal stem cells by using a GAL4 driver, specific to gut stem cells, to ectopically express an active form of Yki [81, 135]. This indicates that other adult stem cells might be suitable for generating tumors when using GAL4 drivers specific of particular stem cell populations, and in combination with temperature-sensitive GAL80 transgenes that allow temporal control of gene expression [160].

One problem to consider with this strategy is that tumors might compromise the function of the organ in which they are being induced and rapidly cause lethality or affect systemic metabolic homeostasis, independently of tumor-secreted factors. Tumors induced in tissues that are not essential for adult viability, such as the germline, might be more compatible with long-term viability, unlike tumors induced, for example, in the brain [16, 92] or in the Malpighian tubules [162]. Nevertheless, the demonstrated ability to induce organ-specific tumors in adult tissues opens the door to testing which tumors are prone to induce systemic organ wasting. More importantly, testing various types of tumors generated in different tissues for their abilities to induce wasting in flies might help understand changes in target tissues during organ wasting, and unravel possible conserved mechanisms that also induce cachexia in humans.

Although neither tumor burden nor tumor type correlates with induction of cachexia [49, 71, 98], tumor-secreted factors can drive lipolysis and/or proteolysis [56, 147]. In flies, Imp-L2 was the first tumor-secreted factor to be identified that induces organ wasting [57, 86], followed by identification of Pvf1 [136]. While both Imp-L2 and Pvf1 were discovered by transcriptomic analysis of tumor samples [136], one additional method to identify novel factors produced by the tumor is to apply proximity-based labeling of proteins specifically in tumors, followed by purification of the labeled proteins from the hemolymph or target tissues of wasting and identification by mass spectrometry [29, 32]. This approach would help identify novel tumor-derived circulating factors with potential to induce organ wasting in peripheral tissues and with a conserved function in humans.

Cachexia is a syndrome that induces general metabolic dysfunction and increased catabolism in muscle and adipose tissue. In addition, muscle tissue can show signs of insulin resistance, an indicator of impaired glucose metabolism [33, 126]. In the two fly models of organ wasting described above, muscle function, adipose tissue mass, and glucose metabolism are affected [57, 86], showing similarities with human patients with cachexia and with rodent models. The development of genetic tools to monitor metabolite levels in flies at cellular resolution will sig-

nificantly facilitate the characterization of metabolic pathways in real time. An ingenious FRET sensor for measuring pyruvate levels [128] was used in the fly brain and unraveled a role for energy consumption in driving long-term memory formation [115]. Additional fluorescent reporters have been developed for measuring the ratio of ATP to ADP [18] and NADH to NAD [75]; however, these need to be tested in *Drosophila*.

In summary, combining *Drosophila* genetic tools with proteomic and transcriptomic approaches in both tumors and in target tissues, as well as with a detailed analysis of the changes in metabolic pathways in muscle and fat body, would produce a more complete and broader picture of the process of organ wasting induced by tumors.

11.9 Conclusion

As there is no treatment for cancer-induced cachexia and because of discrepancies in data obtained from cancer patients with cachexia and rodent models, new models for studying cachexia are needed. In addition, the pace, scale and genetic manipulation of rodent model studies have limitations that a model organism like *Drosophila* does not have. *Drosophila* has been used as a model for studying tumor biology [65] and more recently has emerged as a model to dissect the mechanisms underlying organ wasting induced by tumors [57, 86, 136]. Combining the genetic potential of the fly with protein-labeling techniques may help uncover novel tumor-derived factors with potential to induce organ wasting. More importantly, high-throughput studies of proteomics and metabolomics in *Drosophila* provide a unique opportunity to create a rapid approach to identify the types of tumors that induce metabolic changes similar to organ wasting observed in human patients with cachexia. These studies should help uncover new cellular and molecular mechanisms that drive organ wasting induced by tumors and shed light on the process of cachexia.

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