Developmental Cell
Perspective

Modeling Cancer with Flies and Fish

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https://doi.org/10.1016/j.devcel.2019.04.013

Cancer has joined heart disease as the leading source of mortality in the US. In an era of organoids, patient-derived xenografts, and organs on a chip, model organisms continue to thrive with a combination of powerful genetic tools, rapid pace of discovery, and affordability. Model organisms enable the analysis of both the tumor and its associated microenvironment, aspects that are particularly relevant to our understanding of metastasis and drug resistance. In this Perspective, we explore some of the strengths of fruit flies and zebrafish for addressing fundamental cancer questions and how these two organisms can contribute to identifying promising therapeutic candidates.

Introduction

Drosophila and zebrafish have a growing history of cancer discoveries. A century ago, Mary Stark mapped a *Drosophila* locus linked to tumor emergence; later, *Drosophila* researchers Gateff and Schneiderman identified the first tumor suppressor, lethal giant larvae, in 1967 (Gateff and Schneiderman, 1967; Stark, 1918, 1919). These discoveries—plus a continuing series of oncogenes and tumor suppressors identified and explored in flies and fish—have paired with key discoveries in cell and epithelial biology to help lay the groundwork for our current understanding of cancer.

Why Flies

Drosophila has been especially successful as a pathway discovery platform. Its 10-day life cycle and century's worth of genetic tools has enabled the identification (and naming) of key components of many "core cancer pathways" including RAS, NOTCH, HEDGEHOG, WNT, BMP, HIPPO, JAK/STAT, and TGF-β. In addition to naturally occurring tumors (Salomon and Jackson, 2008), activating these primary cancer pathways in discrete clones can lead to aggressive tumors that interact in complex ways with neighboring normal tissue, which in turn provokes aspects of metastatic progression. *Drosophila* has provided a good context for examining the interactions between tumor cells and their neighbors within epithelia. Such work has shown that as cells transform, their neighbors help remove them from the epithelium: pathways such as JNK, SRC, and even caspases mediate epithelial-to-mesenchymal transition (EMT), cell motility, and distant migration (e.g., Bangi et al., 2016; Ferres-Marco et al., 2006; Pagliarini and Xu, 2003; Stuelten et al., 2018; Vidal et al., 2006; Wu et al., 2010). Conversely, tumors 'push back': elevated levels of MYC and other cellular regulators promote transformed cells to become "supercompetitors," that, through cell competition, expand at the expense of their wild-type neighbors (Rhiner and Moreno, 2009). These studies bring a level of epithelial resolution that can prove useful for understanding how the tumor microenvironment interacts with transforming cells. Many of these molecular mediators represent druggable targets.

Drosophila transgenic models can be established in less than 3 months with minimal cost. As a result, *Drosophila* cancer models have proliferated. These include fly platforms that model aspects of transformation, including proliferation, genome instability, metastasis, and cachexia; diet and other environmental effects on tumor progression have become an active area of focus (reviewed in Herranz and Cohen, 2017; Sonoshita and Cagan, 2017; Warr et al., 2018). Specific tumor types have been modeled including tumors of the lung, colon, thyroid, and brain as well as leukemias. These models have provided important insights into the pathways that direct tumor-specific transformation, but care must be taken in extending these results to mammals; for example, flies differ significantly in their immune system and blood-brain barrier, and they do not have a thyroid.

Why Fish

The zebrafish has always been an excellent model for developmental biology and, over the past 15 years, has taken a prominent role in cancer biology as well. There are several attributes that make it a powerful model for the study of cancer. Transgenics and CRISPR allow the creation of specific and robust cancer models. Mutations with developmental defects can also be studied for cancer development, allowing a quick and facile method to study pathways involved in embryogenesis and cancer. Transgenics allow reporter constructs to be generated to follow cell fate in cancer. Transplantation models that are transparent can be used to watch tumor invasion and spread in real time and even human tumors can be transplanted into zebrafish. The major advantage of the zebrafish system for the study of cancer is the number of vertebrate animals that can be studied simultaneously with cancer. For many studies, up to 100 animals with cancer can be studied for each arm of an experiment to test a new therapy or a certain mutation. It also helps that zebrafish provides a relatively inexpensive model compared to mice.

