The Fruit Fly, *Drosophila melanogaster*: Modeling of Human Diseases (Part II)

Mariateresa Allocca, Sheri Zola and Paola Bellosta

Abstract

The fruit fly, *Drosophila melanogaster* (Meigen, 1830) has been established as a key model organism thanks in part to their considerable biological similarity to mammals and an abundance of available genetic tools. *Drosophila* have been used to model many human disease states and have been critical in elucidating the genetic mechanisms contributing to them. Part I of this chapter covered basic *Drosophila* biology and relevant genetic tools available to *Drosophila* researchers. Here in part II, we review the use of *Drosophila* as a model organism to study neurodegenerative disorders, cardiovascular diseases, kidney diseases, cancer, metabolic disorders, and immune disorders, as well as key findings made in those fields thanks to *Drosophila* research.

**Keywords:** animal model, cancer, diseases, *Drosophila*, genetic techniques, heart, immunology, kidney, metabolic disorders, neurodegeneration

1. Introduction

Please refer to the Introduction of Part I, The fruit fly, *Drosophila melanogaster*: The Making of a Model.

In this two-part chapter, some of the many aspects that make *Drosophila* such a fundamental model organism are covered.

Part I covered the basic fly biology and key genetic tools.

Here, Part II provides an overview of important disease states that *Drosophila* is used to model and some significant advances made in those fields.
2. *Drosophila melanogaster* as model to study human diseases

*Drosophila melanogaster* is a widely used model organism to understand many molecular and developmental processes common to higher eukaryotes. A prerogative for a good model system is to share higher physiology within the molecular pathways with humans, and it is remarkable that approximately 75% of genes associated with human diseases have *Drosophila* homologs and share similarities in their functions, which is of particular interest for medical purposes [2]. Based on this genetic similarity, the fly is a valid tool for understanding the function of genes involved in human disorders. Clearly, *Drosophila* has the limitation of being an invertebrate system, as some biological processes evolved only within the vertebrate lineage. Despite this, *Drosophila* exhibits complex behaviors, and each phenotype observed must be contextualized considering that mammalian physiology is not very different from that of the tiny fly. It is not easy to choose an appropriate organism to model a disease due to the higher complexity of humans, and it is necessary to evaluate the nature of the pathology before choosing. *Drosophila* provides a good background for genetic and biological studies of different pathological conditions such as neurological, cardiac, and metabolic disorders (Table 1).

| Organ system                  | Diseases                                                                 |
|-------------------------------|--------------------------------------------------------------------------|
| **Brain and nervous system**  | • Neurodegeneration                                                       |
|                               |   ○ Huntington’s disease                                                  |
|                               |   ○ Amyotrophic lateral sclerosis                                         |
|                               |   ○ Spinocerebellar ataxia                                               |
|                               |   ○ Alzheimer’s disease                                                   |
|                               |   ○ Parkinson’s disease                                                   |
| **Immune system**             | • Cancer                                                                 |
|                               |   • Wound healing                                                         |
|                               |   • Cancers, including acute myeloid leukemia                              |
|                               |   • Autoimmune diseases                                                  |
|                               |   • Allergies                                                            |
| **Digestive system**          | • Intestinal infections                                                  |
|                               |   • Intestinal inflammation                                               |
|                               |   • Cancer                                                               |
| **Excretory system**          | • Nephrotic syndrome                                                     |
|                               |   • Polycystic kidney disease                                             |
|                               |   • Kidney stones                                                        |

The *Drosophila* brain is two-lobed and contains approximately 100,000 neurons. It is organized into several main structures including: supraesophageal ganglion (optic lobes and cerebrum) and a subesophageal ganglion. Flies also have a segmented nerve cord similar to a mammalian spinal cord (FLYBRAIN neuron Database).

Circulating immune cells called hemocytes (consisting of plasmatocytes, lamellocytes, and crystal cells) fight pathogens by encapsulating them, generating ROS, and/or producing antimicrobial peptides (AMPs). Many tissues are also capable of generating AMPs including the gut and fat body [72].

Consists of mouth parts for chewing, salivary glands to produce saliva, a crop (similar to a stomach), the proventriculus for grinding food, and a gut (midgut and hindgut) for digestion and nutrient and water absorption.

Structures called Malpighian tubules and nephrocytes function similar to kidneys and filter nitrogenous waste from hemolymph. The tubules connect to the hindgut and excretory waste is eliminated along with digestive waste in the form of uric acid.
2.1. Neurodegenerative disorders

The *Drosophila* central nervous system (CNS) is composed of a bilaterally symmetrical brain with two cell types, neurons and glia, both originating from neural progenitors named neuroglioblasts. The fly CNS is considerably simpler than that of vertebrates and the neurodevelopment pattern is conserved among the organisms. Wnt, the mammalian homolog of the *Drosophila* wingless plays an important function during neuronal development [7] and Notch signaling, which plays a pivotal role during neurogenesis and neuronal differentiation, is also evolutionary conserved [8]. Neurons attend to neurotransmission while glia sustain the neurons during development and adult life mainly by providing
trophic factors [9, 10]. When studying neuropathies, it is relevant to consider the interaction between neurons and glia, and research in *Drosophila* is contributing to this. In fact, the power of the neurodegenerative fly model is in the ability to explore the disease in a physiological context. While glia support neuronal survival and promote recovery in cases of neuronal damage, impairment of glial function induces non-autonomous neuronal death. Glial anti-neurodegenerative functions suggest using them as targets in human neurodegeneration [11]. The *Drosophila* brain, in particular the visual system, is widely employed for research related to neurodegenerative diseases [12]. The nervous system of people suffering from these debilitating conditions exhibits the progressive loss of neurons. The origins are disparate, and in many cases, they are unknown so it is necessary to intensify the research, aiming to understand how to treat them. Interestingly, insects lack the human hematoencephalic barrier allowing for pharmacological screening directed at the central nervous system. Depending on the mechanisms inducing the disorder and the symptomatology, we can differentiate several types of human neurodegeneration. Most neurodegenerative disorders are characterized by the presence of protein aggregates in the neurons that are different for the various classes of diseases. Despite identifying many causative factors, it remains to be determined how these proteins become neurotoxic. Thanks to the precious genetic tools available, the fly is an excellent model to explore the function of the genes coding for the proteins involved. In addition, the molecular pathways are remarkably conserved allowing for parallels with humans [13]. The simpler fruit fly CNS allows for a better understanding of the function of a gene involved in a disease and its relationship with the other neuronal patterns.

In order to characterize neuronal dysfunction in *Drosophila*, several approaches can be used including testing motility, individual and social behaviors, hearing, learning, and memory [14–16]. A histological method based on measuring the vacuoles in adult fly brains allows for the quantification of neuronal degeneration [17]; moreover, electrophysiological assays enable the analysis of synapse functionality [18]. Fruit flies affected by neurodegeneration share behavioral defects and reduced lifespans.

*Drosophila* is already used to investigate proteinopathies (protein misfolding diseases) such as Huntington’s disease, amyotrophic lateral sclerosis, and spinocerebellar ataxia [19–21]. The cause of Huntington’s disease (HD) is the expansion of CAG repeats in the *huntingtin* gene, leading to a polyglutamine (poliQ) repeats in the huntingtin (htt) protein. Htt is required for axonal transport and synapsis, and the fly homolog shares the same expression pattern and function [22]. The poliQ expansion is toxic also for *Drosophila* neurons; in fact, the fly gradually loses photoreceptors when human htt is expressed in the eye compartment. When human mutated genes encoding for polyQ are expressed in *Drosophila*, there is a phenotype comparable to the human disease, for instance late onset, progressive loss of neurons and motility, and premature death, and the formation of large protein aggregates of mutant Htt visible also in neurons of *Drosophila* (Figure 1). The *Drosophila* HD model has contributed to some findings, for instance it uncovered that the histone deacetylase (HDAC) controls the level of neurodegeneration, making it an important achievement for
human poliQ diseases [23]. In the fly, as in humans, the neurodegeneration rate is related to polyQ repeat length [24]. Spinocerebellar ataxia (SCA) is another disorder originating from abnormal CAG repeats. Humans can be affected by several types of SCA and ataxin is the mutated gene. Autophagy is a fundamental process to limit the poliQ aggregation, and in a fly model of SCA3, autophagy proteins are overexpressed allowing for a rescue of the toxicity [25]. Amyotrophic lateral sclerosis (ALS) is a disease characterized by loss of cortical and spinal motor neurons [26]. Several genes are involved in ALS and most of them can be expressed in Drosophila to assess their contribution to neurodegeneration. A causative factor of ASL is a mutation in superoxide dismutase SOD1 [27], and interestingly, loss of Drosophila SOD1 causes neuronal death while human SOD1 expression increases the fly lifespan [28, 29].

