Prostate cancer (PC) is the most prevalent cancer affecting older men in developed countries. It is characterized by the uncontrolled, malignant growth of cells in the gland, which ultimately cause symptoms like pain or trouble with urination and ejaculation [1]. Similar to other carcinomas, the first steps of the disease are asymptomatic, rendering early detection difficult without a preventive medical intervention. Therefore, most prostate cancers are detected in late stages, years after disease initiation, and when the benchmark treatment is hormonotherapy. Prostate cancer cells depend on androgens to maintain growth and proliferation, and due to this dependency, androgen deprivation therapy has been shown to be highly effective. However, after an initial response, treatment resistance occurs, and patients develop castration-resistant prostate cancer (CRPC) for which aggressive treatment can slow but not cure the disease. Hence, CRPC ultimately leads to death. For these reasons, improving the prevention and diagnosis of prostate cancer is essential for better patient care. It is now well known that prostate cancer is a heterogeneous cancer, with particularly different evolutions and treatments needed depending on the tumor aggressiveness. Some tumors are indeed very aggressive and will progress quickly, while others are indolent for years or decades, and ideally just require monitoring without curative treatment. Because of this heterogeneity, it is difficult to
determine which genetic abnormalities cause prostate cancer initiation, progression, and ultimately treatment resistance.

In more than 95% of cases, prostate cancer is adenocarcinoma, i.e., of epithelial origin, whereas <2% of cases come from neuroendocrine cells [2]. The formation and evolution of adenocarcinomas follows a classic process that has been summarized in three stages: (1) initiation, in which genetic mutations are thought to be the main driver; (2) promotion, during which an accumulation of more genetic events take place, and where uncontrolled proliferation begins; and (3) progression, which in fact includes different steps of tumor evolution, the most significant being early invasion leading to formation of localized prostate cancer, and then, late invasion. Late invasion corresponds to formation of metastatic prostate cancer, when cells have acquired independence from their environment and are able to form secondary tumors mainly in lymph nodes, bones, brain, lungs, and liver. The evolution of adenocarcinoma through these different phases is the consequence of a succession of genetic alterations (loss of chromosomal fragments, gene fusion, or mutations), and activation of signaling pathways, particularly those involving growth factors, as well as a modification of the microenvironment. Several alterations are associated with prostate cancer. Among them, loss of expression of the tumor suppressor gene NKX3.1 is found in 60–80% of prostate tumors [3,4], and is caused by loss of its heterozygosity or inhibitory epigenetic events, including methylation of its promoter [5]. The most common gene fusion is TMPRSS2-ERG, combining the genes encoding ERG (ETS-related gene) and the serine protease TMPRSS2, found in about 50% of localized prostate cancers [6,7]. Finally, mutation or deletion of genes, such as PTEN, are found in 50-80% of prostate cancers, and result in the overactivation of the PI3K/AKT/mTOR signaling pathway [8–10]. Even though the understanding of the main mechanisms involved in prostate cancer is progressing, it is still necessary to determine and study the key alterations responsible for the initiation and progression of prostate cancer through its different stages. Genomic studies have revealed that primary prostate cancer cells display more than 2000 genetic alterations, whereas around 9000 alterations are detected for CRPC [11]. Furthermore, in some cases, cancer evolution can be correlated to different genomic profiles [12], showing both the actual diversity of these 2000–9000 alterations depending on the considered patient and the necessity to better understand their respective role in order to improve treatments according to a patient’s key alterations. Of course, these studies require pertinent and complementary models to overlap all aspects of prostate cancer biology. Here, we will present the principal cell lines and mouse models that have been developed as specific prostate cancer models, and their relevance for the study of the different stages of the disease. As part of a special issue, we will further present how Drosophila models are increasingly used to better understand fundamental biological processes implicated in tumorigenesis and how they could allow the study of key stages of carcinogenesis that are still poorly understood. Finally, we will discuss the necessity of utilizing multiple and diverse models in order to study the many tumor evolutions that are included in the generic “prostate cancer” term.

2. Cell Lines and Mouse Models of Prostate Cancer Disease

2.1. Two-Dimensional and 3-D Cell Culture for Deciphering Molecular Mechanisms

Since the first cell line derivation in 1975, more than 200 cell lines have been developed and used for PCa studies [13,14]. Each cell line displays a specific molecular signature, and it is important to take it into account in order to choose the most relevant model to answer the chosen biological question. Among the most important molecular markers to consider is the presence or absence of the androgen receptor, which determines if the cells are androgen sensitive, and representative of an earlier stage of tumorigenesis, or androgen insensitive, and representative of late-stage CRPC. Another factor is the expression of PSA (prostate-specific antigen), a protein produced by prostate epithelial cells and used to diagnose prostate cancer. Finally, it is important to consider the presence or absence of genetic alterations characteristic of PCa, such as those previously mentioned (deletion of
NKX3.1, loss of PTEN, etc.). Prostate cell lines can be classified into three main groups: untransformed, androgen-sensitive, and androgen-insensitive/castration-resistant cells.

Only a few prostate cell lines are immortalized but non-transformed, and therefore non-tumoral. The P69SV40-T (P69) cell line is derived from the normal prostate gland of a 63-year-old man. Interestingly, this cell line is immortalized and responsive to IGF1, yet it is not transformed [15]. The RWPE-1 cell line is derived from the peripheral zone of a normal adult human prostate and expresses the androgen receptor as well as PSA [16]. In contrast, the BPH-1 and pRNS-1-1 lines, derived from primary prostate tissue and radical prostatectomy, respectively, express neither the androgen receptor nor PSA [17,18]. These cell lines allow the study of normal growth and development of the prostate [19]. They also allow the study of non-tumorigenic prostatic pathologies, such as benign prostatic hyperplasia (BPH), the most frequent pathology affecting the prostate, which corresponds to a non-malignant excessive growth of the organ [20]

Prostate cancer cell lines are either derived from primary or metastatic tumors, or clonally derived from the previously established cell lines. However, because prostate cancer is characterized by slow growth, it is difficult to obtain cell lines from a primary tumor, and most of the lines are derived from metastases. Furthermore, such cell lines, for example 1013L or E006AA, are less aggressive than those derived from metastases and are rarely used for research purposes [21,22]. Prostate cancer cell lines derived from metastases come from different sites and represent a large panel of the heterogeneity at this stage of disease, including different mutations, gene expression, shape, and metastatic potential. Some cell lines are still sensitive to androgens and thus represent an earlier stage of prostate cancer development. The most representative cells for this stage are LNCaP cells, isolated in 1980 from a lymph node metastasis, and expressing a mutated form of androgen receptor (T877A) [23]. Other androgen-dependent cell lines include LAPC-4, LAPC-9, or LuCaP 23.1, and are derived from lymph node or bone metastases [24–26]. All these cell lines have specific molecular characteristics that must be taken into consideration when used: LNCaP and LAPC-9 are deficient for PTEN, and display a constitutively active PI3K/Akt pathway, while LAPC-4 has a mutation in the P53 tumor suppressor gene. Cell lines that are not sensitive to androgens are representative of a later stage of prostate tumorigenesis, known as CRPC, which generally appears after hormone therapy. This is the case for the PC3 cell line. Isolated from vertebrae metastasis in 1979, this cell line is capable of forming a tumors that metastasize to the lungs, liver, and kidney when injected into mice [27]. The DU145 cell line, derived from a brain metastasis in a 69-year-old white man in 1975, is also androgen independent and tumorigenic when injected in mice [28]. DU145 cells grow well in vitro and can lead to metastasis in mice after injections, depending on the microenvironment [29,30]. Along with LNCaP and PC3, the DU145 constitutes the most utilized PCa cell culture lines. The PC3 and DU145 cell lines are not sensitive to androgens because they no longer express the androgen receptor. However, during the progression of prostate cancer, the acquisition of this androgen independence can occur via loss of androgen receptor expression or via other mechanisms that make the receptor active independently of androgens, i.e., gain-of-function mutations in the receptor [31,32]. In order to study the impact of such mechanisms on prostate cancer evolution, new cell lines can be obtained by androgen deprivation, transfection, co-culture, xenograft, or chemical mutagenesis of the already available cell lines. The LNCaP cells, for example, are androgen dependent and poorly tumorigenic in mice. However, castration-resistant lines expressing the androgen receptor, the C4-2 and C4-2B lines, were derived from them. This was done by xenografts of LNCaP cells combined with osteosarcoma cells in mice, then castration of these mice, and then collection of vertebral and bone metastases, respectively [33–36]. Utilizing this method for generating new cell lines is essential to study the different aspects of resistance to hormonotherapy, while also providing cell lines with higher genetic abnormalities, tumorigenic capacities, and metastatic potential.

The use of cell lines allows for the study of molecular interactions and alterations of cancer cells, and ultimately provides functional and mechanistic insight. They are well
characterized and relatively easy to obtain. They can be manipulated to allow inhibition or overexpression of genes of interest, as well as the expression of fluorescent markers. Furthermore, their proliferation, migration, invasion, and cell adhesion capacities can be analyzed. Thus, they facilitate the study of alterations found in cancer cells, from establishing which molecular mechanisms are altered when these alterations are present, to how these alterations modify the behavior of the cells. In this way, they allow for the in vitro study of certain biological processes that are associated with the passage from an early tumorigenic stage to a more advanced stage in vivo. Commonly used tests are wound healing assays for migration evaluation, spreading assays to assess cell adhesion, and Transwell assays for testing migration and invasion [37].

The main disadvantage of cell lines is their inability to reproduce the pathology found in whole organisms. First, because most cell lines are derived from metastases, they present many genetic alterations, which limits their use for the study of early prostate cancer mechanisms. Moreover, the fact that these cells were cultured for years may have induced cell derivation and altered their initial representative nature of prostate cancer. Finally, complex cellular interactions in the tumor microenvironment are essential for cancer initiation and progression, and traditional bi-dimensional cell culture (2-D), where only one type of cell grows as a monolayer, does not model this rich environment [38,39].

The 2000s witnessed the emergence of three-dimensional (3-D) cell culture models. These models can be closer to a native tumor, with better conservation of its heterogeneity and architecture, and with partial conservation of the tumor microenvironment. Two major 3-D tumor models have been developed: spheroids and organoids [40]. While spheroids are composed of clusters of broad-ranging cells growing as free-floating structures, organoids are more complex and composed of organ-specific cells, intended to assemble in a scaffolding extracellular environment to form microscopic versions of parent organs. This multicellular combination is required for accurate reproduction of the (tumor) microenvironment and allows the conservation of many of the histological features found within the original tissues. Organoids have been obtained for a large number of organs like the colon [41,42], pancreas [43], breast [44], and lung [45]. The first organoid model of prostate cancer was made in 2014 from a patient biopsy [46]. In addition to growth in a more relevant environment, this model allows, when propagated from tumor cells, important molecular signatures of prostate cancer to be retained, such as TMPRSS-ERG fusion or alterations in the p53 and Rb pathways. Other 3-D models of prostate cancer have since been developed from biopsies of lesions [47]. The main advantages of these structures include the conservation of cell morphology, cell–cell/cell–matrix interactions, and that they are generated from patients, allowing conservation of prostate tumor alterations that are specific to individuals. These models can be used for fast therapeutic screens without in vivo models, opening the door for precision medicine where individual patient characteristics are used to improve prevention methods, diagnosis, and personalized treatment. However, the generation of organoids is still in development, and not used as a routine procedure in most labs. Furthermore, it remains an ex vivo procedure, limiting its use for metastatic dissemination, for example. Moreover, these models mostly use aggressive cells representative of advanced-stage prostate cancer. Even though they represent a pertinent model for preclinical and late-stage studies, they are not yet effective for early stages of prostate cancer.

2.2. Mouse Models of Prostate Cancer

In vivo prostate cancer models have been used for decades. They allow global studies with conservation of the interactions occurring between the prostate neoplastic cells, the stroma, and the tumoral microenvironment, including systemic (e.g., hormones, immune system) and local influence (e.g., growth factors, non-epithelial cells). These models allow direct study of genes or group of genes in the tumorigenic process and can be used for both basic and translational research. The first in vivo models used for prostate cancer were rat and dog because they developed spontaneous prostate cancer. However, the frequency for
tumor development is completely random and therefore not optimal for experiments. That is why numerous procedures have been developed to produce tumors, mainly in mice, and through two major ways: introduction of genetic alterations in the prostate epithelium, and xenograft models.

