Chapter 5

Modeling Tumorigenesis in *Drosophila*: Current Advances and Future Perspectives

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http://dx.doi.org/10.5772/55686

1. Introduction

1.1. Tumor suppressor genes, a historical perspective

Cancer is essentially considered as a genetic disease caused by the accumulation of multiple genetic or epigenetic lesions in tumor-suppressor genes and oncogenes [1]. Although the notion that retinoblastoma could be an inherited disease was already formulated at the end of the 19th Century a solid genetic basis was established with the discovery of both proto-oncogenes, whose gain-of function mutations or altered expression is associated with the cancerous state, and tumor suppressor genes (TSGs), whose inactivation releases the "brakes" inhibiting cell proliferation. Analysis of both proto-oncogenes and TSGs revealed also that cancer results from an alteration of the normal pathway of cell fate and differentiation. The hallmarks of cancer, as laid down by Hanahan and Weinberg to explain the complex biology of cancer, comprise six major developmental changes taking successively place in human tumors. These cancer "characteristics" include sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, angiogenesis as well as cell invasion and metastasis. Underlying these hallmarks are genome instability, inflammation, reprogramming of energy metabolism and evading immune destruction [2].

Cancer cells are the foundation of the cancer disease, as they initiate formation of tumors and drive tumor progression forward. Based on the sequence of events in which cells accumulate genetic lesions, tumor progression and metastasis are highly variable, even among tumors of the same type [3]. Previously, cancer cells within tumors were thought to be largely made of homogenous cells until relatively late in the course of tumor progression, when hyperproliferation combined with increased genetic instability spawn distinct clonal populations. Now we know that tumors rather than homogenous masses of proliferating cancer cells are complex

tissues composed of distinct cell types participating in heterotypic interactions with each another. Reflecting such clonal heterogeneity is the finding that many human tumors display a complex histological pattern, characterized by regions exhibiting various degrees of differentiation, proliferation, vascularity, inflammation and invasiveness [2]. In addition, tumors exhibit another dimension of complexity arising from the surrounding normal cells of the "tumor microenvironment" [2] (analyzed in part 3).

Over the last decades the origin of oncogenesis has been the subject of different theoretical "fashions". In the current view taking into account the role of oncogenes and tumor suppressor genes cancer results from a failure occurring more in the control of cell differentiation than in cell proliferation [4, 5]. Nowadays, cancer is generally considered to result from a block or an error in the normal progression of differentiation. As a result, the cancer cells escape the mechanisms controlling normal growth and proliferation. Several decades ago, pioneer studies in the field of *Drosophila*, mouse somatic cells and human genetics revealed that neoplasia may result from a loss of function in regulatory genes controlling cell growth and differentiation [6-9]. In the following years, research in developmental biology has greatly contributed to cancer research. Indeed very often the initiating event in the formation of a malignant tumor is a block of a critical step in normal differentiation, usually through inactivation of a single gene, and can be accompanied by events occurring in parallel. In the case of tumor-suppressor genes, proliferation of tumor cells is suppressed by the same set of genes that suppress the proliferation of normal cells in the same cell type during the process of differentiation. Studies of the *Drosophila lethal [2] giant larvae (lgl)* gene, the first cloned tumor suppressor gene, have shown beyond doubt that tumors can be produced at a defined period of development by the impairment of a gene that normally regulates a critical step of differentiation [5, 10, 11]. At that time, such precise time delimitation in the process of development would not have been possible to achieve with mammalian cells, but the observations made thereafter in mammals were consistent with the conclusion derived from the *Drosophila* study.

1.1.1. Identification of the first tumor suppressor genes in Drosophila melanogaster

Over the past 40 years it has become increasingly evident that cancer is causally related to mutations in specific genes. These genes are instrumental to developmental processes such as cell-cell communication, signal transduction, regulation of gene expression, translation, cytoskeletal organization, protein folding and transport, and differential regulation of cell cycle [12]. The *Drosophila* field has made marked contributions in many of the mechanisms that are fundamental to cancer processes, several of which have been later validated in vertebrates. Less well known is the precursor role of *Drosophila* in the cancer field, as some of the earliest tumor suppressors were identified in flies. The first tumor-containing mutant was recognized in 1967 in a wild type laboratory stock of *Drosophila melanogaster* [6]. The mutant gene was soon identified by Ed Lewis as an allele of the already known *lgl* gene, which was discovered by Bridges in 1933 [13]. During the 1940-50s, this gene has been the subject of a number of developmental studies performed by Hadorn and his collaborators. The phenotypic studies performed by Hadorn's group on *lgl* and other *Drosophila* genes have greatly contributed to the conceptual basis of developmental genetics. In 1955 Hadorn published in German

http://dx.doi.org/10.5772/55686

his seminal monography on "*Letalfaktoren und ihrer Bedeutung für Erbpathologie und Genphysiologie der Entwicklung*" which in 1961 was translated into English as "*Developmental Genetics and Lethal Factors*". Comparative developmental studies conducted thereafter showed that one of the pleiotropic effects of the mutation was the formation of a malignant neuroblastoma in the larval brain and the appearance of imaginal disc tumors [7, 14]. Molecular studies on the *lgl* gene were initiated in 1985 by Mechler and his co-workers by cloning the first tumor suppressor gene [10]. Subsequent analysis of *lgl* has demonstrated unequivocally that the tumorous phenotype results from the lack of *lgl* function and showed that tumorigenesis can be rescued by reintegrating a wild type copy of *lgl* into the genome of *lgl*-deficient animals [15]. Biochemical and genetic analysis of the *lgl* gene and its human homologue *hugl-1*, showed that the encoded proteins are components of the cytoskeleton and interact physically with a domain located near the carboxyl terminal of the non-muscle myosin II heavy chain [11, 16-20]. Further studies also revealed that the Lgl protein can interact with the Nucleosome Assembly Protein-1 (NAP-1), a component of the cyclinB-p34^{cdc2} kinase complex [21, 22] and NAP-1 is intimately associated with the cytoskeletal matrix during interphase, accumulates in the nucleus during prophase where it becomes associated with the spindle apparatus [21].

Recent contributions show that the Lgl protein may directly contribute to genetic regulation in association with the heavy chain of nonmuscle myosin II, or nmMHC [23, 24]. In particular mutations in *lgl* or heteroallelic combinations between *lgl* and *zipper*, encoding nmMHC, were found to block the disintegration of the salivary glands by blocking the program induced by the molting hormone ecdysone [23]. An interaction between both proteins was found to be required for the binding of specific nuclear remodeling proteins to chromatin [24]. Defect in this interaction may result in a block of genetic cascade initiated by the ecdysone hormone and lead to a transcriptionally frozen genome. The outcome of these analyses shed light on the key roles that tumor suppressor genes may play not only in the mechanism of cell shape and tissue organization but also in the regulation of developmental programs.