Tauopathies, including Alzheimer’s, Parkinson’s, and others, refer to disorders caused by aberrant accumulation of the microtubule-associated protein tau [30]. Drosophila has a tau homolog and the pathways involved in tau neurotoxicity such as wnt, JNK, and TOR are shared with humans [31–33]. More than 30 transgenic fly models have been established that express various forms of human wild-type and mutant tau and have uncovered many potential mechanisms for tau toxicity in a variety of neurodegenerative diseases [34]. Alzheimer’s disease (AD) is one of the most common neurodegenerative disorders and yet its pathogenesis is still unclear. The tiny fly is once again a good organism to model this affliction because the AD-associated genes, such as APP and presenilins, are evolutionarily conserved. The brains of Alzheimer’s patients are marked by aggregation of beta-amyloid

Figure 1. Human huntingtin aggregates in neurons. Photograph of a larval brain showing the formation of aggregates of mutant human huntingtin (HTT) with 93-polyQ repeats (red) in neurons using Elav-Gal4 to express UAS-HTTQ93. HTT aggregates are visualized by immunofluorescence with anti-HTT antibodies. OP: optical lobe, CB: central brain, and VNC: ventral nerve cord.
protein and neurofibrillary tangles (NFTs) originating from hyperphosphorylation of Tau [35]. Tau expression induces learning and memory deficits in Drosophila, mimicking AD in humans [36]. Some recent advances uncovered by Drosophila Alzheimer’s models include: explaining the mechanisms behind the phosphorylation of tau and its toxicity [37–40] along with ways to reverse it [41, 42], as well as linking DNA damage and oxidative stress triggered by tau phosphorylation in causing neurotoxicity [33, 43]. Moreover, Drosophila models are helping researchers to uncover the interaction between beta-amyloid proteins and tau and how they cause neuronal death [34]. Parkinson’s disease (PD) is characterized by the progressive loss of dopamine neurons in the substantia nigra, a part of the brain responsible for motor control, as well as the formation of protein accumulations known as Lewy bodies, which are composed primarily of alpha synuclein [44]. Many mechanisms have been proposed for the cause of this neuronal death including disruptions in protein degradation, oxidative stress, mitochondrial dysfunction, autophagy and lysosomal dysfunction, and problems with calcium homeostasis [45] Furthermore, phosphorylated tau has been found to be associated with alpha synuclein in Lewy bodies [46, 47] and the two may function together to destabilize microtubules and damage axonal transport, also contributing to cell death [48]. Many fly models exist to study Parkinson’s disease [49]. The fly dopamine neurotransmitter is similar to the human version and its function in movement is conserved [50]. Homologs of several PD-related genes are present in Drosophila, allowing researchers to model this neurodegenerative disease [51]. Drosophila models are currently being used to test a variety of potential therapeutic approaches, including boosting antioxidant mechanisms, reducing the oxidative stress caused by dopamine metabolites, and using inhibitors for members of the TOR pathway to improve Parkinson’s symptoms [49].

2.2. Cardiovascular diseases

Drosophila melanogaster and humans share some aspects of heart development and function making the fly a good model for studying cardiovascular diseases, which are the leading causes of death worldwide. The heart precursors of Drosophila originate in the lateral mesoderm and converge on the dorsal midline to form a linear tubular structure comparable to the early vertebrate embryo heart. In Drosophila, a simple contractile tube pumps the hemolymph through the larval body cavity in an open cardiovascular system and regulates cardiac rhythm (Figure 2). The cardiovascular system has an anteroposterior polarity and it consists of the posterior portion named the dorsal vessel, corresponding to the heart, and the narrow anterior portion named the aorta, which facilitates the transport of hemolymph to the head [52]. The dorsal vessel is made up of two cell types: the cardiomyocytes, which are the inner contractile muscle cells, and the pericardial non-contractile cells, which flank the cardiomyocytes. The human heart has four distinct chambers, likewise the fly heart is divided into four chambers, each one consisting of six myocardial cells [53] that have a sarcomere structure similar to mammalian cardiac cells. The hemolymph flow moves nutrients, immune cells, and molecules required for homeostasis; however, oxygen is transported through diffusion from spiracles that invaginate from the cuticle into the interior of the animal. Despite the fly dorsal vessel being much simpler than the mammalian looped heart, the signaling
pathways involved are remarkably conserved [54]. Cardiogenic genes required for the proper development of the Drosophila embryonic heart were identified through genome wide screens [55] showing that many molecules important for heart development and morphogenesis are conserved in humans [56]. Tinman is a homeobox transcription factor discovered in Drosophila and it is a master gene of cardiac development conserved in higher organisms [57, 58]. In addition, pannier and hand, which play crucial roles for heart specification as well as neumancer, have counterparts in humans [59–61]. Moreover, these signaling pathways are required for some adult function both in Drosophila and in mammals suggesting that they have a conserved physiology [62].

Even if most studies are based on the embryonic development of the fly heart, nowadays the focus is shifting to the function and structure of the Drosophila adult heart as a model of human heart defects. Indeed, the great availability of genetic tools in Drosophila allows for the identification of elements important for heart functions and facilitates the analysis of mutant isoforms associated with congenital heart defects [63]. The physiological mechanisms are conserved among Drosophila and vertebrates supporting the utility of the fly to investigate cardiomyopathies and arrhythmias [52]. The improvement of techniques for the measurement of cardiac performance in Drosophila also permits the analysis of the effect of aging and the stress response on the heart [64]. Cardiac dysfunction can occur naturally in Drosophila, and this phenotype depends on age, just like in humans [64]. Some strategies allow heart rate monitoring in response to externally applied electric pacing in order to understand the effects of aging in adult flies. Insulin-IGF receptor (InR) and TOR signaling play an important role in regulation of age-dependent cardiac performance [65]. Drosophila is also one of the most efficient model organism used to discern the mechanism underlying channelopathies and cardiomyopathies as many impaired pathways are evolutionarily conserved [66]. Cardiomyopathies affecting Drosophila resemble those of humans both in terms of the genes responsible and the resulting effect. Such a similarity among the fly and humans is also found in the case of channelopathies and arrhythmias.

Several assay systems are helpful in characterizing Drosophila heart function, such as optical coherence tomography (OCT), an imaging of the Drosophila heart tube to observe contraction in vivo similar to clinical echocardiography [62]. In addition, semi-automated measurements allow researchers to record heart function to quantify cardiac impairment in Drosophila.
2.3. Kidney diseases

Despite millions of people suffering from kidney disorders, there is a disconcerting lack of therapies available to patients because the primary causes of kidney disorders are not completely characterized. *Drosophila* is advantageous to model renal disorders since many genes, proteins, and even some functions of the vertebrate kidney have parallels with the fruit fly. Despite many differences due the greater complexity of the human kidney, several orthologous genes have an important role in renal development and function, both in humans and in *Drosophila* [67]. For example, many genes encoding for electrolyte transporting proteins affected in congenital renal disorders have fly counterparts [68, 69].

The insect Malpighian tubules and the nephrocytes are functionally analogous to the vertebrate kidney; in fact, these two organs in *Drosophila* guide the metabolite homeostasis and the excretory process (Figure 3). Nephrocytes, which surround the heart and esophagus, are responsible for filtering the hemolymph, similar to the podocytes in the human glomerulus. In addition, nephrocytes have filtration diaphragms similar to the podocyte slit diaphragms that work as a filtration barrier in higher organisms [70, 71]. The Malpighian tubules, corresponding to the tubular part of nephrons, are two pairs of elongated and thin tubes connected to the hindgut that secrete urine after absorption of water, ions, solutes, and organic metabolites from the hemolymph. The principal cells and the stellate cells are the two main cell types in Malpighian tubules involved in excretion [72].