2.2.1. Xenograft Mouse Models

Xenograft models consist of transplantation of prostate cancer cell lines in a mouse using three different modalities: subcutaneous, orthotopic, or in the SubRenal capsule (SRC). Subcutaneous models were developed in the 1970s and have been widely used for cancer studies. Depending on the injected cell line, the tumor can take several weeks to several months to grow. The tumor develops under the skin, rendering it accessible and easy to monitor its growth without invasive manipulation. However, the subcutaneous microenvironment has low vascularization and presents a low percentage of engraftment success. Moreover, as the subcutaneous microenvironment is very different from the prostatic microenvironment, these models are more likely closer to 3-D cell models than prostate tumor models [48]. SubRenal Capsule (SRC) transplantation has been introduced more recently to increase the efficiency of grafting in mice [49,50]. It allows for a good percentage of efficient transplantation due to the large amount of vascularization within the tissue. Notably, SRC transplantation can be done with benign and malignant tissue, while subcutaneous xenograft can only be established with high-grade tumor cells. Moreover, cells are engrafted in a definite organ where the capsule represents a real frontier. Metastatic potential can be evaluated by the ability for the xenograft cells to cross the capsule and form tumors in other distant organs. The main weakness of this transplantation procedure, like for subcutaneous transplantation, is the microenvironment where the cells are transplanted, which is very different from the prostate microenvironment. Finally, the orthotopic model consists of the introduction of prostate cancer cells directly into the mouse prostate. Unlike previous transplantation models, tumor growth occurs in the prostate microenvironment and allows for the conservation of the interactions between implanted tissue and the prostate tumoral microenvironment [51]. Orthotopic models have existed since the 1990s and can generate metastases, allowing their use for both tumorigenic and metastatic processes [52]. Furthermore, it is possible to mimic and study the acquisition of castration resistance observed in androgen-deprived patients by injection of androgen-dependent cells followed by mouse castration. With many cell lines available today, there are many possibilities to study specific prostate cancer stages.

Overall, xenograft models appear efficient for rapid analyses of both early and late stages of prostate cancer progression, and in some cases including metastatic studies. Moreover, tumor development occurs in a whole organism with physiological processes conserved. However, we now know the major importance of the immune system in cancer development [53,54]. Additionally, as most of the xenografted cells are of human origin, immunodeficient mice are used as a recipient to avoid cell elimination by the mouse immunity system. So, the tumor microenvironment is modified, the immunobiology of prostate cancer cannot be determined with these models, and this immunodeficiency definitely impacts tumor development [55,56]. Further, when cell lines with one combination of genetic abnormalities are injected to obtain these models, the characteristic heterogeneity of prostate tumors is not preserved. More recently, the emergence of PDX models (patient-derived xenograft models), corresponding to patient tissue injection into mice, resolved this problem. The PDX models of prostate cancer are now used for anticancer drug screening [57,58] and could allow, despite the immunodeficiency of the mice, an adaptation of the experiment for a selected patient. This model facilitates the possibility of personalized medicine, with optimization of treatment occurring in the mouse model before it is used in the patient. Still, in any case, cells that are injected into mice are already transformed. As primary tumor cells already display more than 2000 genetic alterations, and CRPC cells around 10,000, it is difficult to study the mechanisms of early tumorigenesis in this context [11].
2.2.2. Genetic Models in Mice

Since the 1990s, numerous genetically engineered mouse models (GEMs) have been developed to introduce specific genetic alterations in specific tissues, such as the prostate epithelium. One complication of this kind of model concerns the organization of the mouse prostate compared to the human prostate. The human prostate is composed of three zones surrounding the urethra: the central, transition, and peripheral zones [59]. The mouse prostate is organized with different lobes, by pairs, morphologically different from the human prostate zone, and also has different levels of sensibility to androgens (reflecting the androgen receptor level of expression) [60,61]. These differences in androgen sensibility can have an impact on genetic mouse models because the most specific promoter used to induce tumorigenesis in these models is based on the probasin gene, which displays prostate-specific expression and is regulated by androgen receptor signaling [62]. Moreover, it is still not completely clear which lobe is the most representative of the human prostate. The stroma surrounding the prostate also appears differently: it is very thin in mice compared to the dense fibromuscular stroma of humans [63]. Nonetheless, GEM models allow tumor growth in an intact prostate microenvironment with the presence of a normal immune system, and the effect of gene manipulations and drug treatment can be well controlled and observed temporally. As for cellular models, there are many mice designed for prostate cancer studies, because a single mouse model is not enough to overlap all aspects of prostate tumorigenesis.

Prostate tumorigenesis is supported by mutations or alterations of tumor suppressor genes and oncogenes. Two major pathways upregulated and driving prostate carcinogenesis are p38/MAPK and PI3K/Akt signaling pathways, which are altered in more than 40% of prostate adenocarcinomas and almost all metastases [8]. It is therefore not surprising that among the most used GEM models are those displaying an inactivation of phosphatase and tensin (PTEN), an inhibitor of the PI3K/Akt pathway, or an overexpression of Ras, inducing overactivation of the p38/MAPK pathway. To generate genetic models, tools like the Cre/Loxp system are used, to have a spatiotemporal control of the genetic modification. The recombinase Cre, a nuclease from a bacteriophage, can recognize conserved sequences, referring to the Loxp sites. It can then excise all genetic information encoded between two of these sites [64]. For example, one largely used model is the PB-Cre4XPtenloxp/loxp, where CRE expression is dependent on the specific probasin prostate-specific promoter, coupled to two androgen receptor response elements (ARR2PB-Cre4). Consequently, PTEN is inactivated specifically in the prostate epithelium, inducing constitutive activation of the PI3K/AKT pathway, and ultimately prostate carcinogenesis. These tumors are similar to what is observed in humans with PIN lesions, including invasive adenocarcinoma and in some case metastases in the lymph nodes and lung [65,66]. This model can then be used to do bi/tri-genic models with, for example, a combination with deletion of known tumor suppressors, such as Nkx3.1 or p27kip1 [67,68], or overexpression of oncogenes, such as k-Ras [69] or Myc [70], to obtain a more aggressive phenotype and explore the role of the same alterations that are found in patients. Other models are developed by expression of oncogenes like the SV40 large T antigen under androgen regulation in prostate epithelium, corresponding to the TRAMP model [71]. This model is widely used because it presents development close to what is observed in humans: from PIN lesion formation to an aggressive tumor with the appearance of metastasis observed at the lymphatic level. However, in many cases, it has been observed that mice instead develop neuroendocrine prostate cancer, limiting its use for prostate adenocarcinoma studies.

The main advantage of using genetic mouse models is the preservation of an intact microenvironment and immune system. Moreover, depending on the model, the histopathological features observed in human pathology are preserved. However, depending on genetic alterations, the growth of tumors can take several weeks or months, which can make their use costly and time-consuming. Contrary to cell lines, animal model constraints limit the number of genetic alterations that can be simultaneously tested. Furthermore, gene redundancy in mammals can complicate the studies about signaling
pathway interactions. There are also ethical concerns, as shown by the growing importance of the 3Rs (replacement, reduction, refinement), which push for a decrease in the use of animal models. Finally, the low rate of success of clinical trials emerging from mouse and cellular models emphasizes the necessity to still improve cancer therapeutics, for which it is necessary to develop additional and novel approaches to complement the current ones.

3. Drosophila, a Model for Human Pathologies and Prostate Cancer

The fruit fly, Drosophila melanogaster, is a reference model for genetic and developmental studies. Drosophila has several advantages compared to other models: a short life cycle (10 days at 25 °C), a large number of offspring per generation, a well-described anatomy, and vast amounts of genetic tools available. A large number of new strains can be generated rapidly for a variety of assays. Moreover, there are few redundancies compared to mammalian genomes, making the loss-of-function studies easier in this model. The fundamental biological mechanisms and signaling pathways are conserved between Drosophila and mammals, and 70% of genes that were found mutated, deleted, or amplified in human pathologies possess an ortholog in Drosophila [72,73]. The physiological function of these genes has been under investigation in Drosophila for the generation of human pathological models [73], and many functional studies have been done in this model to study complex signaling pathways that are relevant in pathologies, and particularly in cancer research [74]. In fact, many intracellular signaling pathways able to drive tumorigenesis and tumor microenvironment implication and interactions were first identified and characterized in Drosophila [75,76]. Drosophila was one of the first experimental models to show a lethal recessive mutation, lethal 7, which induces transplantable malignant tumor leading to lethality [77,78]. As in mammals, tumorigenesis in Drosophila implies cell homeostasis deregulation. The loss of function of tumor suppressor genes, such as Scribble, Disc large, or Merlin, increase cell proliferation [79–81]. Inhibition of the Hippo signaling pathway induces increases the expression of Cyclin E, which enhances the cell cycle, and DIAP1, an inhibitor of apoptosis [82]. It is also possible to modulate the proliferation/apoptosis balance, and consequently, to study the molecular mechanisms implicated during carcinogenesis. Regulatory mechanisms that are essential to maintain genome integrity are also well conserved in Drosophila. It is the case, for example, for the P53 protein, a mediator of one prominent pathway of cell survival, whose loss induces defective cell apoptosis [83]. Considering that interactions between regulatory processes and signaling pathways are at least partially conserved in Drosophila, it is therefore a relevant and powerful model for the study of human pathologies and cancer. It can be used differentially to study prostate cancer: to investigate conserved tumorigenesis mechanisms, for the discovery of new regulator/signaling pathways, for pharmacological screening, and for epithelial carcinogenesis modeling, including early prostate carcinogenesis.

3.1. Drosophila Genetic Tools and Their Use in Tumorigenesis Studies

As described by Hanahan and Weinberg in 2000 and 2011, even if each tumor displays specific features, depending on cell origin, organ, or even genetic mutations, several hallmarks of cancer are common and can be investigated independently of cancer specificity (e.g., sustained proliferation and evasion of apoptosis) [84,85]. Because fundamental processes are well conserved, Drosophila melanogaster can exhibit some classic hallmarks of cancer, and that is why this model is relevant for cancer investigation. In particular, three genetic tools make Drosophila powerful to dissect the role of signaling pathways with spatial and temporal precision: the combination of the UAS/Gal4/Gal80 binary expression system [86,87], the FLP-FRT recombinase system [88,89], and the availability of RNAi transgenic animals. UASs (upstream activation sequences) are nucleic sequences targeted by the yeast transcription activator gene gal4. Gal4 can be expressed time and tissue specifically using native Drosophila gene promoters, and so induces in the same pattern the expression of every sequence placed downstream of a UAS, which can encode for a fluorescent protein, an oncogene, or an RNAi, for example. Gal80 is a Gal4 antagonist that
even exists in a thermosensitive version. Its co-expression permits further limits on Gal4-induced expression at the desired development time by shifting the culture temperature to 29 °C [87]. The Flp-FRT recombinase system is similar to the Cre/Lox system used in mice: the flippase (Flp) recombines flippase recognition target sequences (FRT) and induces either chromosomal recombination or excision of the sequence, which was flanked by the FRTs. Moreover, in *Drosophila*, the flippase expression can be dependent on a heat shock promoter, hsp70, allowing for temporal control of the genetic recombination when *Drosophila* is placed at 37 °C. In this case, the length of the heat shock will determine the quantity of flippase produced, as well as the percentage of cells that will actually have enough flippase to recombine. This gives a variable level of mosaicism in the tissue, representing a unique opportunity to have within the same tissue a vast majority of normal cells alongside a few modified cells, which allows for an accurate portrayal of the microenvironment of tumor cells at the beginning of the tumorigenic process. Mosaic analysis with a repressible marker, the MARCM system [90], is a typical example of the use of this Flp/FRT systems. Amongst others, it allows the generation of spots of cells mutated for tumor suppressor genes in a heterozygous background, mimicking loss of heterozygosity, a fundamental process in tumor progression. Oncogenic activating mutations or specific gene overexpression will also induce tissue overgrowth, invasive, and metastatic behavior [91,92].

3.2. *Drosophila*, the Origin of Signaling Pathways and Gene Discoveries Relevant for Prostate Cancer

Historically, many signaling pathways, regulators, and new genes have been firstly discovered in *Drosophila* and then implicated in mammalian cell biology, including the Hedgehog, Notch, Wnt, Hippo, and Dpp signaling pathways [93–95]. The link between Notch deletion and developmental defects was made firstly in *Drosophila*, and we now know the importance of Notch signaling in carcinogenesis [96,97]. Numerous studies have since allowed for the discovery of new Notch interactors involved in tumorigenesis. This is the case of a study from 2014, which focuses on PTOV1, an adaptor protein able to modulate proliferation and the cell cycle, which is overexpressed during prostate cancer [98]. After showing in human prostate cancer cells that PTOV1 expression correlates with Notch targets’ repression, the authors used *Drosophila* to study the interactions between PTOV1 and Notch. Indeed, in *Drosophila*, Notch-null mutants are associated with notched wings while a Notch gain-of-function mutation is associated with a defect in the development of a wing vein [99,100]. These two phenotypes are easy to observe and allow a rapid functional analysis of Notch signaling. The authors were able to show that PTOV1 acts as a negative regulator of Notch signaling, as its expression in *Drosophila* induces the formation of notched wings and can reverse the development defects induced by a Notch gain-of-function mutation. These results were then supported by mouse experiments and analysis of human prostate tissue. This article is a great example of how complementary models (*Drosophila*, prostate cancer cells, spheroid, mouse, and human prostate samples) can be used, each giving different information, to demonstrate a new regulatory function of a protein on the Notch pathway in vitro and in vivo, and to prove its relevance to prostate cancer progression. Another example concerns the Hippo signaling pathway, which has also emerged from studies on *Drosophila* and is well known as an actor of prostate cancer tumorigenesis [101]. A major target gene of this pathway is MYC, which is overexpressed during prostate carcinogenesis [102,103]. The first tumor suppressor gene identified in *Drosophila* is lgl (lethal giant larvae), which is implicated in epithelial cell polarity, and loss of expression of which is responsible for abnormal growth of the larval brain and imaginal discs. Furthermore, when it is associated with another mutation of the same polarity complex, such as scribble, mutated tissues can induce secondary tumors [104]. A link between lgl and the Hippo signaling pathway has been made in *Drosophila* [105,106]. Moreover, homologs have been found for lgl in mammals: HUGL-1 and HUGL-2 [107], whose roles have now been extensively investigated.