Subsequently to these initial studies, Gateff isolated a series of other recessive mutations in distinct genetic loci, which gave rise to four specific types of tumors. These tumors affected either the developing larval brain, the imaginal discs, the hematopoietic organs, or the germ cells [25, 26]. Shortly after *lgl* was reported [6], a genetic screen assaying imaginal disc morphology identified a mutation in the *discs large* (*dlg*) gene, coding for a septate junction tumor suppressor gene [27] with a second mutation identified few years later [28]. Twenty years later, a third mutation with the strikingly similar phenotypes, called *scribble* (*scrib*), was independently isolated in two different labs. Initially *scrib* was found in a screen for regulators of epithelial architecture [29]. Parallel to this investigation, a P-element mutagenesis screen led to the identification of the recessive *scrib*^{vartul} mutation causing late larval lethality, imaginal disc overgrowth and brain tumors with a complex syndrome reminiscent of that observed in mutations of the other tumor suppressors [30]. Therefore, already at the very early days of tumor genetics, *Drosophila* has been an extremely favorable object of study. Since then a great number of tumor suppressor genes have been identified and *Drosophila* has largely contributed to our understanding of the basic biology and cellular mechanisms of tumorigenesis including cell growth and proliferation, apoptosis, maintenance of cell polarity and architecture.

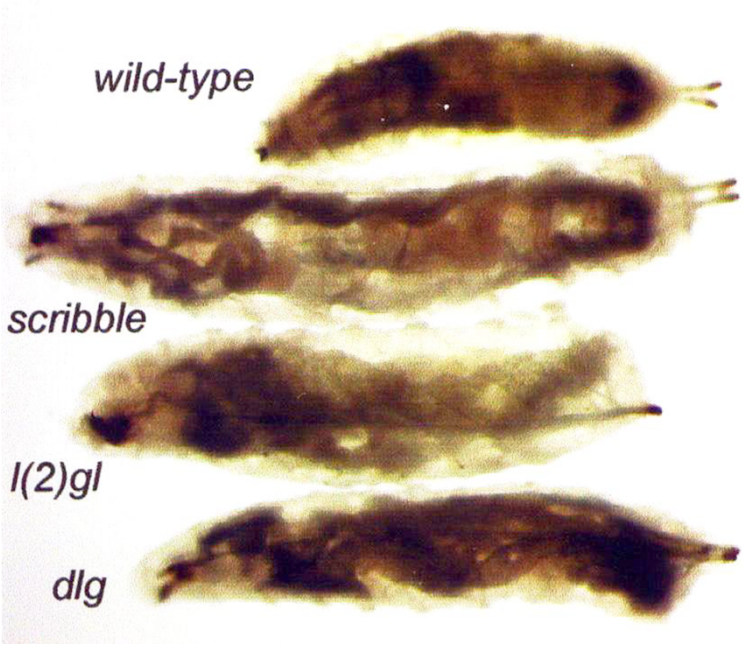

Figure 1. Size comparison between wild type 3rd instar larvae and *scrib, l2gl* and *dlg* giant larvae.

2. *Drosophila* as a unique model system to study tumor suppression

In order for an animal species to serve as a model of human biology it must fulfill two key criteria. The model system should be amenable to a broad set of experimental approaches and to be similar enough to humans so that findings from the model system can be exported to higher organisms and facilitate research in humans. *Drosophila*, being at the forefront of genetic research for the past one hundred years, together with the high degree of conservation with humans at the gene and cellular level, has proved itself as an essential partner in discoveries related to genetics, cell biology, human disease and cancer metastasis.

There are also many technical advantages in using *Drosophila* over vertebrate models. Flies are easy and inexpensive to culture in laboratory conditions, have a much shorter life cycle, produce large number of offsprings with feature-rich morphology and large numbers of externally laid embryos that can be genetically modified in numerous ways. Each female flies lay up to approximately 50 to 100 eggs per day for up to 20 days. It takes approximately 10 days at 25°C for an embryo to develop into a fertile adult fly. Thus, it is easy to generate large numbers of embryos or flies for experimental studies and genome-wide screens. Moreover, there are generally few limited restrictions on their use in the laboratory as there are minimal ethical and safety issues [31, 32].

http://dx.doi.org/10.5772/55686

2.1. *Drosophila* in a century of "tool-building" research

The first documented use of *Drosophila* in the laboratory was in William Castle's group at Harvard in 1901, but the "father" of *Drosophila* research is without doubt Thomas Hunt Morgan [33]. It was almost 100 years ago that Thomas Hunt Morgan reported the identification of the *white* gene in *Drosophila* [34]. Morgan greatly refined the theory of inheritance first proposed by Gregor Mendel, by using *Drosophila* to define genes and establish that they are found within chromosomes, long before it was even established that the DNA is the genetic material [31, 35]. Morgan won the Nobel Prize in Physiology or Medicine in 1933 "for his discoveries concerning the role played by the chromosome in heredity". One of Morgan's students, Hermann Muller, won the Nobel Prize in Physiology or Medicine in 1945 "for the discovery of the production of mutations by means of x-ray irradiation". Using *Drosophila* in 1920s, Muller discovered that x-rays cause massive increase in the rate of mutations and that the mutations can be passed from one generation to the other [36]. The genetics approaches used in the first 50 years of research in *Drosophila* (1910-1960), led to the development of important concepts and tools e.g. balancer chromosomes, that allowed the study of many other biological processes in the years to come [35, 37].

Interestingly, researches realized in the early fifties that genetic approaches could be used to study problems other than heredity. The continuous development of research tools between the years 1960-2010 has driven numerous new discoveries in fruit flies. In the mid-seventies, the available genetic tools in *Drosophila* offered the opportunity to address how embryonic pattern formation is controlled and to identify the genes involved in these processes [37, 38]. By carrying out a systematic chemical mutagenesis screen on the different fly chromosomes and analyzing the larval cuticle patterns, Nüsslein-Volhard and Wieschaus identified 139 genes that affect the development of the fly larva [39-41]. This analysis led to the identification of numerous genes participating in the Hedgehog, Wingless, Decapentaplegic (the *Drosophila* Tumour growth factor-β; TGF-β), and Notch pathways. In 1995, Christiane Nüsslein-Volhard, Eric Wieschaus and Ed Lewis won the Nobel Prize in Physiology or Medicine "for their discoveries concerning the control of early embryonic development". These findings have clearly demonstrated the power of forward genetics in solving complex developmental questions. Further genetic screens shed light on factors involved in various developmental aspects such as neuronal migration and growth cone guidance, circadian rhythms, learning and memory [37]. All these studies proved that despite the great morphological differences between flies and humans, many of the underlying building blocks and cellular processes are strikingly similar and conserved through evolution [31].