Nephrotic syndrome refers to ultrafiltration dysfunction leading mostly to extra protein in the urine and deficiency of protein in blood [73]. Given the evolutionary conservation of the diaphragms and their regulative mechanisms, *Drosophila* is a good option to look into this kind of disease. Some events during the renal development are shared

![Figure 3. Excretory system in larvae. Malpighian tubule and nephrocyte are composing the filtration barrier; hemolymph is filtrated by nephrocyte. Nd: nephrocyte diaphragm, fp: foot process, bm: basal membrane, and el: extracellular lacunae.](image-url)
between the fly and humans and the molecular pathways are conserved. All the genes playing a pivotal role in renal development, such as Kruppel and Cut involved in cell specification, Dwnt in tubulogenesis, and Sns, a nephrin-like protein, in cell differentiation, have a counterpart in mammals. One of the fundamental phases of Malpighian tubules formation is a mesenchymal-to-epithelial transition that resembles the steps of kidney development [74]. This makes the fruit fly organ able to provide insights on disorders affecting the tubular nephrons such as polycystic kidney disease and renal agenesis [75, 76]. Drosophila is also useful to study nephrolithiasis, also known as kidney stones, since insects also produce stone formations like calcium phosphate and calcium oxalate [77]. A simple method exists to score the filtration and the uptake of a secreted fluorescently tagged protein (ANFRFP) that accumulates in nephrocytes to assess the renal function in Drosophila [75].

The similarities among the species definitely allow the use of the Drosophila renal structure as a model to better understand the basis of human kidney impairments and consequently to develop personalized therapeutic agents. Furthermore, immune and inflammatory responses are trigger factors of kidney diseases so they should be taken into account when analyzing these pathologies [78].

2.4. Cancer and growth

The fly is a simple model to improve the understanding of tumor biology and progression [79–83] as the available genetic tools support the analysis of the mechanisms underlying growth regulation in an intact epithelium rather than in cell cultures. The advantage is remarkable since cell-cell and cell-environment interactions contribute to tissue size regulation. The Drosophila cell cycle can escape the normal control system leading to the typical cancer hyperproliferation. Reproducing human tumors in Drosophila allowed for the identification of many oncosuppressor genes that regulate cell division and differentiation [84]. In the fly, the tumor hallmarks mimic the human ones: autonomous proliferation signals and overgrowth, irregular cell morphology, bypassed apoptosis, and metastasis [85]. In spite of these similarities, there are several limitations including lack in flies of processes such as telomere maintenance and angiogenesis that participate in cancer development.

A great conservation across species is detected in regards to the signaling pathways affecting growth. Initial studies using activated proto-oncogenes such as the receptor tyrosine kinase (ret), a gene responsible for medullary thyroid carcinoma (MTC), allowed researchers to perform genetic screens for suppressors or enhancers of the rough eye phenotype, which indicates an overproliferation of cells in the eye [86]. These studies evolved to include tumors that were induced by the activation of growth signaling pathways, such as PI3K and EGFR in glia, which resemble human glioma [87], or studies involving tuberous sclerosis, an autosomal dominant disorder characterized by benign tumors in multiple organs induced by the loss of function activity of the TSC1 and 2 tumor suppressor genes [88]. A large number of studies also demonstrated how the Hippo pathway, which regulates growth through the activation of Yki, is highly conserved and required for cellular proliferation as well as for apoptosis, has a human counterpart that retains sequence
and function, and is mutated within the context of cancer [89, 90]. The same goes for Salvador, a gene promoting apoptosis, and Archipelago [91–94]. The two organisms also share PTEN, a tumor suppressor that plays a crucial role in carcinogenesis both in humans and in flies [95].

New studies defined how the loss of cell polarity could be considered a hallmark of malignancy [96]. Members of discs large (dlg) and lethal giant larvae (lgl) were identified as tumor suppressors in the fly by promoting cell invasion if mutated, with a similar role also seen in human neoplasm [97]. The role of proteins involved in cellular adhesion, such as Rho1 and E-cadherin, was also shown to be conserved and relevant for migration and invasion helping the study of the metastatic process [98, 99]. Other well-studied oncogenes in Drosophila that promote overgrowth and cell survival are Ras and Notch and were also shown to play a role in cellular polarity [100]. Dpp, the homolog of human bone morphogenetic protein/transforming growth factor-beta (BMP/TGF beta), is also responsible for epithelial integrity [101] and implicated in a model for cancer in Drosophila. All these parallelisms provide the potential to dissect in vivo the interacting patterns causing the tumor growth.

As anticipated, the communication between neighboring cells must be taken into consideration when analyzing a tumor tissue. Competitive interactions occur among cells with different growth rates in a process known as cell competition, which was first described in Drosophila using ribosomal proteins [102, 103] and then characterized using dMyc, the fly homolog of human cMyc [104]. Cells expressing higher levels of Myc behave as supercompetitors: they survive and acquire a proliferative advantage inducing apoptosis in the weaker nearby cells, termed losers [105–107]. The mechanisms controlling overproliferation and metastasis are comparable to those involved in cell competition since in human cancer, cells overexpressing Myc acquire the capacity to grow more than normal and to invade the neighboring normal cells. Since then, a few additional oncogenes and tumor suppressor genes have been associated with a competitive behavior, and cell competition is now thought to have an important role in human cancer [108–112]. This similitude underscores the utility of using flies for studying how cells compete for survival.

More studies are arising on the connection between the insurgence of tumors and diet or obesity. Recent studies linked the growth of prostate tumors and the status of obesity [113]. Caloric restriction reduces the growth of tumor cells in rodent models through reduced systemic insulin and IGF-1 signaling [114], while the activation of PI3K induces tumors to be resistant to diet restriction [115] suggesting an important relationship between PI3K signaling in tumors and the nutrients in the tumor environment. The exact link between obesity and cancer has not yet been established and the fly may facilitate this research thanks to the ability to combine obesity and tumor models in Drosophila. Insulin signaling is the main regulator of metabolic homeostasis, and it is also involved in cancer development and progression [116] but we have yet to understand how hyperinsulinemia promotes tumor formation. Interestingly, the oncogenes Src and Ras were overexpressed in a Drosophila model of obesity and increasing the level of insulin exacerbates the malignant phenotype due to wingless activity [117]. The interplay between obesity and cancer is an important area of study to understand the relevance of fat to tumor growth, since fatty acids are unable to penetrate the biological membranes and need to be cleaved by lipases (lipolysis). Recent studies indicate that in the peritumoural area, an increase in adipose triglyceride lipase
(ATGL) that mediates lipolysis results in tumor survival [118, 119]. The ability to manipulate flies genetically and the possibility to change the composition of lipids or nutrients in their food will likely put Drosophila as a key model to investigate the relationship between obesity and cancer and the mechanisms that control cellular overgrowth. Cancer research can only benefit from the ability to create specific disease models in Drosophila. This approach lets researchers detect oncogenes and tumor suppressors, allowing a detailed in vivo analysis of the mechanisms triggering cancer. From these findings, drug therapy compounds can then be developed and tested.

2.5. Metabolic disorders

Hepatic diseases affect a large proportion of the population worldwide making it crucial to investigate the underlying pathogenic mechanisms that still remain unclear. Identification of the molecular defects underlying liver disease requires studies in model organisms, and recently Drosophila has been proposed for this purpose [120].

The use of the fruit fly in the study of hepatic disorders is partially restricted due to the absence of a homologous organ for the liver. The fat body in Drosophila acts as storage for sugar and fat and also performs metabolic functions similar to those of the mammalian hepatocytes, regulated by insulin through an evolutionarily conserved mechanism [121, 122]. During starvation, triglycerides are transported from the fat body into the hemolymph where they are captured by the oenocytes, clusters of hepatocyte-like cells that are important for lipid metabolism [123]. Therefore, some functions of hepatocytes are performed by oenocytes, which are located near the body wall surface and play a prominent role in the fly lipid processing. Drosophila homologs of genes specifically expressed in human hepatocytes are expressed in larval oenocytes and the fat metabolism pathway is similar among the organisms [123]. An interesting aspect regarding lipid metabolism is the interaction between oenocytes and fat cells, as oenocytes control lipolysis in fat cells through a feedback similar to that in mammals [123]. Underfed Drosophila stores many fat droplets resulting in the accumulation of triacylglycerols in the liver, a condition called steatosis, and forms an excellent model for understanding human non-alcoholic fatty liver disease (NAFLD) [124]. Moreover, the relationship between oenocytes and fat cells needs to be elucidated because it contributes to the pathogenesis of metabolic syndrome [125], and fly modeling can be useful for this purpose.