In addition to signaling pathways, some genes have been firstly discovered in *Drosophila*. It is the case for tribbles, a gene that seems to block mitotic progression during fly devel-
opment, and particularly during the gastrulation stage [108]. Mitotic mechanisms and regulation are essential during carcinogenesis. This is why orthologs of this gene have been searched in other species like dog, rat, and human, followed by functional studies. SKIP3, the human ortholog of tribbles, is expressed in human tumors and in the PC3 cell line, and is regulated by hypoxia, giving it an important function during carcinogenesis [108]. Another article investigated the link between Perlecan, a basement membrane component, and the Hedgehog signaling pathway, which was first demonstrated in Drosophila. They show that HSPG2 (perlecan) is a new component of the SHH pathway in prostate tumorigenesis that works independently of the androgen signaling pathway [109]. This gives new perspectives for drug targeting by blocking SHH effects during prostate carcinogenesis.

Another example concerns the use of Drosophila cells, S2 cells, one of the most commonly used Drosophila melanogaster cell lines, derived from a primary culture of late-stage embryos. They have been used to do a genome-wide RNA interference screen to identify new regulators of the androgen receptor [110]. In this study, S2 Drosophila cells were transfected with the human androgen receptor with luciferase as an activity reporter. Then, a genome-wide RNAi screen was performed and combined with R1881 treatment to induce AR activation. The impact of RNAi on androgen receptor activity was then monitored using luciferase to identify inhibitors or enhancers of AR signaling. The existence of RNA interference libraries makes this kind of analysis quick and easy with Drosophila tools, before considering other analyses in human cells or in vivo models, which will be more expensive and time consuming. The discoveries done with this kind of screen allowed the identification of new regulators that are potential new targets in prostate cancer, for drug therapies.

In addition to the intrinsic characteristics of tumors, it is known that the tumor microenvironment is also essential for its progression. Interestingly, the use of imaginal discs for the study of tumorigenic phenomena shows recruitment of immune cells, a major component of the microenvironment during carcinogenesis [111,112]. The partial conservation of the immune system in Drosophila and the evidence of immune cell recruitment in Drosophila-induced tumors highlights the relevance and the numerous possibilities for studies in this model.

So, although Drosophila presents major differences with mammals, the conservation of many genes and signaling pathways makes it possible to translate research done in Drosophila to mammals. In addition, because Drosophila has fewer redundancies than more complex models, studies can be done easily and more quickly. Thus, Drosophila can be the source of studies in mammals that will advance the understanding of tumorigenic mechanisms and provide new potential therapeutic targets.

3.3. Drosophila Tissues Used as Models for Studying Fundamental Tumorigenic Processes

Some specific cellular processes in Drosophila can also be used to study mechanisms related to cancer development. This is the case of the important use of imaginal discs to model general epithelial tumorigenesis or of the tracheal network to study neo-angiogenesis.

3.3.1. The Imaginal Discs

Imaginal discs are embryonic structures composed of epithelial cells able to generate adult organs, such as eyes, legs, wings, mouthparts, antenna, halteres, and the genitalia system. While adult cells are quiescent, imaginal disc cells have intact proliferative capabilities, and that is why they are widely used for carcinogenesis studies. They are determined to become specific structures, but under conditions of damage or gene mis-expression, discs can switch fate, a phenomenon called transdetermination [115]. This is due to a change in cell fate without reversion to an embryonic stage (dedifferentiation) [114]. Use of imaginal discs is interesting in cancer studies, which found that epithelial cells gain new capacities to become invasive and metastatic without complete dedifferentiation. The two main imaginal discs used for tumor growth and invasion studies are the wing and the eye discs because their modification in the larval stage induces visible phenotypes [115,116]. This is
the case for the modification of Notch signaling, which was discussed earlier, and which induces notched wings or defects in the development of the wing vein. Another example related to prostate cancer is a recent study where the authors showed in mice that CDPC1 (CUB domain-containing protein 1), a transmembrane protein that is a substrate for SRC family kinase, can drive prostate cancer progression via activation of the MAPK signaling pathway [117]. In *Drosophila*, increased EGFR/Ras/MAPK signaling in wing imaginal discs induces the formation of bristles located on the dorsal part of the thorax, a tumor-like phenotype. Thus, the authors used this feature to show that CDCP1 overexpression initiates tumorigenesis in vivo. With complementary models, the authors demonstrated that CDCP1 is a powerful driver of prostate cancer progression, opening new potential therapeutic strategies. The possibility to follow rapidly identifiable phenotypes allows the use of *Drosophila* to perform screens for a chemical molecule or even new potential regulators of specific signaling pathways [118,119]. It was illustrated in a study where *Drosophila* was used as the in vivo tumorigenesis model to confirm the effects of radiosensitizing compounds that were screened initially in vitro in DU145 cells, with exploitation of the eye phenotype to evaluate the drug toxicity and effect on tumorigenesis [120]. Genotoxicity can also be done during *Drosophila* wing disc development using the fast SMART (somatic mutation and recombination test), as it was used to evaluate potential risks induced by molecules used in the treatment of benign prostate hyperplasia [121].

The advantage of using imaginal discs as models is that they are an easy-to-manipulate epithelial tissue. Inhibition or expression of specific genes in these discs can be done thanks to already known drivers, and phenotypic observation induced by the manipulation is directly visible by microscopy, due to the small size of the tissue, and ultimately avoids classical histological procedures that denature the tissue structure. However, this size aspect makes it difficult to perform molecular analysis, such as protein analysis, which requires a larger amount of tissue. Moreover, the major inconvenience of using imaginal discs is that they are developing tissues, with their specific activations of signaling pathways and gene expression that are inducing strong proliferation, cell migration, and cell differentiation, all processes that are tightly controlled in adult tissues and strongly targeted by the tumorigenic process. Therefore, it is preferable to use other models to validate the observations made in imaginal discs when studying tumorigenesis.

3.3.2. The Tracheal System

Tumor cell growth depends on nutrient and oxygen availability [122]. In *Drosophila*, nutrient transport relies on hemolymph, a circulatory liquid, similar to blood and invertebrate interstitial liquid. Hemolymph propulsion occurs in the entire organism through the heart. The circulatory system of *Drosophila* is open and allows direct exchange of gases and nutrients/cell byproducts between hemolymph and internal organs [123]. For oxygen, *Drosophila* has an additional, sophisticated system of interconnected tubules: the tracheal system, comparable to the circulatory system of mammals [124]. It is a sensor system of oxygen level and metabolic activity and allows adaptation when environmental changes occur. During development, growth and connection of this tracheal system is supported by Fgf expression (breathless, btl), depending on the detection of hypoxia by HIF1 homolog Sima [125,126]. Neo-tracheogenesis, which can be considered as an equivalent of neoangiogenesis, also occurs in *Drosophila* models of tumorigenesis, reportedly when oxygen levels are lowered by high tumor cell metabolism [127,128].

Tube formation is a universal process conserved in multicellular organisms. A large number of adult mammal organs are tubular, for example, the lung, the digestive system, or even the secretory glands, such as the pancreas or the prostate. The tubulogenesis of the tracheal system in *Drosophila* can be used to study the formation and functionality of other tubular organs. For example, cancers, such as lung cancer, have been modeled by the expression of RasV12 and downregulation of PTEN to induce Ras/MAPK and PI3K/AKT signaling in the tracheal network [129,130]. This model has also been used for a drug screen
to identify chemical compounds able to reduce cell proliferation and to improve tracheal physiological functions.

Mechanisms, such as neo-tracheogenesis or tumor growth, imply microenvironment modification with matrix modification. In mammals, this is especially induced by proteins called metalloproteases. These proteins are conserved in Drosophila with two MMP genes described, DmMmp1 and DmMmp2 [131,132]. These two MMPs possess distinct roles. MMP1 is implicated in trachea elongation and regulation of the circadian rhythm [133,134]. We also recently showed that MMP1 expression is associated with neo-tracheogenesis in Drosophila during accessory gland carcinogenesis induced by RasV12 expression [128]. MMP2 is required for the ovulation process and regulation of WNT signaling [135,136]. MMPs also have common roles in the regulation of motoneurons growth, epidermal healing, coagulation, or basal lamina degradation during metamorphosis [137–139]. Because there are two MMPs in Drosophila, compared to the roughly 20 described in mammals, there is less redundancy, and it is easier to analyze their functions in this model by deletion, for example. Moreover, the presence of proteins able to induce modification of the microenvironment and cell adhesion suggests it is used for a more advanced tumorigenic phenomenon [140].

Drosophila can also be used to study the invasive capacities of tumor cells: Tumor transplantation can be done, where a primary tumor from a donor Drosophila is dissected and transplanted to an adult female abdomen. A few days later, the female host is dissected and the presence of tumor cells outside the abdomen in the thorax, head, legs, wings, muscle, brain, intestine, and ovaries will be considered as proof of cell migration capacities [104,119]. Moreover, if tumoral cells are found in ovarioles, this will be a proof of invasive capacities, as cells need to pass through two successive basement membranes and to reactivate MMP expression in order to do so [141].

3.3.3. Use of Drosophila for Organotypic Models

Finally, Drosophila can be used for specific pathology and as organotypic Drosophila cancer models. We now know the importance of the cancer microenvironment and its interactions with the tumor. Some mutations or signaling pathway deregulations can drive cancer development in some tissues but have a very low impact in other ones [142]. This is what makes studies in a specific tissue so important. Of course, the same tissues in different species can also display different sensitivity to specific modifications, but nonetheless, in Drosophila, organotypic models have been established for glioma, colon, and lung cancer [129,143,144].

The gut has been widely used in Drosophila to model pathologies because it is considered as well conserved, with a similar function as mammals’ intestine (food digestion, nutrient absorption, and defense response against infection), as well as a similar structure [145]. In an article from 2016, Drosophila gut allowed for the production of multigenic models using data from The Cancer Genome Atlas, reproducing key features of human colon cancer and allowing drug screens to identify combinatorial therapy on specific genetic modifications [144]. The midgut also includes stem cells that share many characteristics with human intestinal stem cells, and have been used to identify new homeostasis control mechanisms, implicating stem cells that could be relevant in colorectal cancer [146,147].

Brain cancer is also widely studied in Drosophila. Several models have been made displaying different gene alterations: suppression of Brat expression to model glioma [148] and loss of function of lgl (lethal giant larvae) to model neurogenic brain tumors [149].

In addition to these studies on general processes of carcinogenesis, Drosophila can be used specifically for the study of prostate cancer thanks to the accessory glands, the functional equivalent of the prostate.

4. Accessory Glands as a Model of Epithelial Prostate Carcinogenesis

4.1. The Drosophila Accessory Glands, Functional Equivalents of the Prostate

The Drosophila reproductive tract is composed of structures with a similar function to that in men: two seminal vesicles, two testis, one ejaculatory tract, and two accessory
glands [150]. Epithelial cells from the ejaculatory duct, seminal vesicles, and accessory glands secrete and allow for seminal fluid production. Accessory glands are the functional equivalent of the prostate. The main role of these glands is the secretion of proteins constituting the seminal fluid, such as proteases or glycoproteins [151–154], cysteine-rich proteins [155], and lectins [156,157]. As in men, secreted proteins can modulate bacteria resistance and immunity, particularly in the female genital tract [158,159]. In *Drosophila*, proteins such as Sex-peptide (SP or Acp70A, accessory gland protein 70A), Ovulin (Acp26Aa) [160], or CG33943 [161] have additional functions, such as increasing the female egg-laying rate and decreasing the attractiveness of mated females for other males for a few days.