The range of genetic tools that have become available for *Drosophila* over the past century surpass by far those of any other multicellular organism [31]. Two experimental key features, namely the successful and efficient removal or addition of single genes or gene products, are important for any model organism to be successfully used in the laboratory. In *Drosophila*, genes can be inactivated in a random fashion using chemical mutagenesis followed by screening for specific phenotypes. Current tools allow very rapid mapping of chemically induced mutations that have robust phenotypes, permitting the isolation of null alleles, hypomorphs, neomorphs as well as conditional alleles, making possible the functional analysis

of single genes. These screens, combined with duplications and deletions covering almost all chromosomes, have greatly facilitated gene mapping. The recent improvements in whole genome sequencing techniques and single-cell profiling will enhance even more the speed of accessibility to genomic information [37, 42]. Apart from the imprecise excision of transposable elements [43], one can create mutations by selective removal or replacement of sequences using the "targeted-knockout" technology [44], as well as by using RNA interference (RNAi) to reduce expression on any gene in a tightly regulated temporal and spatial pattern [45, 46].

The use of P-element-mediated transformation, available since 1982 [47] has allowed the insertion of single genes and any DNA fragment of interest in the fly genome, and has opened the field to even more sophisticated genetic manipulations [37]. This technique was significantly improved with the P[acman] technology that allows the insertion DNA fragments in specific docking sites spread throughout the *Drosophila* genome [48, 49]. Efficient transformation has been achieved by using the Flipase-Flipase Recognition Target (FLP-FRT) recombination system, which enables the creation of mutant patches of tissues or cells in an otherwise heterozygous background [50, 51]. This system led to the development of a highly efficient "mitotic recombination system" that knockouts defined genetic function in specific cells, tissues and organs. The yeast site-specific recombinase FLP, coupled with centromere-linked insertions of the FRT target site on all major chromosome arms of *Drosophila* [52], allows the generation of genetic mosaics, within an otherwise wild type organism, by removing almost every fly gene function. Generation of genetic mosaics is particularly useful for elucidating the function of genes which, when mutated, would otherwise kill the organism and subsequently laid the carpet in understanding how cells within tumors can interact with their surrounding microenvironment. A considerable improvement of the FLP-FRT system for analyzing mosaic tissue was the development of the MARCM (Mosaic Analysis with Repressible Cell Marker) technique [53]. Prior to the introduction of MARCM, homozygous mutant cells were identified by the absence of a visible marker such as GFP or *lacZ* in comparison to the surrounding heterozygous environment and the wild type "twin clone". By using the MARCM technique the homozygous clones can be positively marked using e.g. GFP or RFP, which can be of particular importance for the analysis of single cells in a disease model.

Another use of the P-element-mediated transformation facilitated the development of the *UAS-GAL4* system in order to ectopically express or overexpress a gene of interest in almost any tissue or cell [54]. The binary *UAS-GAL4* system allows a gene DNA sequence fused to the UAS (upstream activating sequence) to be ectopically expressed via the enhancer/ promoter of a second gene that drives synthesis of the UAS-binding GAL4 transcription factor. Thousands of *UAS* and *GAL4* fly lines are now available and their use can either modify or even abrogate gene expression in selected cell populations, in a specific temporal and spatial pattern [37, 55, 56]. Moreover, the "*Vienna Drosophila RNAi* Center" and "Bloomington Stock Center" house a collection of transgenic fly lines carrying inducible *UAS-RNAi* constructs against single protein coding genes. Currently they accommodate over 22.000 different transgenic lines, which provide knockdowns for over 90% of *Drosophila* genes. Further development of the *UAS-GAL4* system led to the TARGET (temporal and regional gene expression targeting) system, which utilizes a temperature-sensitive form of GAL80 repressor that binds GAL4 and

http://dx.doi.org/10.5772/55686

blocks its transcription activity at the restrictive temperature, while a shift to a permissive temperature results in GAL80 losing the ability to bind GAL4 [57, 58].

Finally, P-element technology also allowed the tagging of most genes *in vivo* e.g. with GFP, RFP or YFP, permitting sophisticated manipulations in a genomic context [59]. Transgenic flies containing enhancer-trap or protein-trap versions of individual genes allowing the analysis of the gene expression pattern and protein localization are available at "FlyTrap" (http://flytrap.med.yale.edu/index.html) [60-63] and "FlyPROT" (http://www.flyprot.org/index.php) [64].

2.2. *Drosophila* is a model system relevant to human biology

While *Drosophila* has long served as a model for basic biological research, more recently its potential as a model for unraveling molecular mechanisms of human diseases has become widely appreciated, and numerous publications and conferences illustrate the use of *Drosophila* to unravel the mechanisms of human diseases [65-69]. Release of the first sequence of the *Drosophila* genome in March 2000 (11 months ahead of the human genome release) allowed the actual comparison to the human genome. This comparison has greatly consolidated *Drosophila's* legitimacy as a model organism for medical research [31, 70]. The sequence and annotation are freely available in "Flybase", an outstanding online database combining all current knowledge on single *Drosophila* genes including sequence and expression data, mutations, interactions and up to date scientific references. Comparative studies have shown the molecular mechanisms underlying the development of *Drosophila* and humans are highly conserved and that the *Drosophila* genome contains functional homologues of nearly 75% of the human disease genes, we can understand why this aspect of *Drosophila* research continuously expands. Moreover, over 85% of human genes that have been associated with a disease, have a *Drosophila* counterpart. These findings constitute a strong basis for the continuous expansion of *Drosophila* research in relation to human diseases.

What makes *Drosophila* also practical in the analysis of gene function is the nearly absence of genetic redundancy. The duplication of the vertebrate genome during evolution has resulted in the occurrence of multiple paralogs, e.g. Hox genes that control the body plan along the anterior-posterior axis [71], with their subsequent evolution that has generated gene expansion ad diversity in protein function [72, 73]. Genetic expansion means that when knocking down a gene, other genes or homologues can compensate for its function. Yet, the extra genes rarely represent novel functions as they simply allow more complex and subtle regulation of core molecular mechanisms. In this respect, the absence of gene duplication in *Drosophila* provides the advantage of elucidating more readily the fundamental function of single genes, e.g. in tumor development, and then apply it back to vertebrates and humans as the mechanism is very likely conserved [74]. Indeed, lack of redundancy can expose the physiologically relevant phenotype of gene homologues.

The similarities between flies and humans are further supported by the fact that components of signal transduction pathways and the molecular mechanisms involved in specification, development, cell cycle regulation and human diseases were first identified in flies. The genes, which have been characterized in flies, were subsequently studied in mice and humans, and

their names were adopted or adapted from their *Drosophila* homologues. For example, the mammalian Notch 1-4 named after the *Drosophila* Notch (fly wings having a large notch on the wing), "*sonic hedgehog*" named after the *Drosophila* "*hedgehog"* (round larvae with extra bristles) and *Wnt* from the *Drosophila* "*wingless* and *INT*-related" gene [31].

3. Recent advances in modeling tumor progression and metastasis in *Drosophila melanogaster*

Since the discovery of oncogenes and tumor suppressor genes, intense research in many laboratories all over the world has brought us to the point where we are starting to understand the main principles underlying molecular changes in the course of tumor progression [3, 75]. The development of new technologies revealed the complex molecular nature of tumorigenesis in which tumor progression can be envisaged as a network of simultaneous events within both tumor cells and the stroma. Because cancer is an age-associated disease in humans, using *Drosophila* to model cancer development, progression and metastasis was debatable [76, 77]. However, over the last decades the study of *Drosophila* has significantly contributed to the understanding of key cancer events, including the loss of cell polarity, the competition between tumor and normal cells, as well as metastasis [78].