It is necessary to improve assays examining the function of the fat body and oenocytes to solidify Drosophila as a liver disease model. To date, the analyses are based on evaluating lipid accumulation depending on different nutritional conditions. Fly lipid homeostasis can be monitored by Raman scattering microscopy that allows for the visualization of the lipid content in larval oenocytes and in the fat body by in vivo imaging [126]. Oil Red-O and BODIPY are dyes permitting the assessment of lipid content [123, 127].

Several proteins that contribute to lipid metabolism in Drosophila, including proteins responsible for lipid storage, transport, and utilization, have counterparts in higher organisms [128, 129]. This similitude makes the fruit fly helpful in describing the main pathways controlling homeostasis and provides an opportunity to examine metabolic disorders affecting humans such as diabetes and obesity [122]. For example, the main regulator of sugar and
fat metabolism is the nutrient-sensing target of rapamycin (TOR) both in Drosophila and in mammals [130]. Flies are able to regulate carbohydrate metabolism by cellular storage of excess nutrients. The hormone insulin controls hemolymph sugar levels and maintains carbohydrate homeostasis through a phylogenetically conserved signaling pathway [122, 131]. Drosophila insulin induces an increase in fat cell mass, just as in mammals, because insulin acts on triglyceride storage and on fat body cell number. Shaggy is a serine/threonine protein kinase orthologous to glycogen synthase kinase 3 (GSK3), and it is responsible for the lipid accumulation in Drosophila fat cells while the transcription factor Drosophila FOXO (dFOXO) influences the adipocyte cell number [121]. Both of these key factors are regulated by the conserved insulin pathway [121]. Dilp2, 3 and 5, members of the Drosophila insulin-like peptides (Dilps) are expressed in the insulin-producing cells (IPCs), a cluster of cells in the brain that function similarly to human pancreatic β cells [132]. Additionally, the adipokinetic hormone participates in fly glucose regulation with a glucagon-like function [55]. Functional changes to these metabolic regulators in Drosophila cause a phenotype similar to metabolic impairment as well as affecting body size [132, 133]. The resemblance between Drosophila and mammals helps to elucidate the main mechanisms of metabolic homeostasis involved in common pathologies such as type 2 diabetes mellitus (T2DM), which is characterized by insulin resistance, hyperglycemia, and defects in lipid metabolism [134]. High-glycemic diets promote obesity, a disorder characterized by excessive fat storage. Drosophila fed a high fat diet store fat in the fat body and in the midgut [135]. This condition changes the animal physiology and lifespan mainly due to insulin resistance [136, 137]. Moreover, obesity is considered among the risk factors for diabetes, cardiac diseases, and several types of cancer [138, 139]. Insulin resistance is also related to NAFLD, the most frequent chronic hepatic disorder [140]. NAFLD originates from metabolic impairment highlighting the strong relationship between the liver and metabolism and the subsequent need to examine the pathways linking them [124]. 

Drosophila has facilitated the study of metabolic pathways thanks to the availability of several assays of metabolic function, including some that are available for use only in Drosophila, which allow for the quantification of lipids, sugars, ATP, and mitochondria. In spite of the anatomical differences between flies and humans, the identification of novel genes and pathways in the fruit fly could arrange for new therapies to treat metabolic disease in humans.

2.6. Immunological diseases

The mechanism of the innate immune system is fairly conserved across species, and Drosophila is a leading organism for elucidating the process of defense from pathogens and its evolution [141]. Since the adaptive immune response of vertebrates could hide some aspects of the innate immunity, it is beneficial to use Drosophila to detail the regulation of innate immunity because this organism does not have an adaptive one [141]. Pathogenic microorganisms, such as bacteria, fungi, nematodes, and viruses, can infect Drosophila, priming an immune reaction. Despite the greater refinement of mammalian immunity, Drosophila and humans share general defense strategies like epithelial barriers, phagocytosis, and antimicrobial peptides. The fly’s first line of defense against to pathogens is a physical barrier represented by the epithelia of the epidermis, trachea, and gut. Clotting factors in the hemolymph provide a second barrier because they can entrap invaders by means of their protein filaments [142]. Epithelia then
release antimicrobial peptides (AMPs) and reactive oxygen species (ROS), triggering a local immune response [143, 144]. Beside their toxic activity, ROS are involved in wound healing and tissue repair both in Drosophila and mammals [145]. In addition to epithelia, blood cells and the fat body are also required for Drosophila immunity. The external agents are phagocytized by hemocytes; the circulating blood cells and different types of hemocytes are involved in this reaction. Plasmatocytes are monocyte-like cells, which able to phagocytose pathogens, apoptotic bodies, and other foreign particles. Crystal cells, another type of hemocyte, are involved in the production of melanin, a protein involved in both encapsulating and killing microorganisms as well as being implicated in wound healing. Hemocytes differentiate into lamellocytes if a more specialized response is required, and lamellocytes can trap larger parasites, producing a cellular capsule around it in a process named encapsulation [146, 147]. In Drosophila, the majority of blood cells have phagocytic activity.

Some fly macrophages originate via self-renewing and others from progenitor cells that are located in the lymph gland, a specialized hematopoietic organ. The great importance of the lymph gland in controlling the blood cell homeostasis makes this Drosophila organ comparable with the hematopoietic stem cell niche in the bone marrow [148, 149]. ROS levels have a crucial role in the regulation of Drosophila hematopoiesis [150]. Moreover, the signaling pathways regulating blood cell differentiation are conserved from Drosophila to humans [151, 152]. These similarities with vertebrate hematopoiesis underscore the utility of the fly to elucidate the basis of hematopoietic injury, necessary because an impairment in hematopoietic differentiation and homeostasis causes several diseases such as leukemia. Drosophila has already been used to study acute myeloid leukemia, a widespread form of leukemia, in particular to identify the genes promoting the disease. AML1 is one of the transcription factors activating myeloid differentiation and it has a counterpart in the fly [153]. When AML1 is fused with the repressor ETO, the differentiation is inhibited while the proliferation of multilineage progenitors is activated, leading to acute myeloid leukemia. AML1-ETO expression in Drosophila causes the same effect, confirming the fly as a good genetic model for leukemia [153, 154].

The great availability of genetic tools in the fly contributed to defining the innate immune system and to establishing that it is a specific mechanism. In fact, Drosophila can respond specifically to pathogens, discriminating between classes of surface molecules on different intruders. AMPs have different targets, for instance drosomycin acts on fungi, defensin on Gram-positive bacteria, and drosocin on Gram-negative bacteria [155]. Moreover, the sequences of AMPs are conserved between humans and insects [156]. Not only is the defense mechanism evolutionarily conserved, but also is the molecular pattern promoting innate immune reactions. Toll and Imd are the two master genes of Drosophila immunity, but FoxO, JAK/STAT, and JNK transduction also play a part [157]. After pathogen detection, Toll and Imd induce a cascade of events that finally release the antimicrobial peptides in fat body cells through the activation of the NF-κB transcription factors Dif, homolog of Dorsal, and Relish, respectively [155]. Toll encodes an interleukin 1 receptor-like protein that in Drosophila acts in parallel during two different processes: the dorsoventral specification and the immune response regulation [158]. Toll is activated by fungi and most Gram-positive bacteria and has a pivotal function both in the humoral response and in phagocytosis. Dissecting Toll signaling in Drosophila helped to understand toll-like receptors that play an important role
in inflammatory responses [159–161]. The Immune deficiency (Imd) signaling is mainly involved in the Drosophila reaction to Gram-negative bacterial infection [162]. The flies are also helpful in examining the defense against viral infection as they share with humans some proteins, named restriction factors, involved in the reaction to viral infection. Restriction factors, for instance Pastrel in Drosophila, are induced in host cells by virus infection and they can recognize specific viral elements, but the mechanism by which they act in insects is not very clear yet [163].

In order to examine immunity in the fly, an efficient and simple procedure has been developed to elucidate the physiological effect after infection and to quantify the pathogen load. It consists in scoring bacterial load, fly mortality, and also evaluating the effect on immune transcription factors after the direct introduction of bacteria in the fly body cavity, eluding the epithelial barrier [164].