In spite of these similar functions, important differences exist between *Drosophila* and human accessory glands. In *Drosophila*, accessory gland epithelial secretion depends on ecdysone [162], whereas in humans, it depends on testosterone, both of which are steroid hormones. However, ecdysone controls many more processes other than epithelial secretion, and is known as the molting hormone for its major role during the pupal stage of development [163,164]. Furthermore, the ecdysone receptor is more homologous to the liver X receptors than to the androgen receptor [165]. In this regard, it seems difficult to study in *Drosophila* the prostate cancer mechanisms that are directly dependent on androgen receptor signaling. Accessory glands roughly display a similar structure to a human prostate acinus. Each gland is composed of a monolayered epithelium made up of about 1000 cells surrounding a lumen, as compared to multilayered epithelium in humans. The *Drosophila* epithelium is composed of two types of cells: main or primary cells, which are flat and hexagonal, representing 96% of the epithelial secreting cells, and rarer secondary cells (about 4% of the cells), which are spherical and situated mostly at the extremity of the gland [166,167]. These epithelial cells are binucleated, due to incomplete mitosis (without cytokinesis), about 50 h after pupal formation [168]. In humans, the epithelium is mainly composed of two types of epithelial cells: the secretory luminal cells and the less differentiated basal cells. Rare neuroendocrine cells and intermediate epithelial cells are also intercalated between the basal cells. The *Drosophila* epithelial monolayer is surrounded by a thin layer of mononuclear striated muscle cells [169], which is itself enclosed in a basement membrane common to the epithelium [128], and so represents a stroma-like structure enclosing the epithelial compartment. During mating, muscle contraction allows seminal fluid expulsion from the lumen of the accessory gland to the female genital tract [170]. In humans, the fibromuscular stroma also contains endothelial cells, fibroblasts, and immune cells, and both epithelial and stromal compartments also have stem cells to allow for maintenance of the tissues. Epithelial human prostate is composed of three different zones, the peripheral one being the source of most of the cancers. So, morphologically, accessory glands represent a largely simplified version of the prostate, with furthermore a lower cell diversity. This, as for other models, represents both an inconvenience and an advantage: it limits modelization of the complex prostate microenvironment, which is crucial for the evolution of the human pathology. However, it also renders more accessible the interpretation of experiments done with this model. In this regard, it also provides a simple, easy to use in vivo model to study general mechanisms of epithelial tumorigenesis, such as basal extrusion, which is still poorly described due to the scarcity of adequate models to reproduce it experimentally.

Overall, several studies have then shown parallels between *Drosophila* accessory glands and human prostate epithelium, and proven their relevant use to study human prostate pathologies, such as prostate cancer [171–174].

4.2. Secondary Cells to Model Tumor Migration and Progression

In the accessory gland, the secondary cells can migrate by apical delamination, and this ability has been used to do a tissue-specific genetic screen directly in the accessory gland to discover new regulators of human cancer progression that promote growth and migration of secondary cells [171]. After this first screen, the interesting genes were tested
in human prostate cells to confirm their relevance. Moreover, abundant microvesicles are present in these secondary cells, and secreted as exosomes. The accessory gland has been demonstrated as a useful model to study mechanisms regulating these secretions, which should be of interest, considering the importance of exosomes in carcinogenesis [172]. Indeed, these microvesicles secreted in the prostate from the endosomal multivesicular body (MVB) can fuse with sperm to modulate its activity and reinforce its homeostasis [154]. They are implicated in multiple aspects of cancer biology because of their capacity to secrete metabolites and growth factors, ultimately aiding tumor growth. They are also responsible for increased drug resistance by activating mechanisms allowing the elimination of toxic chemicals, such as chemotherapeutic products [175,176]. Moreover, we know that during prostate carcinogenesis, there is a switch from a hormone-dependent to hormone-independent status of prostate cancer cells, and this can lead to CRPC. In Drosophila, this switch from a hormone-dependent to hormone-independent status exists in the secondary cells of accessory glands. A parallel has been made by this model to the hormone-independent status in human prostate cancer progression [173]. Thus, the use of secondary cells in the accessory gland opens many perspectives to decipher the molecular mechanisms implicated in prostate cancer.

4.3. The Drosophila Accessory Glands as a Model for Basal Epithelial Cell Extrusion

Most human cancers present an epithelial origin, such as prostate cancer [85]. A key step in tumorigenesis is the ability of epithelial cells to leave their compartment, allowing the formation of primary tumors, and preceding formal invasion leading to the formation of metastases at distant sites. For this, epithelial cells must cross the basement membrane, a phenomenon known as epithelial basal extrusion. Understanding the mechanisms involved in this key step could help prevent tumor progression and metastasis. As a reminder, the 5-year survival of patients with non-invasive prostate cancer is close to 100% and drops to 30% when prostate cancer has invaded other areas [177]. However, this step is elusive enough, and only rare articles have studied which signaling pathways could be involved in this phenomenon. This is why we recently developed a unique in vivo model of tumorigenesis in the Drosophila accessory glands, allowing the study of basal extrusion [128]. The clonal expression of an oncogene, RasV12, mimicked initiation, and was able to induce a tumorigenic process recapitulating several key features of prostate cancer: cell hyperproliferation and hypertrophy, neo-tracheogenesis facilitating oxygen supply for the tumor cells, and loss of epithelial markers and thus loss of epithelial identity. The latter is a phenomenon notably observed during epithelial-mesenchymal transition (EMT), which is essential in tumorigenesis, and more importantly in basal extrusion. Indeed, the specific shape of the accessory gland allows for easy observation of tumors forming outside the epithelium, following basal extrusion of tumor cells. The use of a large number of animals even allows quantification of this phenomenon. This model has allowed for the more precise description of the role of two major signaling pathways in the initiation of prostate cancer: the RAS/MAPK and PI3K/AKT/TOR pathways. Although these pathways were well known to be deregulated and involved in prostate cancer progression, their involvement in the early phases of tumorigenesis remained poorly understood. We showed that RAS/MAPK and PI3K/AKT/TOR pathways cooperate to induce basal extrusion and thus tumor formation. Their coactivation involves the sequential recruitment of two feedback loops dependent on two growth factors: EGF (Spitz) and IGF (Ilp6), and their respective receptors. These results obtained in Drosophila led to public bioinformatics data analysis and in vitro tests on transformed human prostate cells, validating the possible involvement of the same pathways in early human prostate carcinogenesis.

Finally, due to the almost complete lack of knowledge on basal extrusion and the fact that several hallmarks of cancer are common independently of the origin of this pathology, this model could be of interest for other epithelial cancers.
5. Conclusions

Improving the management of prostate cancer patients requires a better understanding of the players and mechanisms involved at each stage of the disease. For this, studies on in vitro and in vivo models are necessary. Each model, whether cellular, murine, or Drosophila, brings different approaches and different perspectives, making them complementary. In a first approximation, some models seem better suited than others to study specific aspects of prostate cancer biology (see Tables 1 and 2). While cellular models are an excellent first approach for the study of biological processes in which we can easily study molecular interactions, they are still mostly based on 2-D models in which we cannot reproduce human pathology. The 3-D cell models allow better reproduction of the tumor microenvironment by combining the presence of several cell lines and a three-dimensional structure, reproducing essential cellular interactions in human pathology. Moreover, they can be developed directly from patient biopsies, allowing pre-clinical studies to be performed. However, the need to use aggressive cells to obtain them limits their use to the study of late stages of tumorigenesis. Murine models have the advantage of reproducing human pathology with its different stages as well as the interactions between cancer cells, the stroma, and the microenvironment. They are therefore very useful in the study of genes or groups of genes in the tumorigenic process, from initiation to the later stages of tumorigenesis. However, in genetic models of overexpression/deletion, initiation is only imperfectly reproduced as genetic modification occurs in a large proportion of the epithelial cells. When it comes to xenograft models, the tumor microenvironment is not necessarily adequate, especially with an altered immune system. In any case, in mice, the development of tumors can take time, cost a lot of money, and some can argue that a limited number of successful clinical trials have validated this model so far. With the emergence of 3Rs regulation, pressure to decrease the use of such models has increased despite the interest in cancer research. For this, the use of Drosophila provides a new perspective to possibly better understand the mechanisms involved in prostate cancer. Its main strength relies on the fact that it stays an in vivo model in which fundamental cellular processes and signaling pathways are well conserved. It allows for rapid, simple studies thanks to the numerous genetic tools available, and at a lower cost. It also allows for the study of tumorigenic stages that are difficult to study in other models, as it is the case for basal extrusion.

Table 1. Use of different prostate cancer models.

|                         | Prostate Normal Growth and Development | Non Tumorigenic Prostatic Pathologies | Early Prostate Carcinogenesis | Androgen-Insensitive Transition | Late Prostate Cancer (CRPC) and Metastasis | Pre-Clinical Studies |
|-------------------------|----------------------------------------|--------------------------------------|-------------------------------|---------------------------------|--------------------------------------------|---------------------|
| 2-D cell lines          |                                        |                                      |                               |                                 |                                            |                     |
| Untransformed           | RWPE-1, BPH-1, PRNS-1-1, P69            |                                      |                               |                                 |                                            |                     |
| Androgen-sensitive      | LNCaP, LAPC-4, LAPC-9, LuCaP 23.1       |                                      |                               |                                 |                                            |                     |
| Androgen-insensitive    | PC3, DU145                              |                                      |                               |                                 |                                            |                     |
| 3-D models              |                                        |                                      |                               |                                 |                                            |                     |
| Mouse models            | Xenograft                              |                                      |                               |                                 |                                            |                     |
|                          | Genetic models                         |                                      |                               |                                 |                                            |                     |
| Drosophila models       |                                        |                                      |                               |                                 |                                            |                     |

The choice of an appropriate study model is crucial to answer a biological question in a relevant way. Each type of model is represented here with a color code from green to red in order to have an overview of the existing models and their optimal use. Green corresponds to a stage that can be studied in the corresponding model, contrary to red, where the model is not adequate. Orange represents intermediate adequacy of a given model for a given question.
Table 2. Main advantages and disadvantages of the considered models.

|                     | Advantages                                                                 | Disadvantages                                                                 |
|---------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|
| 2-D Cell lines      | - Provide functional and mechanistic insight                                 | - Monolayer culture                                                         |
|                     | - Well characterized                                                         | - Inability to reproduce the pathology                                       |
|                     | - Easy to obtain and manipulate                                               | - Majority of cell lines derived from metastases: limit their use for early prostate cancer |
|                     |                                                                             | - Absence of tumor microenvironment                                         |
| 3-D models          | - Closer to a native tumor                                                   | - Still in development, not a routine procedure                             |
|                     | - Better conservation of heterogeneity                                      | - Remain ex vivo (metastatic studies limitation, limited microenvironment) |
|                     | - Partial tumor microenvironment                                             | - Use of aggressive cells: not adapted for early prostate cancer            |
|                     | - Multilayer culture                                                        |                                                                               |
|                     | - Conservation of cell morphology                                            |                                                                               |
|                     | - Conservation of cell-cell and cell-matrix interactions                     |                                                                               |
| Mouse Xenografts    | - Tumor easily accessible                                                    | - Can take several months to develop a tumor                                |
| models              | - Can be done with benign and malignant tissue (SRC)                        | - Non prostatic microenvironment for subcutaneous and SRC transplantation   |
|                     | - Transplantation in a definite organ, metastatic potential can be evaluated (SRC) | - Only high-grade tissue transplantation for subcutaneous xenograft          |
|                     | - Conserved interactions between implanted tissue and prostate microenvironment (ortho) | - Used of immunodeficient mice                                              |
|                     | - Can generate metastases                                                    | - Loss of prostate tumor heterogeneity                                       |
|                     | - In vivo                                                                    |                                                                               |
|                     | - Conservation of prostate tumor heterogeneity (PDX models)                 |                                                                               |
| Mouse genetic models| - Intact prostate microenvironment                                           | - Use of androgen-dependent promoter                                         |
|                     | - Temporally observation of gene manipulation and drug treatment            | - Differential organization of mouse prostate compared to human             |
|                     | - Preservation of most of histopathological features observed in human pathology | - Tumor development can take several months                                 |
|                     | - In vivo                                                                   | - Limitation of the number of animals that can be used (3Rs).              |
|                     |                                                                             | - Gene redundancy complicating signaling pathways studies                   |
|                     |                                                                             | - Low success of clinical trials emerging from mice                        |
| Drosophila models   | - Short life cycle (10 days at 25 °C)                                       | - Different microenvironment                                                 |
|                     | - Large number of offspring per generation                                  | - Far from mammals: results ought to be confirmed in cell models.           |
|                     | - Well-described anatomy                                                    | - No ortholog of the androgen receptor                                       |
|                     | - Huge amounts of genetic tools available                                   |                                                                               |
|                     | - Few redundancies and good conservation of fundamental biological mechanisms and signaling pathways |                                                                               |
|                     | - Acinus-like organization                                                  |                                                                               |

To conclude, we showed here that the *Drosophila* accessory gland represents a potent new model to modelize prostate tumorigenesis as well as study specific steps of general epithelial tumorigenesis, such as basal extrusion in vivo. It is difficult to summarize, as we tentatively propose in Tables 1 and 2, both the many events associated with tumorigenesis, and the richness of opportunities brought by biological models. We suggest that *Drosophila* will illustrate how new knowledge can be gained in unexpected ways. As said previously, despite having no equivalent of the androgen receptor in this insect, new partners of this receptor were found using the S2 cell line [110]. In the end, the review of the literature indicates that the important thing is to have available the largest panel of models, in the hope of understanding cancer biology in its vast diversity.