In addition to the importance of tumor cells themselves, their neighboring cells and the surrounding stroma are now recognized as important regulators of cancer progression [79]. Cell competition is a type of short-range cell-cell interaction in which cells expressing different levels of a particular protein are able to discriminate between their relative levels so that the one cell, the "loser", disappears from the tissue whereas the other, the "winner", survives and proliferates to cover the space left by the disappearing cell. Some tumor-promoting mutations in *Drosophila* are able to induce cell competition between the cancer cells and their micro- and macroenvironment [80-86]. Metastasis is the latest phase of cancer progression during which cells detach from their original niche to invade distant tissues [87]. For several decades, our understanding of the molecular processes leading to metastasis was largely derived from studies of cancer cell lines *in vitro*, xenografts of human tumors and a limited number of transgenic or knockout mouse models [1, 88, 89]. However, understanding the individual steps leading to tumorigenesis or analyzing multiple genetic interactions in mice is difficult. Existing models are currently being re-evaluated given the increasing awareness of tumor complexity. Therefore, using a model system that allows the efficient analysis of the combinations of genetic events that trigger tumor initiation and metastasis during cancer development is the major challenge in cancer research at the moment. *Drosophila melanogaster* provides a model of choice for cancer analysis as the collection of sophisticated genetic manipulation techniques have been invaluable for dissecting signaling pathways that affect cell specification, differentiation and growth [90-93]. Indeed, *Drosophila* cancer models are very helpful in unraveling the chronological sequence of events leading to human cancer. For example, in metastasized human tumors elucidating the identity of the initial mutations is often tedious as oncologists are in most cases looking at the end point of the disease progression. Finally, *Drosophila* genetics is

http://dx.doi.org/10.5772/55686

very powerful as we can dissect the triggering events initiating cancer and the subsequent steps leading to the progression of the disease [94].

3.1. Modeling cell competition and metastasis

With these added complexities in mind, the analysis of cancer-disposing mutations in only a subset of cells or in clones within the context of a wild type surrounding tissue is gaining more interest because it offers a reasonable approximation of the clonal nature of human cancers, compared to the analysis of the multi-step model of tumor progression in the context of a whole organism. A great number of very interesting publications provided us with information about new and unexpected findings on the role of the polarity genes *scrib*, *lgl* and *dlg* in cancer initiation, progression and metastasis. Nowadays, it becomes obvious that they play a broader role than initially thought, through the cooperation with individual partners and signaling pathways and have helped us to understand the role of cell competition and of the tumor microenvironment during tumor survival and progression.

Analysis of *scrib*$^{-}$ mutant clones in the *Drosophila* eye imaginal discs has shown that tumor development is suppressed by the JNK-mediated apoptotic pathway activated by the surrounding wild type cells, whereas the neoplastic and metastatic potential is regained through the synergistic effect of a simultaneous up-regulation of Ras signalling within the same clones [84, 95, 96]. These results underline the effect of the surrounding normal cells on the transformed *scrib*$^{-}$ clonal cells, which leads to a cell competition similar to the one observed in the mammalian cancers [1, 96-100]. In a model for *scrib* tumorigenesis, the analysis of the downstream pathways in *scrib*$^{-}$ epithelial clones revealed that excessive cell proliferation is restrained by JNK-mediated apoptosis. Upon simultaneous activation of either Ras or Notch, JNK-mediated apoptosis is blocked, and Ras/Notch together with JNK cooperatively promote tumor growth and invasion [96]. In other words, whereas JNK activity normally promotes the apoptosis of *scrib*-deficient cells, it becomes a driver of cellular overgrowth, tumorigenesis and invasion in the presence of oncogenically activated Ras or Notch signalling [76, 101]. These tumors present similar characteristics to human cancers that lack Scrib, including basement membrane and extracellular matrix degradation, loss of E-cad expression, combined with migration, invasion and secondary tumor formation [101] Another report provided a molecular link between loss of polarity and tumorigenesis, since *scrib*$^{-}$, *dlg*$^{-}$ and *lgl*$^{-}$ clonal cells in a wild type surrounding become metastatic only in combination with Ras^{v12} activation, resulting in JNK activation and E-cad inactivation [102]. The analysis of the JNK-mediated tumorigenesis, which in *Drosophila* cells reveals a cooperation with Ras similar to that taking place in mammalian breast epithelial cells, indicates sthat the knowledge gained from analysis in *Drosophila* can help us elucidate tumor formation in the mammalian system.

Mutations inactivating the Salvator-Warts-Hippo (Sav-Wts-Hpo) pathway can also cause super-competition, contributing to the overgrowth of cells expressing these mutations in the presence of wild type cells [80, 103]. Since the first discovery of the Sav-Wts-Hpo pathway in *Drosophila*, the role of these genes in restricting cell growth and proliferation, and inducing apoptosis has triggered a great interest in its study. The components of this pathway act as important tumor suppressors that regulate tissue growth by promoting cell cycle exit and

apoptosis [80, 104-112]. Recent data from mammals and *Drosophila* show the occurrence of a very conserved pathway that links the pathway of cell polarity to the regulation of tissue growth.

In the model of Scrib tumorigenesis, induction of apoptosis in *scrib*-clones could not explain how *scrib*$^-$ cells are prevented from overproliferating. This was answered by the finding that cell competition between *scrib* and wild type cells prevents overproliferation by suppressing Yorkie (Yki; a transcription factor, which is suppressed by the Sav/Wts/Hpo pathway) activity in *scrib*$^-$ cells [113, 114]. Suppressing Yki activation is critical for *scrib*$^-$ clone elimination by cell competition. Cell competition leads to activation of JNK in *scrib*$^-$ cells and JNK antagonises Yki activity, which leads to elimination of the clone. Experimental Yki elevation is sufficient to promote neoplastic growth in *scrib*$^-$ cells [114]. Along the same line, when *lgl* is mutated in a mosaic tissue, the *lgl*$^-$ clonal cells become the "losers" in cell competition. However, simultaneous overexpression of the Ras signalling pathway or of *yki* in *lgl*$^-$ clones, causes overgrowths and JNK-mediated apoptosis at the periphery of the transformed clones [115-120]. Moreover, JNK-mediated elimination of *lgl*$^-$ clonal cells is relieved and the overgrowth potential is re-established by upregulation of c-Myc, demonstrating the the death of *lgl*$^-$ clones is essentially driven mainly by c-Myc-induced cell competition [121]. Simultaneous downregulation of the *lgl* and the JNK pathway in the whole-animal system results in a phenotypic reversion of tumor growth, absence of the giant larvae and recurrence of pupariation, thereby showing that JNK activity is essential for overgrowth and invasion of *lgl* tumorous discs [122]. Moreover, in the developing *Drosophila* eye and imaginal disc epithelia Lgl, αPKC and Crumbs proteins regulate cell proliferation and survival by controlling the activity of the Sav-Wts-Hpo pathway [115, 123-125].