The innate immunity contributes to Drosophila homeostasis and it is regulated by endocrine and metabolic systems. Since immune dysfunction leads to several human diseases, including autoimmune disorders, allergy, and intestinal infections, it is fruitful to use this model organism to better understand how all these systems are regulated. The fruit fly is also used to investigate the association between the microbiome and host, trying to characterize the resistance and tolerance mechanisms that are conserved in humans [165–167]. Circadian rhythms also participate in immune regulation both in Drosophila and in humans providing another similarity between organisms [168].

3. Conclusions

As illustrated throughout these two chapters, Drosophila melanogaster has been an invaluable tool for unlocking mechanisms contributing to the pathogenesis of many diseases such as cancer, diabetes, obesity, neurodegenerative disorders, kidney disease, immunological impairments, and many others. Given the advances in the field of genetics, new tools and techniques are continually being developed that will keep flies at the forefront of biomedical research.

Acknowledgements

We thanks the Confocal facility at IFOM-Milan, Matteo Cascinelli, Matteo Frattaroli, Valeria Lupi, and John Benedict Pollard from Liceo Scientifico “A. Einstein Milano” and Zhasmine Mirzoyan University of Milan for helping with the images. Funding from Cariplo Foundation and EHDN to PB and from CiBio to MTC.

Conflict of interest

The authors declare no conflict of interest.
Author details

Mariateresa Allocca, Sheri Zola and Paola Bellosta*

*Address all correspondence to: paola.bellosta@unitn.it

Center of Integrated Biology, University of Trento, Trento, Italy

References

[1] Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, et al. The genome sequence of *Drosophila melanogaster*. Science. 2000;287(5461):2185-2195

[2] Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Pharmacological Reviews. 2011;63(2):411-436

[3] Millburn GH, Crosby MA, Gramates LS, Tweedie S, FlyBase C. FlyBase portals to human disease research using *Drosophila* models. Disease Models & Mechanisms. 2016;9(3):245-252

[4] Kenney DE, Borisy GG. Thomas hunt Morgan at the marine biological laboratory: Naturalist and experimentalist. Genetics. 2009;181(3):841-846

[5] Bilder D, Irvine KD. Taking stock of the *Drosophila* research ecosystem. Genetics. 2017;206(3):1227-1236

[6] Patel S, Prokop A. The Manchester fly facility: Implementing an objective-driven long-term science communication initiative. Seminars in Cell & Developmental Biology. 2017;70:38-48

[7] Bejsovec A. Wnt pathway activation: New relations and locations. Cell. 2005;120(1):11-14

[8] Kopan R, Ilagan MX. The canonical notch signaling pathway: Unfolding the activation mechanism. Cell. 2009;137(2):216-233

[9] Bergmann A, Tugentman M, Shilo BZ, Steller H. Regulation of cell number by MAPK-dependent control of apoptosis: A mechanism for trophic survival signaling. Developmental Cell. 2002;2(2):159-170

[10] Chotard C, Salecker I. Glial cell development and function in the *Drosophila* visual system. Neuron Glia Biology. 2007;3(1):17-25

[11] Evans JR, Barker RA. Neurotrophic factors as a therapeutic target for Parkinson's disease. Expert Opinion on Therapeutic Targets. 2008;12(4):437-447

[12] Marsh JL, Thompson LM. *Drosophila* in the study of neurodegenerative disease. Neuron. 2006;52(1):169-178
[13] Bellen HJ, Tong C, Tsuda H. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: A history lesson for the future. Nature Reviews Neuroscience. 2010; 11(7):514-522

[14] Branson K, Robie AA, Bender J, Perona P, Dickinson MH. High-throughput ethomics in large groups of *Drosophila*. Nature Methods. 2009;6(6):451-457

[15] Inagaki HK, Kamikouchi A, Ito K. Protocol for quantifying sound-sensing ability of *Drosophila melanogaster*. Nature Protocols. 2010;5(1):26-30

[16] McGuire SE, Deshazer M, Davis RL. Thirty years of olfactory learning and memory research in *Drosophila melanogaster*. Progress in Neurobiology. 2005;76(5):328-347

[17] Sunderhaus ER, Kretzschmar D. Mass histology to quantify Neurodegeneration in *Drosophila*. Journal of Visualized Experiments: JoVE. 2016;118

[18] Broadie K, Bate M. Activity-dependent development of the neuromuscular synapse during *Drosophila* embryogenesis. Neuron. 1993;11(4):607-619

[19] Jackson GR, Salecker I, Dong X, Yao X, Arnheim N, Faber PW, MacDonald ME, Zipursky SL. Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. Neuron. 1998;21(3):633-642

[20] Watson MR, Lagow RD, Xu K, Zhang B, Bonini NM. *A drosophila* model for amyotrophic lateral sclerosis reveals motor neuron damage by human SOD1. The Journal of Biological Chemistry. 2008;283(36):24972-24981

[21] Casci I, Pandey UB. A fruitful endeavor: Modeling ALS in the fruit fly. Brain Research. 2015;1607:47-74

[22] Li Z, Karlovich CA, Fish MP, Scott MP, Myers RM. A putative *Drosophila* homolog of the Huntington’s disease gene. Human Molecular Genetics. 1999;8(9):1807-1815

[23] Steffan JS, Bodai L, Pallos J, Poelman M, McCamphell A, Apostol BL, Kazantsev A, Schmidt E, Zhu YZ, Greenwald M, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature. 2001;413(6857):739-743

[24] Marsh JL, Walker H, Theisen H, Zhu YZ, Fielder T, Purcell J, Thompson LM. Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in *Drosophila*. Human Molecular Genetics. 2000;9(1):13-25

[25] Bilen J, Bonini NM. *Drosophila* as a model for human neurodegenerative disease. Annual Review of Genetics. 2005;39:153-171

[26] Boilée S, Vande Velde C, Cleveland DW. ALS: A disease of motor neurons and their nonneuronal neighbors. Neuron. 2006;52(1):39-59

[27] Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. Annals of Neurology. 2009;65(Suppl 1):S3-S9

[28] Phillips JP, Tainer JA, Getzoff ED, Boulianne GL, Kirby K, Hilliker AJ. Subunit destabilizing mutations in *Drosophila* copper/zinc superoxide dismutase: Neuropathology
and a model of dimer dysequilibrium. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(19):8574-8578

[29] Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL. Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. Nature Genetics. 1998;19(2):171-174

[30] Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. Annual Review of Neuroscience. 2001;24:1121-1159

[31] Jackson GR, Wiedau-Pazos M, Sang TK, Wagle N, Brown CA, Massachi S, Geschwind DH. Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. Neuron. 2002;34(4):509-519

[32] Dias-Santagata D, Fulga TA, Duttaroy A, Feany MB. Oxidative stress mediates tau-induced neurodegeneration in Drosophila. The Journal of Clinical Investigation. 2007;117(1):236-245

[33] Khurana S, Robinson BG, Wang Z, Shropshire WC, Zhong AC, Garcia LE, Corpuz J, Chow J, Hatch MM, Precise EF, et al. Olfactory conditioning in the third instar larvae of Drosophila melanogaster using heat shock reinforcement. Behavior Genetics. 2012;42(1):151-161

[34] Fernandez-Funez P, de Mena L, Rincon-Limas DE. Modeling the complex pathology of Alzheimer’s disease in Drosophila. Experimental Neurology. 2015;274(Pt A):58-71

[35] Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer’s disease and related disorders. Nature Reviews Neuroscience. 2007;8(9):663-672

[36] Mershin A, Pavlopoulos E, Fitch O, Braden BC, Nanopoulos DV, Skoulakis EM. Learning and memory deficits upon TAU accumulation in Drosophila mushroom body neurons. Learning & Memory. 2004;11(3):277-287

[37] Kosmidis S, Grammenoudi S, Papanikolopoulou K, Skoulakis EM. Differential effects of tau on the integrity and function of neurons essential for learning in Drosophila. The Journal of Neuroscience. 2010;30(2):464-477

[38] Nishimura I, Yang Y, Lu B. PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in Drosophila. Cell. 2004;116(5):671-682

[39] Steinhilb ML, Dias-Santagata D, Mulkearns EE, Shulman JM, Biernat J, Mandelkow EM, Feany MB. S/P and T/P phosphorylation is critical for tau neurotoxicity in Drosophila. Journal of Neuroscience Research. 2007;85(6):1271-1278