**Funding:** This research was funded by Ligue contre le cancer, C. d. J. grant.

**Acknowledgments:** The authors thank James Wilmouth for proofreading the manuscript.
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Rebello, R.J.; Oing, C.; Knudsen, K.E.; Loeb, S.; Johnson, D.C.; Reiter, R.E.; Gillessen, S.; Van der Kwast, T.; Bristow, R.G. Prostate Cancer. Nat. Rev. Dis. Prim. 2021, 7, 1–27. [CrossRef] [PubMed]
2. Grignon, D.J. Unusual Subtypes of Prostate Cancer. Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc. 2004, 17, 316–327. [CrossRef]
3. Behel, C.R.; Faith, D.; Li, X.; Guan, B.; Hicks, J.L.; Lan, F.; Jenkins, R.B.; Bieberich, C.J.; De Marzo, A.M. Decreased NKX3.1 Protein Expression in Focal Prostatic Atrophy, Prostatic Intraepithelial Neoplasia, and Adenocarcinoma: Association with Gleason Score and Chromosome 8p Deletion. Cancer. Res. 2006, 66, 10683–10690. [CrossRef]
4. Abate-Shen, C.; Shen, M.M.; Gelmann, E. Integrating Differentiation and Cancer: The Nkx3.1 Homeobox Gene in Prostate Organogenesis and Carcinogenesis. Differ. Res. Biol. Divers. 2008, 76, 717–727. [CrossRef]
5. Asatiani, E.; Huang, W.-X.; Wang, A.; Rodriguez Ortner, E.; Cavalli, L.R.; Haddad, B.R.; Gelmann, E.P. Deletion, Methylation, and Expression of the NKX3.1 Suppressor Gene in Primary Human Prostate Cancer. Cancer. Res. 2005, 65, 1164–1173. [CrossRef] [PubMed]
6. Albadine, R.; Latour, M.; Toubaji, A.; Haffner, M.; Isaacs, W.B.; Platz, E.A.; Meeker, A.K.; Demarzo, A.M.; Epstein, J.I.; Netto, G.J. TMPRSS2-ERG Gene Fusion Status in Minute (Minimal) Prostatic Adenocarcinoma. Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc. 2009, 22, 1415–1422. [CrossRef]
7. Baca, S.C.; Prandi, D.; Lawrence, M.S.; Mosquera, J.M.; Romanel, A.; Drier, Y.; Park, K.; Kitabayashi, N.; MacDonald, T.Y.; Ghandi, M.; et al. Punctuated Evolution of Prostate Cancer Genomes. Cell 2013, 153, 666–677. [CrossRef]
8. Taylor, B.S.; Schwartz, N.; Hiebremys, H.; Gopalan, A.; Xiao, Y.; Carver, B.S.; Arora, V.K.; Kaushik, P.; Cerami, E.; Reva, B.; et al. Integrative Genomic Profiling of Human Prostate Cancer. Cancer. Cell. 2010, 18, 11–22. [CrossRef] [PubMed]
9. Salmena, L.; Carcacco, A.; Pandolfi, P.P. Tenets of PTEN Tumor Suppression. Cell 2008, 133, 403–414. [CrossRef]
10. Ruscetti, M.; Wu, H.-X. PTEN in prostate cancer. In Prostate Cancer: Biochemistry, Molecular Biology and Genetics; Dehm, S., Tindall, D.J., Eds.; Springer International Publishing: Cham, Switzerland, 2013; pp: 87–137. ISBN 978-1-4614-6827-1.
11. van Dessel, L.F.; van Riet, J.; Smits, M.; Zhu, Y.; Hamberg, P.; van der Heijden, M.S.; Bergman, A.M.; van Oort, I.M.; de Wit, R.; Voest, E.E.; et al. The Genomic Landscape of Metastatic Castration-Resistant Prostate Cancers Reveals Multiple Distinct Genotypes with Potential Clinical Impact. Nat. Commun. 2019, 10, 5251. [CrossRef] [PubMed]
12. Rubin, M.A.; Girelli, G.; Demichelis, F. Genomic Correlates to the Newly Proposed Grading Prognostic Groups for Prostate Cancer. Eur. Urol. 2016, 69, 557–560. [CrossRef] [PubMed]
13. Sobel, R.E.; Sadar, M.D. CELL LINES USED IN PROSTATE CANCER RESEARCH: A COMPRENDIUM OF OLD AND NEW LINES—PART 1. J. Urol. 2005, 173, 342–359. [CrossRef] [PubMed]
14. Sobel, R.E.; Sadar, M.D. Cell Lines Used in Prostate Cancer Research: A Compendium of Old and New Lines—Part 2. J. Urol. 2005, 173, 360–372. [CrossRef] [PubMed]
15. Plymate, S.R.; Tennant, M.; Birnbaum, R.S.; Thrasher, J.B.; Chatta, G.; Ware, J.L. The Effect on the Insulin-like Growth Factor System in Human Prostate Epithelial Cells of Immortalization and Transformation by Simian Virus-40 T Antigen. J. Clin. Endocrinol. Metab. 1996, 81, 3709–3716. [CrossRef] [PubMed]
16. Webber, M.M. Normal and Benign Human Prostatic Epithelial Cell Culture. I. Isolation. In Vitro 1979, 15, 967–982. [CrossRef]
17. Hayward, S.W.; Daihya, R.; Cunha, G.R.; Bartek, J.; Deshpande, N.; Narayan, P. Establishment and Characterization of an Immortalized but Non-Transformed Human Prostate Epithelial Cell Line: BPH-1. In Vitro Cell. Dev. Biol. Anim. 1995, 31, 14–24. [CrossRef] [PubMed]
18. Lee, M.; Garkovenko, E.; Yun, J.; Weijerman, P.; Peehl, D.; Chen, L.; Rhim, J. Characterization of Adult Human Prostatic Epithelial-Cells Immortalized by Polybrene-Induced DNA Transfection with a Plasmid Containing an Origin-Defective SV40-Genome. Int. J. Oncol. 1994, 4, 821–830. [CrossRef]
19. Dent, M.P.; Madnick, S.J.; Hall, S.; Vanantpol Policelli, M.; Bars, C.; Li, H.; Amin, A.; Carmichael, P.L.; Martin, P.L.; Boekelheide, K. A Human-Derived Prostate Co-Culture Microtissue Model Using Epithelial (RWPE-1) and Stromal (WPMM-1) Cell Lines. Toxicol. Vitr. Int. J. Publ. Assoc. IBIRA 2019, 60, 203–211. [CrossRef] [PubMed]
20. McNeal, J.E. Origin and Evolution of Benign Prostatic Enlargement. Invest. Urol. 1978, 15, 340–345. [PubMed]
21. Williams, R.D. Human Urologic Cancer Cell Lines. Invest. Urol. 1980, 17, 359–363. [PubMed]
22. Koochekpour, S.; Maresh, G.A.; Katner, A.; Parker-Johnson, K.; Lee, T.-J.; Hebert, F.E.; Kao, Y.S.; Skinner, J.; Rayford, W. Establishment and Characterization of a Primary Androgen-Responsive African-American Prostate Cancer Cell Line, E006AA. Prostate 2004, 60, 141–152. [CrossRef]
23. Veldscholte, J.; Ris-Stalpers, C.; Kuiper, G.G.; Jenster, G.; Berrevoets, C.; Claassen, E.; van Rooij, H.C.; Trapman, J.; Brinkmann, A.O.; Mulder, E. A Mutation in the Ligand Binding Domain of the Androgen Receptor of Human LNCaP Cells Affects Steroid Binding Characteristics and Response to Anti-Androgens. Biochem. Biophys. Res. Commun. 1990, 173, 534–540. [CrossRef]
24. Klein, K.A.; Reiter, R.E.; Redula, J.; Moradi, H.; Zhu, X.L.; Brothman, A.R.; Lamb, D.J.; Marcelli, M.; Beldegrun, A.; Witte, O.N.; et al. Progression of Metastatic Human Prostate Cancer to Androgen Independence in Immunodeficient SCID Mice. Nat. Med. 1997, 3, 402–408. [CrossRef] [PubMed]
Cells 2021, 10, 2387

25. Craft, N.; Chhor, C.; Tran, C.; Belledegrün, A.; DeKernion, J.; Witte, O.N.; Said, J.; Reiter, R.E.; Sawyers, C.L. Evidence for Clonal Outgrowth of Androgen-Independent Prostate Cancer Cells from Androgen-Dependent Tumors through a Two-Step Process. Cancer Res. 1999, 59, 5030–5036. [PubMed]

26. Whang, Y.E.; Wu, X.; Suzuki, H.; Reiter, R.E.; Tran, C.; Vessella, R.L.; Said, J.W.; Isaacs, W.B.; Sawyers, C.L. Inactivation of the Tumor Suppressor PTEN/MMAC1 in Advanced Human Prostate Cancer through Loss of Expression. Proc. Natl. Acad. Sci. USA 1998, 95, 5246–5250. [CrossRef]

27. Kailg, M.E.; Narayan, K.S.; Ohnuki, Y.; Lechner, J.F.; Jones, L.W. Establishment and Characterization of a Human Prostatic Carcinoma Cell Line (PC-3). Invest. Urol. 1979, 17, 16–23.

28. Stone, K.R.; Mickey, D.D.; Wunderli, H.; Mickey, G.H.; Paulson, D.F. Isolation of a Human Prostate Carcinoma Cell Line (DU 145). Int. J. Cancer. 1978, 21, 271–281. [CrossRef]

29. Mickey, D.D.; Stone, K.R.; Wunderli, H.; Mickey, G.H.; Vollmer, R.T.; Paulson, D.F. Heterotransplantation of a Human Prostatic Adenocarcinoma Cell Line in Nude Mice. Cancer Res. 1977, 37, 4049–4058. [PubMed]

30. Lange, T.; Ullrich, S.; Müller, L.; Nentwich, M.F.; Stübke, K.; Feldhaus, S.; Knie, C.; Hellwing, O.J.C.; Vessella, R.L.; Abramjuk, C.; et al. Human Prostate Cancer in a Clinically Relevant Xenograft Mouse Model: Identification of β(1,6)-Linked Oligosaccharides as a Marker of Tumor Progression. Clin. Cancer Res. Off. J. Am. Assoc. Cancer. Res. 2012, 18, 1364–1373. [CrossRef]

31. Buchanan, G.; Greenberg, N.M.; Scher, H.I.; Harris, J.M.; Marshall, V.R.; Tilley, W.D. Collocation of Androgen Receptor Gene Mutations in Prostate Cancer. Clin. Cancer Res. Off. J. Am. Assoc. Cancer. Res. 2001, 7, 1273–1281.