Among the wide palette of cellular events leading to JNK activation is the dTNF (tumor necrosis factor)/Eiger. Eiger is the only *Drosophila* member of the TNF superfamily and its deregulated expression in imaginal disc cells results in JNK-mediated apoptosis [76, 126]. JNK-dependent cell death in *scrib* and *dlg* clones requires dTNF, acting as a "tumor death factor" [127]. On the other hand, in tumors deficient for *scrib* and *dlg* that also express *Ras*, the TNF signal is converted into a signal, which promotes tumor growth and invasion [126]. More precisely, upon dTNF downregulation, cell death in *dlg* and *scrib* clones was blocked and *in situ* outgrowths appeared, probably by TNF-mediated extra-cellular matrix (ECM) remodelling [76, 126]. When generated in an *eiger*$^-$ mutant background, *Ras*^{v12}*scrib*$^-$ clones displayed non-invasive *in situ* overgrowth. Similarly, in whole *Ras*^{v12}*scrib*$^-$*dTNF*$^-$ animals, development proceeded up to pupal stages, overcoming the "giant larvae" phenotype [76, 126]. These recent results suggest that several of the critical overgrowth phenotypes of *scrib, dlg* and *lgl* in the clonal and whole-tissue context are mediated by dTNF and that dTNF pro-tumor function depends partially on JNK activation in tumor cells, which provides a switch from *in situ* to invasive growth. Immunostaining experiments that detected dTNF in a punctuated, intracellular vesicle pattern at the periphery of hemocytes in association with the *dlg*$^-$ clones, indicating that dTNF expression in hemocytes is sufficient for dTNF/JNK pathway activation within the *dlg*$^-$ clones, and mark the importance of hemolymph and non-cell autonomous immune response in tumor progression [76, 126].

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Until very recently, the mechanism by which surrounding normal tissue exerts antitumor effects against *dlg, scrib* or *lgl* clones remained elucive. New results from clonal analysis in *Drosophila* imaginal discs have shown that JNK activation from the wild type surrounding leads to upregulation of PVR, the *Drosophila* PDGF/VEGF receptor, which subsequently activates the ELMO/Mbc phagocytic pathway, which in turn eliminates the oncogenic clonal cells by engulfment [128]. From an evolutionary point of view, the development of such mechanism, which senses and eliminates "neoplastic" tumor-suppressor mutant cells such as those of *scrib*$^-$ and *dlg*$^-$ but not "hyperplastic" ones (in which despite of overproliferation, cells are normally shaped and retain a differentiated epithelial monolayer, such as those of the Hippo pathway and PTEN) [128], shows the necessity to specifically eliminate the high-risk malignant neoplastic cells before they confer any harm to the organism. Taken together, these studies demonstrate that hemocytes together with the tumor microenvironment act as regulators of epithelial delamination required for tumor invasion. Due to the ease of genetic manipulations, *Drosophila* research can bring meaningful insights to our understanding of the mechanisms of communication between cancerous and normal cells as well as between tumor tissue and the immune system [87].

3.2. *Drosophila* provides critical insights on how conserved mechanisms contribute in cancer and tumorous development

Drosophila research has also contributed to cancer analysis by identifying genes and signaling pathways later found to be critical for tumorigenesis in mammalian systems. In several cases *Drosophila* has been used to establish specific model systems in order to understand processes that seem to be more complex in vertebrates and mammals [81, 101, 129, 130]. One of the most extensively studied *Drosophila* models of tumor biology is the asymmetric cell division of neuroblasts, the *Drosophila* neuronal stem cells. In *Drosophila* neuroblasts (NB), the PAR3-PAR6-atypical protein kinase C (aPKC) complex segregates apically and recruits the adaptor protein Inscutable (Insc), which connects this complex to the partner of Insc (Pins), guanine nucleotide associated protein-α_1 (Gα_1), mushroom body defect (MUD), and p150^{glued} to the crescent directing the orientation of the mitotic spindle during asymmetric cell divisions. aPKC promotes the exclusion of partner of numb (PON), Lgl and Numb, which along with Miranda (Mira), Brain Tumor (Brat) and Prospero (Pros), localize to the basal crescents [101, 130, 131]. Interestingly, most of these genes have functional mammalian homologues and a very recent study points out the role of the Par3 in mammalian skin tumorigenesis [132]. In mouse skin tumorigenesis, Par3 deficiency results in reduced papilloma formation and growth. Furthermore, Par3 expression is reduced in both mouse and human keratoacanthomas, indicating the tumor-suppressive properties of Par3. More insights into tumor physiology came from a very interesting study in *Drosophila* NBs that indicates the critical role of starvation in promoting the overgrowth *pins* larval brains. Energy stress, mediated by loss of TOR and PI3K components, in combination with loss of *pins* results in loss of asymmetric NB division and brain overgrowth (Rossi, 2012). Since the PI3K and TOR signaling pathways are vital to the growth and survival of mammalian cancers [133, 134] using *Drosophila* in order to dissect the cross talk of these pathways to preexisting tumor susceptibility defects e.g. polarity defects, in a simple animal model is of great importance.

The usefulness of *Drosophila* is further illustrated in the development of a *Drosophila* model for human brain cancer. Glioblastoma (GMB) is the most frequent and malignant form of high-grade glioma that infiltrate the brain, grow rapidly and are refractory to current therapies [135]. One key to development of new and effective therapies against these tumors is to understand the fundamental genetic, cellular and molecular logic underlying gliomagenesis. Signature genetic lesions in glioblastomas include mutation of the epidermal growth factor receptor tyrosine kinase (EGFR) and mutations activating components of the PI3K pathway. *Drosophila* studies using lineage analysis combined with cell-type specific markers demonstrated that EGFR-Ras and PI3K can induce fly glial neoplasia through activation of a combinatorial genetic network composed in part of other genetic pathways also commonly mutated in human glioblastomas [135]. In the future, large-scale forward genetic screens with this model may reveal new insights into the origins of glioblastoma and may also provide new therapeutic strategy for the treatment of this form of human tumor.

Drosophila has been also used to elucidate the molecular mechanisms of human hereditary diffuse gastric cancer (HDGC) [136]. Gastric cancer, as several human cancers, originates from epithelial cells. Mutations in the CDH1 gene, which encodes the cell adhesion molecule E-cadherin (E-cad), are associated with HDGC in humans. In order to understand the role of E-cad in this disease, a *Drosophila* model has been developed in which mutated forms of E-cad can be studied *in vivo* [136, 137]. Moreover, genetic and molecular studies of *Drosophila* hematopoiesis can also contribute to our knowledge of the hematopoietic niche and hematopoietic malignancies in humans. Vertebrate hematopoietic stem cells give rise to an hierarchically organized set of progenitors and deregulation of the hematopoietic differentiation program can lead to numerous pathologies including leukemias. With the discovery that many transcriptional regulators and signaling pathways controlling blood development are conserved between humans and flies, *Drosophila* is particularly suitable model for investigating the mechanisms underlying the generation of blood cell lineages and blood cell homeostasis [138].