[40] Chatterjee S, Sang TK, Lawless GM, Jackson GR. Dissociation of tau toxicity and phosphorylation: Role of GSK-3beta, MARK and Cdk5 in a Drosophila model. Human Molecular Genetics. 2009;18(1):164-177

[41] Cowan CM, Bossing T, Page A, Shepherd D, Mudher A. Soluble hyper-phosphorylated tau causes microtubule breakdown and functionally compromises normal tau in vivo. Acta Neuropathologica. 2010;120(5):593-604
Mudher A, Shepherd D, Newman TA, Mildren P, Jukes JP, Squire A, Mears A, Drummond JA, Berg S, MacKay D, et al. GSK-3beta inhibition reverses axonal transport defects and behavioural phenotypes in Drosophila. Molecular Psychiatry. 2004;9(5):522-530

Frost B, Hemberg M, Lewis J, Feany MB. Tau promotes neurodegeneration through global chromatin relaxation. Nature Neuroscience. 2014;17(3):357-366

Dauer W, Przedborski S. Parkinson's disease: Mechanisms and models. Neuron. 2003;39(6):889-909

Michel PP, Hirsch EC, Hunot S. Understanding dopaminergic cell death pathways in Parkinson disease. Neuron. 2016;90(4):675-691

Arima K, Hirai S, Sunohara N, Aoto K, Izumiyama Y, Ueda K, Ikeda K, Kawai M. Cellular co-localization of phosphorylated tau- and NACP/alpha-synuclein-epitopes in Lewy bodies in sporadic Parkinson's disease and in dementia with Lewy bodies. Brain Research. 1999;843(1-2):53-61

Ishizawa T, Mattila P, Davies P, Wang D, Dickson DW. Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. Journal of Neuropathology and Experimental Neurology. 2003;62(4):389-397

Lei P, Ayton S, Finkelstein DI, Adlard PA, Masters CL, Bush AI. Tau protein: Relevance to Parkinson's disease. The International Journal of Biochemistry & Cell Biology. 2010;42(11):1775-1778

Hewitt VL, Whitworth AJ. Mechanisms of Parkinson's disease: Lessons from Drosophila. Current Topics in Developmental Biology. 2017;121:173-200

Monastirioti M. Biogenic amine systems in the fruit fly Drosophila melanogaster. Microscopy Research and Technique. 1999;45(2):106-121

Shulman JM, De Jager PL. Evidence for a common pathway linking neurodegenerative diseases. Nature Genetics. 2009;41(12):1261-1262

Bier E, Bodmer R. Drosophila, an emerging model for cardiac disease. Gene. 2004;342(1):1-11

Lehmacher C, Abeln B, Paululat A. The ultrastructure of Drosophila heart cells. Arthropod Structure & Development. 2012;41(5):459-474

Ahmad SM. Conserved signaling mechanisms in Drosophila heart development. Developmental Dynamics: An Official Publication of the American Association of Anatomists. 2017;246(9):641-656

Kim SK, Rulifson EJ. Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. Nature. 2004;431(7006):316-320

Neely GG, Kuba K, Cammarato A, Isobe K, Amann S, Zhang L, Murata M, Elmen L, Gupta V, Arora S, et al. A global in vivo Drosophila RNAi screen identifies NOT3 as a conserved regulator of heart function. Cell. 2010;141(1):142-153
[57] Bodmer R. The gene tinman is required for specification of the heart and visceral muscles in *Drosophila*. Development. 1993;118(3):719-729

[58] Olson EN. Gene regulatory networks in the evolution and development of the heart. Science. 2006;313(5795):1922-1927

[59] Qian L, Bodmer R. Partial loss of GATA factor pannier impairs adult heart function in *Drosophila*. Human Molecular Genetics. 2009;18(17):3153-3163

[60] Qian L, Mohapatra B, Akasaka T, Liu J, Ocorr K, Towbin JA, Bodmer R. Transcription factor neuromancer/TBX20 is required for cardiac function in *Drosophila* with implications for human heart disease. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(50):19833-19838

[61] Hallier B, Hoffmann J, Roeder T, Togel M, Meyer H, Paululat A. The bHLH transcription factor hand regulates the expression of genes critical to heart and muscle function in *Drosophila melanogaster*. PLoS One. 2015;10(8):e0134204

[62] Choma MA, Suter MJ, Vacoc BJ, Bouma BE, Tearney GJ. Physiological homology between *Drosophila melanogaster* and vertebrate cardiovascular systems. Disease Models & Mechanisms. 2011;4(3):411-420

[63] Amodio V, Tevy MF, Traina C, Ghosh TK, Capovilla M. Transactivation in *Drosophila* of human enhancers by human transcription factors involved in congenital heart diseases. Developmental Dynamics: An Official Publication of the American Association of Anatomists. 2012;241(1):190-199

[64] Ocorr KA, Crawley T, Gibson G, Bodmer R. Genetic variation for cardiac dysfunction in *Drosophila*. PLoS One. 2007;2(7):e601

[65] Wessells R, Fitzgerald E, Piazza N, Ocorr K, Morley S, Davies C, Lim HY, Mitchell L, Hayes M, Oldham S, et al. d4eBP acts downstream of both dTOR and dFoxo to modulate cardiac functional aging in *Drosophila*. Aging Cell. 2009;8(5):542-552

[66] Wolf MJ, Amrein H, Izatt JA, Choma MA, Reedy MC, Rockman HA. *Drosophila* as a model for the identification of genes causing adult human heart disease. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(5):1394-1399

[67] Dow JA, Romero MF. *Drosophila* provides rapid modeling of renal development, function, and disease. American Journal of Physiology Renal Physiology. 2010;299(6):F1237-F1244

[68] Wang J, Kean L, Yang J, Allan AK, Davies SA, Herzyk P, Dow JA. Function-informed transcriptome analysis of *Drosophila* renal tubule. Genome Biology. 2004;5(9):R69

[69] Hatton-Ellis E, Ainsworth C, Sushama Y, Wan S, VijayRaghavan K, Skaer H. Genetic regulation of patterned tubular branching in *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(1):169-174

[70] Weavers H, Prieto-Sanchez S, Grawe F, Garcia-Lopez A, Artero R, Wilsch-Brauninger M, Ruiz-Gomez M, Skaer H, Denholm B. The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm. Nature. 2009;457(7227):322-326
[71] Zhuang S, Shao H, Guo F, Trimble R, Pearce E, Abmayr SM. Sns and Kirre, the Drosophila orthologs of Nephrin and Neph1, direct adhesion, fusion and formation of a slit diaphragm-like structure in insect nephrocytes. Development. 2009;136(14):2335-2344

[72] Sozen MA, Armstrong JD, Yang M, Kaiser K, Dow JA. Functional domains are specified to single-cell resolution in a Drosophila epithelium. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(10):5207-5212

[73] Nielsen R, Christensen EI. Proteinuria and events beyond the slit. Pediatric Nephrology. 2010;25(5):813-822

[74] Singh SR, Hou SX. Lessons learned about adult kidney stem cells from the malpighian tubules of Drosophila. Journal of the American Society of Nephrology: JASN. 2008;19(4):660-666

[75] Zhang Q, Taulman PD, Yoder BK. Cystic kidney diseases: All roads lead to the cilium. Physiology. 2004;19:225-230

[76] Perin L, Giuliani S, Sedrakyan S, Das S, De Filippo RE. Stem cell and regenerative science applications in the development of bioengineering of renal tissue. Pediatric Research. 2008;63(5):467-471

[77] Miller J, Chi T, Kapahi P, Kahn AJ, Kim MS, Hirata T, Romero MF, Dow JA, Stoller ML. Drosophila melanogaster as an emerging translational model of human nephrolithiasis. The Journal of Urology. 2013;190(5):1648-1656

[78] Imig JD, Ryan MJ. Immune and inflammatory role in renal disease. Comprehensive Physiology. 2013;3(2):957-976

[79] Hariharan IK, Bilder D. Regulation of imaginal disc growth by tumor-suppressor genes in Drosophila. Annual Review of Genetics. 2006;40:335-361

[80] Brumby AM, Richardson HE. Using Drosophila melanogaster to map human cancer pathways. Nature Reviews Cancer. 2005;5(8):626-639