32. Sharifi, N. Mechanisms of Androgen Receptor Activation in Castration-Resistant Prostate Cancer. Endocrinology 2013, 154, 4010–4017. [CrossRef]

33. Pfitzenmaier, J.; Quinn, J.E.; Odman, A.M.; Zhang, J.; Keller, E.T.; Vessella, R.L.; Corey, E. Characterization of C4-2 Prostate Cancer Bone Metastases and Their Response to Castration. J. Bone Miner. Res. Off. J. Am. Soc. Bone. Miner. Res. 2003, 18, 1882–1888. [CrossRef] [PubMed]

34. Thalmann, G.N.; Anezinis, P.E.; Chang, S.M.; Zhou, H.E.; Kim, E.E.; Hopwood, V.L.; Pathak, S.; von Eschenbach, A.C.; Chung, L.W. Androgen-Independent Cancer Progression and Bone Metastasis in the LNCaP Model of Human Prostate Cancer. Cancer Res. 1994, 54, 2577–2581. [PubMed]

35. Wu, H.C.; Hsieh, J.T.; Gleave, M.E.; Brown, N.M.; Pathak, S.; Chung, L.W. Derivation of Androgen-Independent Human LNCaP Prostatic Cancer Cell Sublines: Role of Bone Stromal Cells. Int. J. Cancer. 1994, 57, 406–412. [CrossRef]

36. Thalmann, G.N.; Sikes, R.A.; Wu, T.T.; Degeorges, A.; Chang, S.-M.; Ozen, M.; Pathak, S.; Chung, L.W.K. LNCaP Progression Model of Human Prostate Cancer: Androgen-Independence and Ossseous Metastasis. Prostate 2000, 44, 91–103. [CrossRef]

37. Pijuan, J.; Barceló, C.; Moreno, D.F.; Maiques, O.; Sisò, P.; Martí, R.M.; Macià, A.; Panosa, A. In Vitro Cell Migration, Invasion, and Adhesion Assays: From Cell Imaging to Data Analysis. Front. Cell. Dev. Biol. 2019, 0. [CrossRef] [PubMed]

38. Thienger, P.; Rubin, M.A. Prostate Cancer Hijacks the Microenvironment. Nat. Cell. Biol. 2021, 23, 3–5. [CrossRef] [PubMed]

39. Bahmad, H.F.; Jalloul, M.; Azar, J.; Moubarak, M.M.; Samad, T.A.; Mukherji, D.; Al-Sayegh, M.; Abou-Kheir, W. Tumor Microenvironment in Prostate Cancer: Toward Identification of Novel Molecular Biomarkers for Diagnosis, Prognosis, and Therapy Development. Front. Genet. 2021, 0. [CrossRef] [PubMed]

40. Gunti, S.; Hoke, A.T.K.; Vu, K.P.; London, N.R. Organoid and Spheroid Tumor Models: Techniques and Applications. Cancers 2021, 13, 874. [CrossRef] [PubMed]

41. Sato, T.; Stange, D.E.; Ferrante, M.; Vries, R.G.J.; Van Es, J.H.; Van den Brink, S.; Van Houdt, W.J.; Pronk, A.; Van Gorp, J.; Siersema, P.D.; et al. Long-Term Expansion of Epithelial Organoids from Human Colon, Adenocarcinoma, and Barrett’s Epithelium. Gastroenterology 2011, 141, 1762–1772. [CrossRef]

42. van de Wetering, M.; Francies, H.E.; Francis, J.M.; Bounova, G.; Iorio, F.; Pronk, A.; van Houdt, W.; van Gorp, J.; Taylor-Weiner, A.; Kester, L.; et al. Prospective Derivation of a Living Organoid Biobank of Colorectal Cancer Patients. Cell 2015, 161, 933–945. [CrossRef] [PubMed]

43. Boj, S.F.; Hwang, C.-J.; Baker, L.A.; Chio, I.I.C.; Engle, D.D.; Corbo, V.; Jager, M.; Ponz-Sarvise, M.; Tiiriac, H.; Spector, M.S.; et al. Organoid Models of Human and Mouse Ductal Pancreatic Cancer. Cell 2015, 160, 324–338. [CrossRef] [PubMed]

44. Sachs, N.; de Ligt, J.; Kopper, O.; Gogola, E.; Bounova, G.; Weeber, F.; Balgobind, A.V.; Wind, K.; Gracanin, A.; Begthel, H.; et al. A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. Cell 2018, 172, 373–386.e10. [CrossRef]

45. Sachs, N.; Papasyropoulos, A.; Zomer-van Ommen, D.D.; Heo, I.; Böttiger, L.; Klay, D.; Weeber, F.; Huelsz-Prince, G.; Jakobchavilli, N.; Amatngalim, G.D.; et al. Long-Term Expanding Human Airway Organoids for Disease Modeling. EMBO J. 2019, 38. [CrossRef]

46. Gao, D.; Vela, I.; Storer, A.; Iaquinta, P.J.; Karthaus, W.R.; Gopalan, A.; Dowling, C.; Wanjala, J.N.; Undvall, E.A.; Arora, V.K.; et al. Organoid Cultures Derived from Patients with Advanced Prostate Cancer. Cell 2014, 159, 176–187. [CrossRef] [PubMed]

47. Puca, L.; Bareja, R.; Prandi, D.; Shaw, R.; Benelli, M.; Karthaus, W.R.; Hess, J.; Sigourous, M.; Donoghue, A.; Kossai, M.; et al. Patient Derived Organoids to Model Rare Prostate Cancer Phenotypes. Nat. Commun. 2018, 9, 2404. [CrossRef]

48. van Weerden, W.M.; Romijn, J.C. Use of Nude Mouse Xenograft Models in Prostate Cancer Research. Prostate 2000, 43, 263–271. [CrossRef]

49. Stack, A.; Kassis, A.P.; Olshen, A.; Wang, Y.; Wu, D.; Carroll, P.R.; Grossfeld, G.D.; Cunha, G.R.; Hayward, S.W. Quantitation of Apoptotic Activity Following Castration in Human Prostatic Tissue in Vivo. Prostate 2003, 54, 212–219. [CrossRef] [PubMed]
50. Wang, Y.; Revelo, M.P.; Sudilovsky, D.; Cao, M.; Chen, W.G.; Goetz, L.; Xue, H.; Sadar, M.; Shappell, S.B.; Cunha, G.R.; et al. Development and Characterization of Efficient Xenograft Models for Benign and Malignant Human Prostate Tissue. *Prostate* 2005, 64, 149–159. [CrossRef] [PubMed]

51. Rembrink, K.; Romijn, J.C.; van der Kwast, T.H.; Rübben, H.; Schröder, F.H. Orthotopic Implantation of Human Prostate Cancer Cell Lines: A Clinically Relevant Animal Model for Metastatic Prostate Cancer. *Prostate* 1997, 31, 168–174. [CrossRef]

52. An, Z.; Wang, X.; Geller, J.; Moossa, A.R.; Hoffman, R.M. Surgical Orthotopic Implantation Allows High Lung and Lymph Node Metastatic Expression of Human Prostate Carcinoma Cell Line PC-3 in Nude Mice. *Prostate* 1998, 34, 169–174. [CrossRef]

53. Zhao, S.G.; Lehrer, J.; Chang, S.L.; Das, R.; Erho, N.; Liu, Y.; Sjöström, M.; Den, R.B.; Freedland, S.J.; Klein, E.A.; et al. The Immune Landscape of Prostate Cancer and Nomination of PD-L2 as a Potential Therapeutic Target. *JNCI J. Natl. Cancer. Inst.* 2019, 111, 301–310. [CrossRef]

54. Stultz, J.; Fong, L. How to Turn up the Heat on the Cold Immune Microenvironment of Metastatic Prostate Cancer. *Prostate. Cancer. Prostatic. Dis.* 2021, 1–21. [CrossRef]

55. Roussos, L.; Septier, A.; Bunay, J.; Voisin, A.; Guiton, R.; Damon-Soubeyrant, C.; Renaud, Y.; Haze, A.D.; Sapin, V.; Fogli, A.; et al. Absence of Nuclear Receptors LXRs Impairs Immune Response to Androgen Deprivation and Leads to Prostate Neoplasia. *PLoS. Biol.* 2020, 18, e3000948. [CrossRef] [PubMed]

56. Gonzalez, H.; Hagerling, C.; Werb, Z. Roles of the Immune System in Cancer: From Tumor Initiation to Metastatic Progression. *Genes. Dev.* 2018, 32, 1267–1284. [CrossRef] [PubMed]

57. Nguyen, H.M.; Vessella, R.L.; Morrisey, C.; Brown, L.G.; Coleman, I.M.; Higano, C.S.; Mostaghel, E.A.; Zhang, X.; True, L.D.; Lam, H.-M.; et al. LuCaP Prostate Cancer Patient-Derived Xenografts Reflect the Molecular Heterogeneity of Advanced Disease and Serve as Models for Evaluating Cancer Therapeutics. *Prostate 2017*, 77, 654–671. [CrossRef] [PubMed]

58. Navone, N.M.; van Weerden, W.M.; Vessella, R.L.; Williams, E.D.; Wang, Y.; Isaacs, J.T.; Nguyen, H.M.; Culig, Z.; van der Pluijm, G.; Rentsch, C.A.; et al. November GAP1 PDX Project: An International Collection of Serially Transplantable Prostate Cancer Patient-Derived Xenograft (PDX) Models. *Prostate 2018*, 78, 1262–1282. [CrossRef] [PubMed]

59. McNeal, J.E. Normal Histology of the Prostate. *Am. J. Surg. Pathol.* 1988, 12, 619–633. [CrossRef] [PubMed]

60. Shappell, S.B.; Thomas, G.V.; Roberts, R.L.; Herbert, R.; Ittmann, M.M.; Rubin, M.A.; Humphrey, P.A.; Sundberg, J.P.; Rozengurt, N.; Barrios, R.; et al. Prostate Pathology of Genetically Engineered Mice: Definitions and Classification. The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer. Res.* 2004, 64, 2270–2305. [CrossRef]

61. Berquin, I.M.; Min, Y.; Wu, R.; Wu, H.; Chen, Y.Q. Expression Signature of the Mouse Prostate*. J. Biol. Chem.* 2005, 280, 36442–36451. [CrossRef] [PubMed]

62. Abbott, D.E.; Pritchard, C.; Clegg, N.J.; Ferguson, C.; Dumpit, R.; Sikes, R.A.; Nelson, P.S. Expressed Sequence Tag Profiling Identifies Developmental and Anatomic Partitioning of Gene Expression in the Mouse Prostate. *Genome. Biol.* 2003, 4, R79. [CrossRef] [PubMed]

63. Roy-Burman, P.; Wu, H.; Powell, W.C.; Hagenkord, J.; Cohen, M.B. Genetically Defined Mouse Models That Mimic Natural Aspects of Human Prostate Cancer Development. *Endocr. Relat. Cancer.* 2004, 11, 225–254. [CrossRef] [PubMed]

64. Abremski, K.; Hoess, R. Bacteriophage P1 Site-Specific Recombination. Purification and Properties of the Cre Recombinase Protein. *J. Biol. Chem.* 1984, 259, 1509–1514. [CrossRef]

65. Wang, S.; Gao, J.; Li, Q.; Rozengurt, N.; Pritchard, C.; Jiao, J.; Thomas, G.V.; Li, G.; Roy-Burman, P.; Nelson, P.S.; et al. Prostate-Specific Deletion of the Murine Pten Tumor Suppressor Gene Leads to Metastatic Prostate Cancer. *Cancer. Cell* 2003, 4, 209–221. [CrossRef]

66. Kulkarni, M.K.; Johnson, D.T.; Zhu, C.; Lee, S.H.; Ye, D.W.; Luong, R.; Sun, Z. Conditional Deletion of the Pten Gene in the Mouse Prostate Induces Prostatic Intraepithelial Neoplasms at Early Ages but a Slow Progression to Prostate Tumors. *PloS ONE* 2013, 8, e53476. [CrossRef] [PubMed]

67. Di Cristofano, A.; De Acetis, M.; Koff, A.; Cordon-Cardo, C.; Pandolfi, P.P. Pten and P27KIP1 Cooperate in Prostate Cancer Tumor Suppression in the Mouse. *Nat. Genet.* 2001, 27, 222–224. [CrossRef] [PubMed]

68. Kim, M.J.; Cardiff, R.D.; Desai, N.; Banach-Petrosky, W.A.; Parsons, R.; Shen, M.M.; Abate-Shen, C. Cooperativity of Nkx3.1 and Pten Loss of Function in a Mouse Model of Prostate Carcinogenesis. *Proc. Natl. Acad. Sci. USA.* 2002, 99, 2884–2889. [CrossRef]

69. Mulholland, D.J.; Kobayashi, N.; Russetti, M.; Zhi, A.; Tran, L.M.; Huang, J.; Gleave, M.; Wu, H. Pten Loss and RAS/RAF MAPK Activation Cooperate to Promote EMT and Metastasis Initiated from Prostate Cancer Stem/Progenitor Cells. *Cancer Res.* 2012, 72, 1878–1889. [CrossRef]

70. Hubbard, G.K.; Mutton, L.N.; Khalili, M.; McMullin, R.P.; Hicks, J.L.; Bianchi-Frias, D.; Horn, L.A.; Kulac, I.; Moubarek, M.S.; Nelson, P.S.; et al. Combined MYC Activation and Pten Loss Are Sufficient to Create Genomic Instability and Lethal Metastatic Prostate Cancer. *Cancer. Res.* 2016, 76, 283–292. [CrossRef] [PubMed]

71. Hurwitz, A.A.; Foster, B.A.; Allison, J.P.; Greenberg, N.M.; Kwon, E.D. The TRAMP Mouse as a Model for Prostate Cancer. *Curr. Protoc. Immunol.* 2001, 45, 20.5. [CrossRef] [PubMed]

72. Bernards, A.; Hariharan, I.K. Of Flies and Men—Studying Human Disease in Drosophila. *Curr. Opin. Genet. Dev.* 2001, 11, 274–278. [CrossRef]

73. Bier, E. Drosophila, the Golden Bug. Emerges as a Tool for Human Genetics. *Nat. Rev. Genet.* 2005, 6, 9–23. [CrossRef] [PubMed]
74. Millburn, G.H.; Crosby, M.A.; Gramates, L.S.; Tweedie, S. FlyBase Consortium FlyBase Portals to Human Disease Research Using Drosophila Models. *Dis. Model. Mech.* 2016, 9, 245–252. [CrossRef] [PubMed]

75. Perrimon, N.; Pitsouli, C.; Shiolo, B.-Z. Signaling Mechanisms Controlling Cell Fate and Embryonic Patterning. *Cold Spring Harb. Perspect. Biol.* 2012, 4. [CrossRef]

76. Patel, P.H.; Edgar, B.A. Tissue Design: How Drosophila Tumors Remodel Their Neighborhood. *Semin. Cell Dev. Biol.* 2014, 28, 86–95. [CrossRef]

77. Gateff, E. Malignant Neoplasms of Genetic Origin in Drosophila Melanogaster. *Science* 1978, 200, 1448–1459. [CrossRef] [PubMed]

78. Gateff, E.; Schneiderman, H.A. Neoplasms in Mutant and Cultured Wild-Type Tissues of Drosophila. *Natl. Cancer. Inst. Monogr.* 1969, 31, 365–397.