Interesting results using a *Drosophila* cancer model demonstrated that apoptosis activation in differentiation-compromised cells constitutes a mechanism for early prevention of cancer [139]. Apoptosis is a highly conserved cellular function to remove excessive or unstable cells in diverse developmental processes and disease-responses. An important example is the elimination of cells unable to differentiate, which have the potential to generate tumors. Using cell-type specification in *Drosophila* Ingrid Lohmann and her colleagues identified a conserved regulatory mechanism that underlies cell-type specific removal of uncommitted cells by apoptosis as a cancer prevention mechanism [139]. Under normal conditions the transcription factor Cut activates differentiation, while it simultaneously represses cell death via the direct regulation of a pro-apoptotic gene. However, loss of Cut and subsequent release of apoptosis leads to overproliferation of the mispecified cells that can acquire metastatic potential in a sensitized background. Importantly, this regulatory wiring is also found in vertebrates in which other cell-type specification factors may similarly be employed to suppress tumor formation. Thus, coupling of differentiation and apoptosis by individual transcription factors

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1	*Drosophila* flies are easy and inexpensive to culture in laboratory conditions, have relatively low set-up and maintenance costs
2	Short life cycle
3	High fecundity (produce large number of off-springs with feature-rich morphology)
4	The *Drosophila* community is open and generous in sharing reagents within the community.
5	No ethical issues and regulatory considerations.
6	Genetic advantages • flies have only 4 pairs of chromosomes • males lack genetic recombination, making genetic crosses easier • flies tend to lack genetic redundancy
7	Signaling pathways controlling growth, differentation and development, which are involved in tumor formation in the fly are largely conserved between *Drosophila* and humans
8	Availability of numerous genetic tools & reagents for generating mutants and analysis of gene expression by using methods producing over- & ectopic- expression. • The use of "balancer chromosomes" with multiple DNA inversions prevent female recombination and allows the perdurability of mutations on the original carrier chromosome • Wide variety of gene targeting strategies, e.g. UAS-GAL4 system combined with RNAi knock-down allow the tissue-specific analysis of tumor suppressor gene and oncogene function • Mosaic analysis of animals containing mutant clones next to wild type tissue, using FLP-FRT and MARCM recombination systems, allows the analysis of tumor microenvironment in invasion, metastasis & inflammation.
9	Possibility to perform genome-wide screens using chemical mutagenesis, tissue-specific RNAi knockdown, effectors and modifier screens to identify genes involved in specific developmental pathways and assign and validate new gene functions.
10	Use of *Drosophila* tumor models for pharmacological screening and development of new therapeutic strategies for cancer.

Table 1. Advantages of *Drosophila melanogaster* for the analysis of cancer.

is a widely used and evolutionarily conserved cancer prevention module, which is hard-wired into the developmental program [139].

Furthermore, genetic analysis of border cell migration in the *Drosophila melanogaster* ovary provides clues that improve our understanding of ovarian cancer metastasis at the molecular level that might also lead to therapeutic targets [140]. The border cells of the *Drosophila* ovary provide a particularly simple example of cell migration in which cells derived from an epithelium invade a neighboring tissue. The large numbers of genes that control border cell migration identified in genetic screens emphasized also the requirement of multiple extracellular signals for border cell motility [141]. Interestingly, the motile and invasive properties of the border cells seem to share common characteristics with mammalian ovarian carcinoma cells. Based on work done in *Drosophila,* the function of some mammalian proteins such as myosin VI, have been tested for their ability to regulate motility of ovarian carcinoma cells.

Another example can be found in the EGFR pathway, which functions redundantly to PVR to stimulate border cell migration [142]. Overexpression of EGFR has been reported in up to 70% of ovarian carcinomas [143]. The role of E-cad is also here critical. Normal cells of ovarian surface epithelium express little or no E-cad. However, many primary ovarian carcinomas, similar to border cells, express E-cad at the cell surface and in the cytoplasm [144, 145]. Although cell-surface expression of E-cad is reduced in many metastatic carcinomas, these tumors frequently still have detectable intracellular E-cad [146], indicating that these cells, similar to border cells, have acquired the ability to downregulate E-cad activity at a post-transcriptional level [140].

Numerous molecules identified in *Drosophila* genetic screens have proven to be important to human cancers [140, 147]. For example, the Hedgehog gene and the Wnt homologue Wingless were originally identified in genetic screens for mutations that disrupt embryonic patterning. Subsequent studies of signaling proteins such as Hh, Wnt [140, 148-150], Notch [151, 152] and RhoGTPases [153] as well as of integrin-related adaptor proteins [154, 155] and Hox genes [156, 157], revealed crucial functions not only in normal mammalian development but also in various cancers. It is therefore well accepted that genetic approaches to the study of normal cellular behavior in simple model organisms can yield fundamental insights into the molecular underpinnings of cancer.

4. New perspectives in modeling tumorigenesis in *Drosophila melanogaster*

Drosophila is emerging as a valuable system for use in clinical drug discovery and therapeutic process [52, 129, 158-160]. So far, *Drosophila* was not a favorable model in drug discovery. The main reason was that "Drosophilists" were mainly concerned with answering fundamental development and cell biology questions, and elucidating principles of basic mechanisms and not practical issues of therapeutics [129]. This view is slowly changing as interest in therapeutics and pressure for practical outcomes increases and combined with the development of powerful tools allows *Drosophila* to catch-up in the field.

The remarkable degree in conservation of biochemical pathways that control processes such as cell proliferation, differentiation and migration as well as nervous system function in behavior and cognition, sustains perfectly the use of invertebrate model genetic organisms as tools for drug discovery and validation [52]. *Drosophila's* genetic and genomic tools can be adapted to build sophisticated disease models for studying cancer and metastasis, and for therapeutic development. While testing with mammalian models is essential prior to approval for human trials, the use of invertebrate animal models that are amenable to molecular genetic manipulations, provides experimental and biological advantages that can streamline drug discovery and testing process. Among the benefits of a genetics-based approach is the ability to screen for proteins that may be novel drug targets, and in genetic backgrounds that could more accurately reflect a specific disease state [52]. New drugs can be tested in *Drosophila* much

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faster than in mammalian models and can even be used for high throughput screening processes as an alternative to cell culture [31].

Drosophila may constitute an appropriate model system that can be used for screening a "whole animal setting" as an alternative to cell-cultured based methods. In most pharmaceutical industries the discovery of new compounds with potential positive pharmacological effect relies on the screening of small molecule libraries for interactions with purified proteins, or for the ability to induce a desired physiological response in cultured cells [52]. One of the main problems is that complex processes such as tumor metastasis cannot be recapitulated in a cell or an organ culture. Moreover, cells and organs are physiologically connected and their interplay could be critical in the development of some diseases. Furthermore, the time component of the disease progression is not cannot be easily recapitulated *in vitro* [161]. The second problem is that after the initial screen, the next phase of drug testing, which requires the use of intact mammalian animal models to assay the effectiveness of candidate compounds, usually fails. If in this step of drug discovery process, the compounds isolated *in vitro* or in cell culture, are invalid in mouse, the result is an enormous waste of funds and efforts [158]. At the same time, whole animal vertebrate models are not particularly suitable for high-throughput methodologies and if then only at an extremely high cost. To by pass these limitations, efforts are now being made to screen chemical libraries on whole-animals like *Drosophila* with genetic amenability, low cost and culture conditions compatible with large-scale high-throughput screening.