[81] Gonzalez C. Drosophila melanogaster: A model and a tool to investigate malignancy and identify new therapeutics. Nature Reviews Cancer. 2013;13(3):172-183

[82] Miles WO, Dyson NJ, Walker JA. Modeling tumor invasion and metastasis in Drosophila. Disease Models & Mechanisms. 2011;4(6):753-761

[83] Vidal M, Larson DE, Cagan RL. Csk-deficient boundary cells are eliminated from normal Drosophila epithelia by exclusion, migration, and apoptosis. Developmental Cell. 2006;10(1):33-44

[84] Gateff E. Tumor suppressor and overgrowth suppressor genes of Drosophila melanogaster: Developmental aspects. The International Journal of Developmental Biology. 1994;38(4):565-590

[85] Mechler BM, Strand D. Tumor suppression in Drosophila. Immunology Series. 1990;51:123-144
[86] Das TK, Cagan RL. A *Drosophila* approach to thyroid cancer therapeutics. Drug Discovery Today Technologies. 2013;10(1):e65-e71

[87] Read RD, Cavenee WK, Furnari FB, Thomas JB. A *drosophila* model for EGFR-Ras and PI3K-dependent human glioma. PLoS Genetics. 2009;5(2):e1000374

[88] Pan D, Dong J, Zhang Y, Gao X. Tuberous sclerosis complex: From *Drosophila* to human disease. Trends in Cell Biology. 2004;14(2):78-85

[89] Yu J, Zheng Y, Dong J, Klusza S, Deng WM, Pan D. Kibra functions as a tumor suppressor protein that regulates hippo signaling in conjunction with Merlin and Expanded. Developmental Cell. 2010;18(2):288-299

[90] Pan D. The hippo signaling pathway in development and cancer. Developmental Cell. 2010;19(4):491-505

[91] Tapon N, Harvey KF, Bell DW, Wahrer DC, Schiripo TA, Haber DA, Hariharan IK. Salvador promotes both cell cycle exit and apoptosis in *Drosophila* and is mutated in human cancer cell lines. Cell. 2002;110(4):467-478

[92] Udan RS, Kango-Singh M, Nolo R, Tao C, Halder G. Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. Nature Cell Biology. 2003;5(10):914-920

[93] Wu S, Huang J, Dong J, Pan D. Hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. Cell. 2003;114(4):445-456

[94] Moberg KH, Bell DW, Wahrer DC, Haber DA, Hariharan IK. Archipelago regulates Cyclin E levels in *Drosophila* and is mutated in human cancer cell lines. Nature. 2001;413(6853):311-316

[95] Hafen E. Cancer, type 2 diabetes, and ageing: News from flies and worms. Swiss Medical Weekly. 2004;134(49-50):711-719

[96] Grzeschik NA, Amin N, Secombe J, Brumby AM, Richardson HE. Abnormalities in cell proliferation and apico-basal cell polarity are separable in *Drosophila* lgl mutant clones in the developing eye. Developmental Biology. 2007;311(1):106-123

[97] Naora H, Montell DJ. Ovarian cancer metastasis: Integrating insights from disparate model organisms. Nature Reviews Cancer. 2005;5(5):355-366

[98] Brumby AM, Goulding KR, Schlosser T, Loi S, Galea R, Khoo P, Bolden JE, Aigaki T, Humbert PO, Richardson HE. Identification of novel Ras-cooperating oncogenes in *Drosophila melanogaster*: A RhoGEF/rho-family/JNK pathway is a central driver of tumorigenesis. Genetics. 2011;188(1):105-125

[99] Navarro C, Nola S, Audebert S, Santoni MJ, Arsanto JP, Ginestier C, Marchetto S, Jacquemier J, Isnardon D, Le Bivic A, et al. Junctional recruitment of mammalian scribble relies on E-cadherin engagement. Oncogene. 2005;24(27):4330-4339

[100] Pagliarini RA, Xu T. A genetic screen in *Drosophila* for metastatic behavior. Science. 2003;302(5648):1227-1231
[101] Cordero JB, Larson DE, Craig CR, Hays R, Cagan R. Dynamic decapentaplegic signaling regulates patterning and adhesion in the Drosophila pupal retina. Development. 2007;134(10):1861-1871

[102] Morata G, Ripoll P. Minutes: Mutants of drosophila autonomously affecting cell division rate. Developmental Biology. 1975;42(2):211-221

[103] Simpson P, Morata G. Differential mitotic rates and patterns of growth in compartments in the Drosophila wing. Developmental Biology. 1981;85(2):299-308

[104] Gallant P, Shioi Y, Cheng PF, Parkhurst SM, Eisenman RN. Myc and max homologs in Drosophila. Science. 1996;274(5292):1523-1527

[105] Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P. Drosophila myc regulates cellular growth during development. Cell. 1999;98(6):779-790

[106] de la Cova C, Abril M, Bellotta P, Gallant P, Johnston LA. Drosophila myc regulates organ size by inducing cell competition. Cell. 2004;117(1):107-116

[107] Moreno E, Basler K. dMyc transforms cells into super-competitors. Cell. 2004;117(1):117-129

[108] Tamori Y, Deng WM. Cell competition and its implications for development and cancer. Journal of Genetics and Genomics. 2011;38(10):483-495

[109] Wagstaff L, Kolahgar G, Piddini E. Competitive cell interactions in cancer: A cellular tug of war. Trends in Cell Biology. 2013;23(4):160-167

[110] Baker NE, Li W. Cell competition and its possible relation to cancer. Cancer Research. 2008;68(14):5505-5507

[111] Moreno E. Is cell competition relevant to cancer? Nature Reviews Cancer. 2008;8(2):141-147

[112] Di Giacomo S, Sollazzo M, de Biase D, Ragazzi M, Bellotta P, Pession A, Grifoni D. Human cancer cells signal their competitive fitness through MYC activity. Scientific Reports. 2017;7(1):12568

[113] Pettersson A, Lis RT, Meisner A, Flavin R, Stack EC, Fiorentino M, Finn S, Graff RE, Penney KL, Rider JR, et al. Modification of the association between obesity and lethal prostate cancer by TMPRSS2:ERG. Journal of the National Cancer Institute. 2013;105(24):1881-1890

[114] Breese CR, Ingram RL, Sonntag WE. Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. Journal of Gerontology. 1991;46(5):B180-B187

[115] Kalaany NY, Sabatini DM. Tumours with PI3K activation are resistant to dietary restriction. Nature. 2009;458(7239):725-731

[116] Donadon V, Balbi M, Zanette G. Hyperinsulinemia and risk for hepatocellular carcinoma in patients with chronic liver diseases and Type 2 diabetes mellitus. Expert Review of Gastroenterology & Hepatology. 2009;3(5):465-467
[117] Hirabayashi S, Baranski TJ, Cagan RL. Transformed Drosophila cells evade diet-mediated insulin resistance through wingless signaling. Cell. 2013;154(3):664-675

[118] Gnerlich JL, Yao KA, Fitchev PS, Goldschmidt RA, Bond MC, Cornwell M, Crawford SE. Peritumoral expression of adipokines and fatty acids in breast cancer. Annals of Surgical Oncology. 2013;20(Suppl 3):S731-S738

[119] Martinez-Outschoorn UE, Pestell RG, Howell A, Tykocinski ML, Nagajyothi F, Machado FS, Tanowitz HB, Sotgia F, Lisanti MP. Energy transfer in “parasitic” cancer metabolism: Mitochondria are the powerhouse and Achilles’ heel of tumor cells. Cell Cycle. 2011;10(24):4208-4216

[120] Ugur B, Chen K, Bellen HJ. Drosophila tools and assays for the study of human diseases. Disease Models & Mechanisms. 2016;9(3):235-244

[121] Diangelo JR, Birnbaum MJ. The regulation of fat cell mass by insulin in Drosophila melanogaster. Molecular and Cellular Biology. 2009;(24):6341-6352

[122] Baker KD, Thummel CS. Diabetic larvae and obese flies-emerging studies of metabolism in Drosophila. Cell Metabolism. 2007;6(4):257-266

[123] Gutierrez E, Wiggins D, Fielding B, Gould AP. Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. Nature. 2007;445(7125):275-280

[124] Samuel VT, Shulman GI. Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. Cell Metabolism. 2017;(17):30487-30494