79. Bilder, D.; Li, M.; Perrimon, N. Cooperative Regulation of Cell Polarity and Growth by Drosophila Tumor Suppressors. *Science* 2000, 289, 113–116. [CrossRef]

80. McCartney, B.M.; Kulikauskas, R.M.; LaJeunesse, D.R.; Fehon, R.G. The Neurofibromatosis-2 Homologue, Merlin, and the Tumor Suppressor Expanded Function Together in Drosophila to Regulate Cell Proliferation and Differentiation. *Dev. Camb. Engl.* 2000, 127, 1315–1324.

81. Gardiol, D.; Zacchi, A.; Petera, F.; Stanta, G.; Banks, L. Human Discs Large and Scrib Are Localized at the Same Regions in Colon Mucosa and Changes in Their Expression Patterns Are Correlated with Loss of Tissue Architecture during Malignant Progression. *Int. J. Cancer* 2006, 119, 1285–1290. [CrossRef]

82. Hay, B.A.; Guo, M. Coupling Cell Growth, Proliferation, and Death. Hippo Weighs In. *Nature* 2011, 472, 480–487. [CrossRef] [PubMed]

83. Logsdon, M.; Edgar, B.A. Tissue Design: How Drosophila Tumors Remodel Their Neighborhood. *Semin. Cell Dev. Biol.* 2014, 28, 86–95. [CrossRef]

84. Hanahan, D.; Weinberg, R.A. Hallmarks of Cancer: The next Generation. *Cell* 2011, 144, 646–674. [CrossRef] [PubMed]

85. McGuire, S.E.; Mao, Z.; Davis, R.L. Spatiotemporal Gene Expression Targeting with the TARGET and Gene-Switch Systems in Drosophila. *Dev. Camb. Engl.* 1993, 118, 401–415.

86. Xu, T.; Rubin, G.M. Analysis of Genetic Mosaics in Developing and Adult Drosophila Tissues. *Dev. Camb. Engl.* 1993, 117, 1223–1237.

87. Lee, T.; Luo, L. Mosaic Analysis with a Repressible Cell Marker for Studies of Gene Function in Neuronal Morphogenesis. *Cell* 1993, 75, 103–112. [CrossRef]

88. Golic, K.G.; Lindquist, S. The FLP Recombinase of Yeast Catalyzes Site-Specific Recombination in the Drosophila Genome. *Cell* 1989, 59, 499–509. [CrossRef]

89. Xu, T.; Rubin, G.M. Analysis of Genetic Mosaics in Developing and Adult Drosophila Tissues. *Dev. Camb. Engl.* 1993, 117, 1223–1237.

90. Neto-Silva, R.M.; de Beco, S.; Johnston, L.A. Evidence for a Growth-Stabilizing Regulatory Feedback Mechanism between Myc and Yorkie, the Drosophila Homolog of Yap. *EMBO J.* 2014, 33, 996–1006. [CrossRef] [PubMed]

91. Neto-Silva, R.M.; de Beco, S.; Johnston, L.A. Evidence for a Growth-Stabilizing Regulatory Feedback Mechanism between Myc and Yorkie, the Drosophila Homolog of Yap. *EMBO J.* 2014, 33, 996–1006. [CrossRef] [PubMed]

92. Gateff, E.; Schneiderman, H.A. Neoplasms in Mutant and Cultured Wild-Type Tissues of Drosophila. *Natl. Cancer. Inst. Monogr.* 1969, 31, 365–397.

93. Poulsen, D.F. Chromosomal Control of Embryogenesis in Drosophila. *Biol. Reproduc.* 1950, 168–274.

94. Poulsen, D.F. Chromosomal Control of Embryogenesis in Drosophila. *Am. Nat.* 1945, 79, 340–363. [CrossRef]

95. Poulsen, D.F. Histogenesis, Organogenesis, and Differentiation in the Embryo of Drosophila Melanogaster Meigen. *Biol. Reproduc.* 1950, 168–274.

96. Gateff, E.; Schneiderman, H.A. Neoplasms in Mutant and Cultured Wild-Type Tissues of Drosophila. *Natl. Cancer. Inst. Monogr.* 1969, 31, 365–397.

97. Miller, G.H.; Crosby, M.A.; Gramates, L.S.; Tweedie, S. FlyBase Consortium FlyBase Portals to Human Disease Research Using Drosophila Models. *Dis. Model. Mech.* 2016, 9, 245–252. [CrossRef] [PubMed]

98. Coffey, K. Targeting the Hippo Pathway in Prostate Cancer: What’s New? *Cancers* 2021, 13, 611. [CrossRef]

99. Neto-Silva, R.M.; de Beco, S.; Johnston, L.A. Evidence for a Growth-Stabilizing Regulatory Feedback Mechanism between Myc and Yorkie, the Drosophila Homolog of Yap. *Dev. Cell* 2010, 19, 507–520. [CrossRef]

100. Coffey, K. Targeting the Hippo Pathway in Prostate Cancer: What’s New? *Cancers* 2021, 13, 611. [CrossRef]

101. Neto-Silva, R.M.; de Beco, S.; Johnston, L.A. Evidence for a Growth-Stabilizing Regulatory Feedback Mechanism between Myc and Yorkie, the Drosophila Homolog of Yap. *Dev. Cell* 2010, 19, 507–520. [CrossRef]

102. Neto-Silva, R.M.; de Beco, S.; Johnston, L.A. Evidence for a Growth-Stabilizing Regulatory Feedback Mechanism between Myc and Yorkie, the Drosophila Homolog of Yap. *Dev. Cell* 2010, 19, 507–520. [CrossRef]

103. Gurel, B.; Iwata, T.; Koh, C.M.; Jenkins, R.B.; Lan, F.; Van Dang, C.; Hicks, J.L.; Morgan, J.; Cornish, T.C.; Sutcliffe, S.; et al. Nuclear MYC Protein Overexpression is an Early Alteration in Human Prostate Carcinogenesis. *Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc* 2008, 21, 1156–1167. [CrossRef] [PubMed]

104. Woodhouse, E.; Hersperger, E.; Shearn, A. Growth, Metastasis, and Invasiveness of Drosophila Tumors Caused by Mutations in Specific Tumor Suppressor Genes. *Dev. Genes Evol.* 1998, 207, 542–550. [CrossRef] [PubMed]
Grzeschik, N.A.; Parsons, L.M.; Allott, M.L.; Harvey, K.F.; Richardson, H.E. Lgl, APKC, and Crumbs Regulate the Salvador/Warts/Hippo Pathway through Two Distinct Mechanisms. *Curr. Biol. CB* **2010**, *20*, 573–581. [CrossRef]

Parsons, L.M.; Grzeschik, N.A.; Richardson, H.E. Lgl Regulates the Hippo Pathway Independently of Fat/Dachs, Ki- bbra/Expanded/Merlin and DRASSF/DSTRIPAK. *Cancers* **2014**, *6*, 879–896. [CrossRef]

Grifoni, D.; Garoia, F.; Schimanski, C.C.; Schmitz, G.; Laurenti, E.; Galle, P.R.; Pession, A.; Cavicchi, S.; Strand, D. The Human Protein Hugl-1 Substitutes for Drosophila Lethal Giant Larvae Tumour Suppressor Function in Vivo. *OncoGene* **2004**, *23*, 8688–8694. [CrossRef]

Bowers, A.J.; Scully, S.; Boylan, J.F. SKIP3, a Novel Drosophila Tribbles Ortholog, Is Overexpressed in Human Tumors and Is Regulated by Hypoxia. *OncoGene* **2003**, *22*, 2823–2835. [CrossRef]

Datta, M.W.; Hernandez, A.M.; Schlicht, M.J.; Kahler, A.J.; de Gueme, A.M.; Dhir, R.; Shah, R.B.; Farach-Carson, C.; Barrett, A.; Datta, S. Perlecan, a Candidate Gene for the CAPB Locus, Regulates Prostate Cancer Cell Growth via the Sonic Hedgehog Pathway. *Mol. Cancer* **2006**, *5*, 9. [CrossRef] [PubMed]

Imberger-Kazdan, K.; Ha, S.; Greenfield, A.; Poultnay, C.S.; Bonneau, R.; Logan, S.K.; Carabedian, M.J. A Genome-Wide RNA Interference Screen Identifies New Regulators of Androgen Receptor Function in Prostate Cancer Cells. *Genome. Res.* **2013**, *23*, 581–591. [CrossRef]

Herranz, H.; Eichenlaub, T.; Cohen, S.M. Cancer in Drosophila: Imaginal Discs as a Model for Epithelial Tumor Formation. *Curr. Top. Dev. Biol.* **2016**, *116*, 181–199. [CrossRef]

Muzzopappa, M.; Murcia, L.; Milán, M. Feedback Amplification Loop Drives Malignant Growth in Epithelial Tissues. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E7291–E7300. [CrossRef]

Hadorn, E. Transdetermination in Cells. *Sci. Am.* **2002**, *287*, 115. [CrossRef]

Hadorn, E.; Gsell, R.; Schultz, J. Stability of a Position-Effect Variegation in Normal and Transdetermined Larval Blastemas from Drosophila Melanogaster. *Proc. Natl. Acad. Sci. USA* **1970**, *65*, 633–637. [CrossRef]

Gladstone, M.; Su, T.T. Chemical Genetics and Drug Screening in Drosophila Cancer Models. *J. Genet. Genom. Yi Chuan Xue Bao* **2011**, *38*, 497–504. [CrossRef]

Gao, G.; Chen, L.; Huang, C. Anti-Cancer Drug Discovery: Update and Comparisons in Yeast, Drosophila, and Zebrafish. *Curr. Mol. Pharmacol.* **2014**, *7*, 44–51. [CrossRef]

Alajati, A.; D’Ambrosio, M.; Troiani, M.; Mosole, S.; Pellegrini, L.; Chen, J.; Revandkar, A.; Bolis, M.; Theurillat, J.-P.; Guccini, I.; et al. CDCP1 Overexpression Drives Prostate Cancer Progression and Can Be Targeted in Vivo. *J. Clin. Invest.* **2020**, *130*, 2435–2450. [CrossRef]

Tipping, M.; Perrimon, N. Drosophila as a Model for Context-Dependent Tumorigenesis. *J. Cell. Physiol.* **2014**, *229*, 27–33. [CrossRef]

Pagliarini, R.A.; Xu, T. A Genetic Screen in Drosophila for Metastatic Behavior. *Science* **2003**, *302*, 1227–1231. [CrossRef]

Fu, S.; Yang, Y.; Tirtha, D.; Yen, Y.; Zhou, B.; Zhou, M.-M.; Xu, D.; Yu, X.; Tian, Y. Advanced Research on Vasculogenic Mimicry in Cancer. *Sci. Rep.* **2015**, *5*, 9061. [CrossRef]

Medioni, C.; Sénatore, S.; Salmand, P.-A.; Lalevée, N.; Perrin, L.; Séméria, M. The Fabulous Destiny of the Drosophila Heart. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 6161–6166. [CrossRef] [PubMed]

Grifoni, D.; Sollazzo, M.; Fontana, E.; Froldi, F.; Pession, A. Multiple Strategies of Oxygen Supply in Drosophila Malignancies Identify Tracheogenesis as a Novel Cancer Hallmark. *J. Appl. Toxicol.* **2013**, *33*, 209–213. [CrossRef]

Datta, M.W.; Hernandez, A.M.; Schlicht, M.J.; Kahler, A.J.; DeGueme, A.M.; Dhir, R.; Shah, R.B.; Farach-Carson, C.; Barrett, A.; Datta, S. Perlecan, a Candidate Gene for the CAPB Locus, Regulates Prostate Cancer Cell Growth via the Sonic Hedgehog Pathway. *Mol. Cancer* **2006**, *5*, 9. [CrossRef] [PubMed]