Furthermore, performing drug screening in the *Drosophila* "whole animal system" does not dependent on the prior identification of a target and permits the selection of compounds with an improved safety profile. A screen based on a phenotypic observation has the advantage of being independent of the specific molecular target involved. Then, depending on the readout used to assay the effectiveness of the candidate drug compound, a large variety of bio-active molecules may be detected in the same screen [158]. Finally, similar to the established "enhancer" or "suppressor" screens, *Drosophila* gives the possibility to test chemical libraries in the genetic background of a disease for their ability to reverse the abnormal phenotype to wild type or partially rescue the disease phenotype [158, 161].

Several groups today develop the associated technology to use *Drosophila* in the first phase of screening for drug compounds, and subsequently test them in more expensive mammalian models. Moreover, the fact that it is usually easier and more straightforward to manipulate the genetic background of *Drosophila* and mimic a disease state, opens also new possibilities for efficient drug testing in a disease-related content. For example, the development of genetically modified animals with fluorescently tagged proteins would allow the use of standard plate-reader spectrofluorometer for whole-animal screens [161].

When the development of mosaic tissues is essential for the analyses of a disease model, the use of MARCM provides notable advantages for effective drug discovery. One is the ability to follow the morphology of mutant cells and tissues which could be useful for assessing the efficacy of a therapeutic compound [52]. When a mutation in a given gene causally produces a disease, it is possible that this mutation elicits a change in expression of other genes and in the function of proteins. These alterations may contribute to the pathologies associated with

the disease. The characterization of these changes constitutes then the first step needed to develop rational therapeutic strategies. Finally, the MARCM methodology should provide ways to identify mutant cells or tissues for a given gene, isolate and subject them to proteomic and genomic analyses which would determine modifications in gene expression and protein interaction profiles [52].

The phenotypes of complex trait diseases such as obesity, heart disease and cancer are the result of modifications occurring in multiple biochemical pathways. The disease phenotype can be caused by improper activation or inhibition of a protein that acts in any of the contributing pathways. Restoration of the normal phenotype would be expected if the output from the primary biochemical pathway affected is rescued via drug-based therapy [52]. However, if multiple pathways contribute to a phenotype, it stands to reason that modifying the activity of a parallel pathway could also elicit a positive therapeutic effect. The use of genetic model organisms has long been a successful means for elucidation of biochemical and physiological pathways, and one of the most powerful strategies available for uncovering genes that act together in producing a phenotype is a search for genetic interaction or a modifier screen. Modifier screens work by generating animals with a mutation in a gene of interest that elicits a sensitized phenotype, and then screening for mutations in progeny that enhance or suppress (i.e., modify) the primary phenotype [52]. *Drosophila* disease models are currently used in drug screening for treatment of diseases such as Alzheimer's and Huntingtons's disease, Fragile X Syndrome and muscular dystrophy [160, 161]. The use of drug discovery especially for muscle diseases is of particular importance as the muscles are difficult to reconstitute *in vitro* and elucidation of the physiology of muscle related diseases and relevant treatment is still poorly understood However, the similarity in architecture, composition and function between *Drosophila* and vertebrates may trigger studies in the fruit fly and provide these diseases with some valuable therapeutic answers [161].

Drug discovery has also proved very effective for the identification of cancer treatments such as the multiple endocrine neoplasia type-2 (MEN2) [129, 162]. MEN2 is a "one-hit" solid tumor syndrome, characterized by mutations in the Ret protein, a transmembrane receptor tyrosine kinase. Patients with mutated oncogenic isoforms of Ret, develop medullary thyroid carcinomas (MTC) that lead to metastasis, which seem to be resistant to traditional chemotherapies. To develop a whole-animal transgenic model, various oncogenic Ret isoforms were targeted to the developing fly eye epithelium. The fly "rough-eye" phenotype is characterized by eye overgrowth, switch in cell fate and other aspects, proving the effectiveness of fly as model and useful readout for screening. The screening for clinical relevant compounds led in the tumorous developing flies by Cagan and his group resulted in the identification of Vandetanib, a broad kinase inhibitor, which Cagan called "the worst kinase inhibitor". Although not very specific and effective, this kinase inhibitor was indeed effective in rescuing the fly phenotype because it regulated the activity of other kinases such as Ras, Src and PI3K (all of which are involved in cancer). Other compounds were more capable of rescuing the rough eye phenotype but reduced animal viability. Yet, Vandetanib displayed little toxicity to the animal as a whole, indicating that tumors might display a lower tolerance threshold for drugs than the entire animal. Obviously the "off-target" effects of Vandetanib, by suppressing kinases other than

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Ret, are important for its effectiveness. Classical drug screenings would reject Vandetanib as too inefficient to the target and too low in its specificity [129, 162]. Cell-culture and subsequent fly work proved to be extremely valuable, as Vandetanib was shown to be efficient in Phase II clinical trials, Currently Vandetanib is in Phase III of clinical assays. This further proves the power of *Drosophila* not only for clinical relevant drug discovery but also for shaping how we should approach drug discovery for treating diseases.

A new *in vivo* drug screening in *Drosophila* has been performed to target cancer stem cells (CSCs) in the group of Norbert Perrimon [163]. Cancer cells represent a small number of cells within tumors with a self-renewing capacity that can regenerate tumor cells types through their stem cell-like renewal capacity. Their resistance to chemotherapy is the main reason why chemotherapeutic treatments are ineffective and the disease often relapses. In order to cope with the challenge of finding drugs that specifically target the CSCs, the Perrimon laboratory uses the *Drosophila* gut as the stem cell system to develop novel methods and approaches to screen for anti-cancer drugs that target CSCs *in vivo* in the gut microenvironment. By directing the expression of oncogenes in *Drosophila* transgenic fly models combined with a screening method that involves monitoring of tumor size by luciferase reporter activity, they identified 25 compounds that reduced tumor size. Further confirmation was validated with dissection of the gut, histochemical-imaging of the gut specific cells and determination of the specificity of the drugs. For example, some drugs were targeting only the CSCs whereas others were targeting CSCs but at the same time promoted growth of the wild type stem cells and in some cases also affected stress pathways in the daughter cells.