[125] Oike Y, Akao M, Kubota Y, Suda T. Angiopoietin-like proteins: Potential new targets for metabolic syndrome therapy. Trends in Molecular Medicine. 2005;11(10):473-479

[126] Chien CH, Chen WW, JT W, Chang TC. Investigation of lipid homeostasis in living Drosophila by coherent anti-stokes Raman scattering microscopy. Journal of Biomedical Optics. 2012;17(12):126001

[127] Kohyama-Koganeya A, Kim YJ, Miura M, Hirabayashi Y, Drosophila A. Orphan G protein-coupled receptor BOSS functions as a glucose-responding receptor: Loss of boss causes abnormal energy metabolism. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(40):15328-15333

[128] Canavoso LE, Jouni ZE, Karnas KJ, Pennington JE, Wells MA. Fat metabolism in insects. Annual Review of Nutrition. 2001;21:23-46

[129] Gronke S, Muller G, Hirsch J, Fellert S, Andreou A, Haase T, Jackle H, Kuhnlein RP. Dual lipolytic control of body fat storage and mobilization in Drosophila. PLoS Biology. 2007;5(6):e137

[130] Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell. 2006;124(3):471-484

[131] Garofalo RS. Genetic analysis of insulin signaling in Drosophila. Trends in Endocrinology and Metabolism: TEM. 2002;13(4):156-162
[132] Rulifson EJ, Kim SK, Nusse R. Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. Science. 2002;296(5570):1118-1120

[133] Rajan A, Perrimon N. Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. Cell. 2012;151(1):123-137

[134] Musselman LP, Fink JL, Narzinski K, Ramachandran PV, Hathiramani SS, Cagan RL, Baranski TJ. A high-sugar diet produces obesity and insulin resistance in wild-type Drosophila. Disease Models & Mechanisms. 2011;4(6):842-849

[135] Birse RT, Choi J, Reardon K, Rodriguez J, Graham S, Diop S, Ocorr K, Bodmer R, Oldham S. High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in Drosophila. Cell Metabolism. 2010;12(5):533-544

[136] Skorupa DA, Dervisefendic A, Zwiener J, Pletcher SD. Dietary composition specifies consumption, obesity, and lifespan in Drosophila melanogaster. Aging Cell. 2008;7(4):478-490

[137] Woodcock KJ, Kierdorf K, Pouchelon CA, Vivancos V, Dionne MS, Geissmann F. Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in Drosophila fed a lipid-rich diet. Immunity. 2015;42(1):133-144

[138] Na J, Cagan R. The Drosophila nephrocyte: Back on stage. Journal of the American Society of Nephrology: JASN. 2013;24(2):161-163

[139] Renehan AG, Roberts DL, Dive C. Obesity and cancer: Pathophysiological and biological mechanisms. Archives of Physiology and Biochemistry. 2008;114(1):71-83

[140] Younossi Z, Henry L. Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. Gastroenterology. 2016;150(8):1778-1785

[141] Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. Phylogenetic perspectives in innate immunity. Science. 1999;284(5418):1313-1318

[142] Scherfer C, Karlsson C, Loseva O, Bidla G, Goto A, Havemann J, Dushay MS, Theopold U. Isolation and characterization of hemolymph clotting factors in Drosophila melanogaster by a pullout method. Current Biology: CB. 2004;14(7):625-629

[143] Bulet P, Hetru C, Dimarcq JL, Hoffmann D. Antimicrobial peptides in insects; structure and function. Developmental and Comparative Immunology. 1999;23(4-5):329-344

[144] Ha EM, CT O, Ryu JH, Bae YS, Kang SW, Jang IH, Brey PT, Lee WJ. An antioxidant system required for host protection against gut infection in Drosophila. Developmental Cell. 2005;8(1):125-132

[145] Juarez MT, Patterson RA, Sandoval-Guillen E, McGinnis W. Duox, Flotillin-2, and Src42A are required to activate or delimit the spread of the transcriptional response to epidermal wounds in Drosophila. PLoS Genetics. 2011;7(12):e1002424

[146] Carton Y, Nappi AJ. Immunogenetic aspects of the cellular immune response of Drosophila against parasitoids. Immunogenetics. 2001;52(3-4):157-164
[147] Gold KS, Bruckner K. Macrophages and cellular immunity in Drosophila melanogaster. Seminars in Immunology. 2015;27(6):357-368

[148] Krzemen J, Dubois L, Makki R, Meister M, Vincent A, Crozatier M. Control of blood cell homeostasis in Drosophila larvae by the posterior signalling centre. Nature. 2007;446(7133):325-328

[149] Mandal L, Martinez-Agosto JA, Evans CJ, Hartenstein V, Banerjee U. A hedgehog- and Antennapedia-dependent niche maintains Drosophila haematopoietic precursors. Nature. 2007;446(7133):320-324

[150] Owusu-Ansah E, Banerjee U. Reactive oxygen species prime Drosophila haematopoietic progenitors for differentiation. Nature. 2009;461(7263):537-541

[151] Lebestky T, Jung SH, Banerjee U. A serrate-expressing signaling center controls Drosophila hematopoiesis. Genes & Development. 2003;17(3):348-353

[152] Jung SH, Evans CJ, Uemura C, Banerjee U. The Drosophila lymph gland as a developmental model of hematopoiesis. Development. 2005;132(11):2521-2533

[153] Sinenko SA, Hung T, Moroz T, Tran QM, Sidhu S, Cheney MD, Speck NA, Banerjee U. Genetic manipulation of AML1-ETO-induced expansion of hematopoietic precursors in a Drosophila model. Blood. 2010;116(22):4612-4620

[154] Osman D, Gobert V, Ponthan F, Heidenreich O, Haenlin M, Waltzer L, Drosophila A. Model identifies calpains as modulators of the human leukemogenic fusion protein AML1-ETO. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(29):12043-12048

[155] Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annual Review of Immunology. 2007;25:697-743

[156] Hoffmann JA, Reichhart JM. Drosophila innate immunity: An evolutionary perspective. Nature Immunology. 2002;3(2):121-126

[157] Varma H, Cheng R, Voisine C, Hart AC, Stockwell BR. Inhibitors of metabolism rescue cell death in Huntington’s disease models. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(36):14525-14530

[158] Lemaitre B. The road to toll. Nature Reviews Immunology. 2004;4(7):521-527

[159] Manfruelli P, Reichhart JM, Steward R, Hoffmann JA, Lemaitre B. A mosaic analysis in Drosophila fat body cells of the control of antimicrobial peptide genes by the Rel proteins Dorsal and DIF. The EMBO Journal. 1999;18(12):3380-3391

[160] Tauszig S, Jouanguy E, Hoffmann JA, Imler JL. Toll-related receptors and the control of antimicrobial peptide expression in Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(19):10520-10525

[161] Zettervall CJ, Anderl I, Williams MJ, Palmer R, Kurucz E, Ando I, Hultmark D. A directed screen for genes involved in Drosophila blood cell activation. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(39):14192-14197
Lemaitre B, Kromer-Metzger E, Michaut L, Nicolas E, Meister M, Georgel P, Reichhart JM, Hoffmann JA. A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the *Drosophila* host defense. Proceedings of the National Academy of Sciences of the United States of America. 1995;92(21):9465-9469

Cogni R, Cao C, Day JP, Bridson C, Jiggins FM. The genetic architecture of resistance to virus infection in *Drosophila*. Molecular Ecology. 2016;25(20):5228-5241

Khalil S, Jacobson E, Chambers MC, Lazzaro BP. Systemic bacterial infection and immune defense phenotypes in *Drosophila melanogaster*. Journal of Visualized Experiments: JoVE. 2015;99:e52613

Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B. *Drosophila* intestinal response to bacterial infection: Activation of host defense and stem cell proliferation. Cell Host & Microbe. 2009;5(2):200-211

Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. Lactobacillus plantarum promotes *Drosophila* systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. Cell Metabolism. 2011;14(3):403-414

Ferrandon D. The complementary facets of epithelial host defenses in the genetic model organism *Drosophila melanogaster*: From resistance to resilience. Current Opinion in Immunology. 2013;25(1):59-70

Lee JE, Edery I. Circadian regulation in the ability of *Drosophila* to combat pathogenic infections. Current Biology: CB. 2008;18(3):195-199