Gao, G.; Chen, L.; Huang, C. Anti-Cancer Drug Discovery: Update and Comparisons in Yeast, Drosophila, and Zebrafish. *Curr. Mol. Pharmacol.* **2014**, *7*, 44–51. [CrossRef]

Medioni, C.; Sénatore, S.; Salmand, P.-A.; Lalevée, N.; Perrin, L.; Séméria, M. The Fabulous Destiny of the Drosophila Heart. *Curr. Opin. Genet. Dev.* **2009**, *19*, 518–525. [CrossRef]

Grigioni, D.; Sollazzo, M.; Fontana, E.; Froldi, F.; Pession, A. Multiple Strategies of Oxygen Supply in Drosophila Malignancies Identify Tracheogenesis as a Novel Cancer Hallmark. *Sci. Rep.* **2015**, *5*, 9061. [CrossRef]

Rambur, A.; Lours-Calet, C.; Beaudoin, C.; Buñay, J.; Vialat, M.; Mirouse, V.; Trousson, A.; Renaud, Y.; Lobaccaro, J.-M.A.; Baron, S.; et al. Sequential Ras/ERK and PI3K/AKT/MTOR Pathways Recruitment Drives Basal Extrusion in the Prostate-like Gland of Drosophila. *Nat. Commun.* **2020**, *11*, 2300. [CrossRef]

Levine, B.D.; Cagan, R.L. Drosophila Lung Cancer Models Identify Trametinib plus Statin as Candidate Therapeutic. *Cell. Rep.* **2016**, *14*, 1477–1487. [CrossRef]

Andrew, D.J.; Ewald, A.J. Morphogenesis of Epithelial Tubes: Insights into Tube Formation, Elongation, and Elaboration. *Dev. Biol.* **2010**, *341*, 34–55. [CrossRef]

Llano, E.; Adam, G.; Pendás, A.M.; Quesada, V.; Sánchez, L.M.; Santamaria, I.; Noselli, S.; López-Otin, C. Structural and Enzymatic Characterization of Drosophila Dm2-MMP, a Membrane-Bound Metalloproteinase with Tissue-Specific Expression. *J. Biol. Chem.* **2002**, *277*, 23321–23329. [CrossRef]
132. Page-Mccaw, A.; Serano, J.; Santé, J.M.; Rubin, G.M. Drosophila Matrix Metalloproteinases Are Required for Tissue Remodeling, but Not Embryonic Development. Dev. Cell 2003, 4, 95–106. [CrossRef]
133. Glasheen, B.M.; Robbins, R.M.; Pieten, C.; Beitel, G.J.; Page-Mccaw, A. A Matrix Metalloproteinase Mediates Airway Remodeling in Drosophila. Dev. Biol. 2010, 344, 772–783. [CrossRef]
134. Depetriz-Chauvin, A.; Fernandez-Gamba, A.; Gorostiza, E.A.; Herrera, A.; Castaño, E.M.; Ceriani, M.F. Mmp1 Processing of the PDF Neuropeptide Regulates Circadian Structural Plasticity of Pacemaker Neurons. PLoS Genet. 2014, 10, e1004700. [CrossRef]
135. Wang, X.; Page-Mccaw, A. A Matrix Metalloproteinase Mediates Long-Distance Attenuation of Stem Cell Proliferation. J. Cell Biol. 2014, 206, 923–936. [CrossRef]
136. Deady, L.D.; Shen, W.; Mosure, S.A.; Spradling, A.C.; Sun, J. Matrix Metalloproteinase 2 Is Required for Ovulation and Corpus Luteum Formation in Drosophila. PLoS Genet. 2015, 11, e1004989. [CrossRef]
137. Miller, C.M.; Page-Mccaw, A.; Brohier, H.T. Matrix Metalloproteinases Promote Motor Axon Fasciculation in the Drosophila Embryo. Dev. Camb. Biol. 2008, 135, 95–109. [CrossRef] [PubMed]
138. Srivastava, A.; Pastor-Pareja, J.C.; Igaki, T.; Pagliarini, R.; Xu, T. Basement Membrane Remodeling Is Essential for Drosophila Disc Eversion and Tumor Invasion. Proc. Natl. Acad. Sci. USA 2007, 104, 2721–2726. [CrossRef]
139. Stevens, L.J.; Page-Mccaw, A. A Secreted MMP Is Required for Reepithelialization during Wound Healing. Mol. Biol. Cell 2012, 23, 1068–1079. [CrossRef]
140. Page-Mccaw, A. Remodeling the Model Organism: Matrix Metalloproteinase Functions in Invertebrates. Semin. Cell Dev. Biol. 2008, 19, 14–23. [CrossRef]
141. Miles, W.O.; Dyson, N.J.; Walker, J.A. Modeling Tumor Invasion and Metastasis in Drosophila. Dis. Model. Mech. 2011, 4, 753–761. [CrossRef]
142. Torkamani, A.; Schork, N.J. Identification of Rare Cancer Driver Mutations by Network Reconstruction. Genome. Res. 2009, 19, 1570–1578. [CrossRef]
143. Read, R.D.; Cavenee, W.K.; Furnari, F.B.; Thomas, J.B. A Drosophila Model for EGFR-Ras and PI3K-Dependent Human Glioma. PLoS Genet. 2009, 5, e1000574. [CrossRef] [PubMed]
144. Bangi, E.; Murgia, C.; Teague, A.G.S.; Sansom, O.J.; Cagan, R.L. Functional Exploration of Colorectal Cancer Genomes Using Drosophila. Nat. Commun. 2016, 7. [CrossRef] [PubMed]
145. Tian, A.; Benchabane, H.; Ahmed, Y. Wingless/Wnt Signaling in Intestinal Development, Homeostasis, Regeneration and Tumorigenesis: A Drosophila Perspective. J. Dev. Biol. 2018, 6, 8. [CrossRef] [PubMed]
146. Panayidou, S.; Apidianakis, Y. Regenerative Inflammation: Lessons from Drosophila Intestinal Epithelium in Health and Disease. Pathog. Basel Switz. 2013, 2, 209–231. [CrossRef]
147. Nászai, M.; Carroll, L.R.; Cordero, J.B. Intestinal Stem Cell Proliferation and Epithelial Homeostasis in the Adult Drosophila Midgut. Insect Biochem. Mol. Biol. 2015, 67, 9–14. [CrossRef]
148. Mukherjee, S.; Tucker-Burden, C.; Zhang, C.; Moberg, K.; Read, R.; Hadijapanayis, C.; Brat, D.J. Drosophila Brat and Human Ortholog TRIM3 Maintain Stem Cell Equilibrium and Suppress Brain Tumorigenesis by Attenuating Notch Nuclear Transport. Cancer. Res. 2016, 76, 2443–2452. [CrossRef]
149. Paglia, S.; Sollazzo, M.; Di Giacomo, S.; de Biase, D.; Pession, A.; Grifoni, D. Failure of the PTEN/APKC/Lgl Axis Primes a Pro-Cathepsin and a Soluble Gamma-Glutamyl Transpeptidase in Drosophila Male Accessory Gland Proteins, Including a pro-Cathepsin and a Soluble Gamma-Glutamyl Transpeptidase. Proteome. Sci. 2006, 4, 9. [CrossRef]
150. Page-Mccaw, A.; Howell, G.J.; Shirras, A.D.; Isaac, R.E. Proteomic Identification of Drosophila Melanogaster Male Accessory Gland Proteins, Including a pro-Cathepsin and a Soluble Gamma-Glutamyl Transpeptidase. J. Exp. Biol. 2007, 210, 3601–3606. [CrossRef]
151. Wilson, C.; Leiblich, A.; Goberdhan, D.C.I.; Hamdy, F. The Drosophila Accessory Gland as a Model for Prostate Cancer and Other Pathologies. Curr. Top. Dev. Biol. 2017, 121, 339–375. [CrossRef]
152. Krätzschmar, J.; Haendler, B.; Eberspaecher, U.; Roosterman, D.; Donner, P.; Schleuning, W.D. The Human Cysteine-Rich Secretory Protein (CRISP) Family. Primary Structure and Tissue Distribution of CRISP-1, CRISP-2 and CRISP-3. Eur. J. Biochem. 1996, 236, 827–836. [CrossRef]
153. Ram, K.R.; Wolfrin, M.F. A Network of Interactions among Seminal Proteins Underlies the Long-Term Postmating Response in Drosophila. Proc. Natl. Acad. Sci. USA 2009, 106, 15384–15389. [CrossRef] [PubMed]
154. Garénaux, E.; Kanagawa, M.; Tsuchiyama, T.; Horii, K.; Kanazawa, T.; Goshima, A.; Chiba, M.; Yasue, H.; Ikeda, A.; Yamaguchi, Y.; et al. Discovery, Primary, and Crystal Structures and Capacitation-Related Properties of a Prostate-Derived Heparin-Binding Protein WGA16 from Boar Sperm. J. Biol. Chem. 2015, 290, 5484–5501. [CrossRef]
155. Peng, J.; Zipperlen, P.; Kubli, E. Drosophila Sex-Peptide Stimulates Female Innate Immune System after Mating via the Toll and Immune Pathways. Curr. Biol. CB 2005, 15, 1690–1694. [CrossRef]
159. Short, S.M.; Wolfner, M.F.; Lazzaro, B.P. Female Drosophila Melanogaster Suffer Reduced Defense against Infection Due to Seminal Fluid Components. *J. Insect. Physiol.* 2012, 58, 1192–1201. [CrossRef]

160. Herndon, L.A.; Wolfner, M.F. A Drosophila Seminal Fluid Protein, Acp26Aa, Stimulates Egg Laying in Females for 1 Day after Mating. *Proc. Natl. Acad. Sci. USA* 1995, 92, 10114–10118. [CrossRef]

161. Ram, K.R.; Wolfner, M.F. Sustained Post-Mating Response in Drosophila Melanogaster Requires Multiple Seminal Fluid Proteins. *PloS Genet.* 2007, 3, e238. [CrossRef]

162. Sharma, V.; Pandey, A.K.; Kumar, A.; Misra, S.; Gupta, H.P.K.; Gupta, S.; Singh, A.; Buehner, N.A.; Ravi Ram, K. Functional Male Accessory Glands and Fertility in Drosophila Require Novel Ecdysone Receptor. *PloS Genet.* 2017, 13, e1006788. [CrossRef]

163. Truman, J.W.; Riddiford, L.M. Endocrine Insights into the Evolution of Metamorphosis in Insects. *Annu. Rev. Entomol.* 2002, 47, 467–500. [CrossRef] [PubMed]

164. Fourche, J. The determination of the molting and metamorphosis of Drosophila melanogaster: Effects of starvation and of ecdysone supply. *Arch. Anat. Microsc. Morphol. Exp.* 1967, 56, 141–152.

165. King-Jones, K.; Thummel, C.S. Nuclear Receptors—A Perspective from Drosophila. *Nat. Rev. Genet.* 2005, 6, 311–323. [CrossRef] [PubMed]

166. Bairati, A. Struttura ed ultrastruttura dell’apparato genitale maschile di Drosophila melanogaster Meig. *Z. Für Zellforsch. Mikrosk. Anat.* 1967, 76, 56–99. [CrossRef]

167. Kalb, J.M.; DiBenedetto, A.J.; Wolfner, M.F. Probing the Function of Drosophila Melanogaster Accessory Glands by Directed Cell Ablation. *Proc. Natl. Acad. Sci. USA* 1993, 90, 8093–8097. [CrossRef]

168. Taniguchi, K.; Kokuryo, A.; Imano, T.; Minami, R.; Nakagoshi, H.; Adachi-Yamada, T. Isoform-Specific Functions of Mud/NuMA Mediate Binucleation of Drosophila Male Accessory Gland Cells. *BMC Dev. Biol.* 2014, 14, 46. [CrossRef]

169. Susic-Jung, L.; Hornbruch-Freitag, C.; Kuckva, J.; Rexer, K.-H.; Lammel, U.; Renkawitz-Pohl, R. Multinucleated Smooth Muscles and Mononucleated as Well as Multinucleated Striated Muscles Develop during Establishment of the Male Reproductive Organs of Drosophila Melanogaster. *Dev. Biol.* 2012, 370, 86–97. [CrossRef]

170. Xue, L.; Noll, M. Dual Role of the Pax Gene Paired in Accessory Gland Development of Drosophila. *Development* 2002, 129, 339–346. [CrossRef]

171. Ruivo, C.F.; Adem, B.; Silva, M.; Melo, S.A. The Biology of Cancer Exosomes: Insights and New Perspectives. *Cancer. Res.* 2017, 77, 6480–6488. [CrossRef]

172. American Cancer Society. Cancer Facts & Figures. 2021. Available online: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2021.html (accessed on 27 July 2021).