5. Limitations in using *Drosophila* as a model system: how far can we go?

Drosophila melanogaster, as a model system for studying tumorigenesis and human disease has certain limitations, especially in regard to the biological processes that evolved only within the vertebrate lineage [164]. The main limitation of *Drosophila* arises from the fact that some diseases cannot be modeled because the corresponding genes and organs present in humans are missing in flies [161]. For example, *Drosophila* has a single cardiac chamber that functions as a heart in the context of an open circulatory system. Moreover, the *Drosophila* myocardium receives oxygenation through diffusion and does not have coronary arteries [165]. A second limitation arises from differences in cellular and molecular processes of *Drosophila* in comparison to humans. For example, there are cases in which one or several key molecules mediating a human disease-specific pathway are missing in flies [160]. Ultimately, some areas such as learning, endocrine function and mammary gland development, may prove difficult to study in a simple invertebrate like *Drosophila* and so the study of these particular disorders may not benefit substantially from *Drosophila* genetics [166]. Another example is modeling tumor metastasis. In mammals malignant cells undergoing metastasis enter the local blood or lymph vessel before colonizing a distant tissue and forming metastatic tumors. This is very difficult to model in *Drosophila* as flies have a rudimentary hematopoietic system and a dramatic different lymphatic system compared to mammals [78]. However, one should point out that despite these differences the “master regulators” of heart, eye, kidney etc. have proved to be

remarkably conserved [162]. This means that in the case of e.g. spinal malformation, flies could be used to model bone formation *per se*, but as Notch signaling has a pivotal role in regulating this process, any knowledge obtained about interactions between components of this pathway in *Drosophila* could be relevant to processes that these genes control during spinal formation in humans [164].

Are there limitations in using *Drosophila* for treatment-relevant drug discovery? The limitations in this case results from the anatomical and molecular differences of small model organisms in comparison to humans, as this may cause the elimination of a significant fraction of the hits generated The use of *Drosophila* models should be viewed as complementary alternatives to cellular assays and *in vitro* screenings made in mammalian cells, rather than the absolute shortcut to screen drugs for human treatments [158]. Their added value for drug discovery varies from disease to disease, and mainly depends on the availability of other options. Indeed, assays in *Drosophila* are complementary to *in vitro* and cellular systems, and in comparison to rodent model systems *Drosophila's* small size and culture conditions fulfill the requirements for large-scale screens [158]. Furthermore, another limitation also results from the dose-dependence of the drug treatment. In rodent model systems the drug dosage may differ according to the mode of penetration and the nature of the drug. In *Drosophila* dose-response experiments are easily feasible but the compounds are essentially delivered to the fruit flies through the media [167]. Thus it is important that the results obtained with *Drosophila* are compared with data obtained on laboratory rodents and, when possible, in humans. Furthermore, in numerous cases the results will be more qualitative than quantitative. Although the conclusions derived from *Drosophila* studies may remain too uncertain for pharmacologists, the data obtained from invertebrate-based screening could lead to important breakthrough, particularly for those diseases in which the pathophysiology is poorly understood [158, 161].

Often model systems are used to understand life and basic biological and cellular mechanisms. A better understanding of a specific human disease often comes as a consequence of the better overall understanding of biological processes [159]. Within this context, *Drosophila* is a valuable tool to categorize putative candidate genes for further downstream functional analysis in vertebrates. It can be used to dissect the likelihood that individual genes in a gene-cluster contribute to disease susceptibility, identify the relevance of a gene to a disease-pathway and get insights on gene specific functions manifested at the level of a tissue or involving cell-cell communication. Using *Drosophila* models, preliminary experiments with other model systems (such as mice) may be reduced and experiments in higher organisms can be better focused. Using *Drosophila* in a systematic approach together with other models and tools, seems promising in order to significantly reduce the turnout time from genetic results into biologically meaningful data [168]. Conclusively, although *Drosophila* will probably not always serve as a perfect model for human disease modeling, the common underlying molecular interactions and signaling pathways between flies and humans will continue to allow researchers to use *Drosophila* in order to answer existing questions, pose new hypotheses, get entry points in elucidating cancer and personalized cancer therapy, and complement studies from other model systems and vertebrate organisms [164].

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6. The expanding role of *Drosophila* in cancer research: Bridging past, present and future

Undeniably the study of *Drosophila* has brought major contributions to the understanding to the fundamentals of cancer and has further given strong impulses not only in basic but also in applied research. The unrivalled advantages and tools offered by *Drosophila* will ensure that it will remain a premier research organism for many years to come. The advantages of *Drosophila* as a model system includes the availability of genetic tools developed in a century of "tool-building" research, its short life cycle and ability to unravel the basic function of genes in a straightforward way. The use of visible mutations and chromosome mapping coupled to currently available complex genetic databases including genomic and proteomic sequences, together with help of systematic gene disruption (RNAi libraries), microarray analysis, protein interaction maps and Flybase, lay the carpet for a renewed new age of research in *Drosophila*, and will allow scientists to address new questions on biological processes which were previously inaccessible. In turn the new discoveries will foster new research and answer to more precise questions about signaling pathways and behavior of individual cells in cancer. The *Drosophila* research will continuously contribute to a better understanding of tumor formation and progression, and may thus improve therapies in treating cancer.

Could *Drosophila* still be a valid model for understanding tumor formation and could it still provide a lead for curing cancer? Although the fruit fly does not appear to be suited for studying vertebrate-specific tissues, such as brain and heart development or neural crest migration [37], *Drosophila* may still help to identify critical key-genes, discover new biological pathways, define new research approaches, and therefore pioneer numerous fields in the understanding of cancer, including vertebrate biology. *Drosophila* can also be used to unravel the sequence of events leading to tumor formation and to trace the initial stages of tumor formation. One of the main outcomes of the genome analysis has been the finding of a high degree of conservation among genes. Subsequent analyses revealed that 75% of the genes involved in human diseases have homologues in *Drosophila*. There is also a high degree of functional conservation between the signal transduction pathways and a high degree of structural and functional conservation between cell adhesion proteins of *Drosophila* and humans showing that the fundamental biological processes have a common origin and has been relatively conserved during the 600 million years of evolutionary divergence between invertebrates and vertebrates. New emerging challenges in the study of tumorigenic inflammation, in *in vivo* screening for drug acting on cancer stem cells, in the therapeutic drug design and discovery will provide us with new insights into a "multi-target" approach for treating cancer. Finally, innovative technologies such as microarrays and nanotechnology, combined with novel methods in computation and bioinformatics, could be used in combination with genome-wide analysis in *Drosophila*, functional maps of the chromatin landscape [169-171], cis-regulatory map of the *Drosophila* genome and pattern of co-binding partners in transcription [172], as well as high-resolution of transcriptome dynamics throughout development [173, 174] to define more accurately the network of genes and pathways that would permit initial cancer cells to build expanding tumors. These new directions highlight not only the value of

basic research but also the intrinsic advantages of *Drosophila* as a model organism for studying the complexity of cancer.

Acknowledgements

We apologize to all whose work has not been sited due to space limitations. This work was supported by the "Zentraler Forschungspool" funding of the University of Heidelberg to F.P. and the Czech Grant Foundation P302/11/1640, the Charles University Center UNCE204022 and the First Faculty of Medicine Prvouk/1LF/1 to B.M.M.

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