The zebrafish has a long history as a model for cancer, beginning even before the development of the most recent genetic approaches. Treatment with carcinogens was long ago observed to produce tumors in these animals (Pliss et al., 1982), and treatment with mutagens such as ethylnitrosourea (ENU), dimethylbenz(a)anthracene (DMBA), and N-methyl-nitrosoguanadine (MNNG) was known to lead to a wide variety of tumor types (Beckwith et al., 2000; Spitsbergen et al., 2000a, 2000b). The

zebrafish model is also amenable to many types of unbiased screens for genes and drugs that alter cancer biology. One of the first "cancer" screens looked for mutant fish that had cell-cycle defects, yielding mutations in tumor suppressor genes (Shepard et al., 2005; Stern et al., 2005). The screen made use of the phospho-H3 antibody (pH3) that stains mitotic cells in the zebrafish embryo. Using ENU mutagenesis, mutations causing abnormal patterns of pH3 staining were identified, including a *c-myb* loss-of-function mutation that led to a higher rate of cancer when heterozygotes animals were treated with carcinogens. Another mutation, in the separase gene—required for entry into anaphase—caused development of polyploid cells during embryogenesis. Animals heterozygous for separase had a higher rate of epithelial cancers compared to control fish treated with a carcinogen (Shepard et al., 2007). This work established the zebrafish as a cancer model.

Other screens have employed transgenic zebrafish approaches. It was known, for example, that 1q21 was a genomic region recurrently amplified in melanoma (Lin et al., 2008), but this region contained ~30 genes, making it difficult to identify the relevant cancer driver. To evaluate their functional role, the genes in the critical interval were screened one by one by overexpressing the corresponding human cDNAs in concert with the human BRAFV600E oncogene, in the background of p53 loss (Patton et al., 2005; Ceol et al., 2011). Because BRAF is the most commonly mutated driver oncogene in melanoma, the screen was designed to identify genes on 1q21 that cooperated with BRAF. This led to the discovery that SETDB1, an epigenetic regulator, was the major driver gene in the interval and that it plays similar roles in human melanoma. Similar transgenic systems were used to overexpress genes involved in other cancers such as rhabdomyosarcoma, pancreatic adenocarcinoma, thyroid cancers, and leukemia (Anelli et al., 2017; Langenau et al., 2007; Lobbardi et al., 2017; Park et al., 2008), leading to a better understanding of cancer development and resistance.

Technologies in Flies and Fish for Exploring Cancer Biology

A key advantage of model systems such as flies and fish is the ability to explore cancer biology at a sophisticated, single-cell level in the context of the whole-animal. A brief overview of some of the relevant technologies provides insight into the speed and scale that can be achieved with these models.

Transgenesis in Flies

The *Drosophila* community has developed an astonishing array of tools for genetic manipulation in the context of the whole-animal. Extensive reviews describing the myriad of transgenic approaches (Enomoto et al., 2018; Parvy et al., 2018) are available, and here, we provide only a brief overview.

Most importantly, transgenic tools in flies permit the knockdown or overexpression of any gene in nearly any tissue—or in single cells—at any stage of development or adulthood. Furthermore, fly lines harboring individual mutations or inducible knockdown constructs for most predicted genes are available by mail order for a nominal fee from the Bloomington *Drosophila* Stock Center. Cell lines and transformation constructs are similarly easy to obtain.

An especially important tool is the GAL4/UAS targeting system (Brand and Perrimon, 1993). Paired with hundreds of characterized promoters and promoter fragments, this system allows fine control of transgene expression. For example, to model a glioblastoma tumor in the nervous system with the oncogene EGFR plus the tumor suppressor PTEN (Read et al., 2009, 2013) required establishing a fly with a glial-specific "driver" (*repo-GAL4*) and GAL4-responsive transgenes (e.g., *UAS-EGF [activated]* plus *UAS-PTEN[RNA-interference-knockdown]*). The resulting fly models exhibited many of the features of glioblastoma including strong transformation in the brain (Figure 1A). Recent work has pushed this technology further: colorectal cancer tumors with up to four simultaneous tumorigenic mutations ("hits") were recently reported (Figure 1B), and most recently, a "nine hit" model—in which one oncogene was paired with knockdown of eight tumor suppressors—was used to model an individual patient (see below; Bangi et al., 2016, 2019).

These transgenic models are powerful, but they do have important caveats. Aggressive metastatic tumors typically evolve genomically, whereas fly transgenic cancer models are likely genomically stable. Furthermore, large genomic alterations such as copy-number variation are poorly modeled in flies and fish (and most other transgenic models). The value in these transgenic cancer models comes from their ability to generate hypotheses by exploring aspects of transformation in a whole-animal context.

Transgenesis in Fish

The zebrafish also offers a wonderful system for creating cancer models, for instance using tissue-specific transgenic fish that overexpress oncogenes. The field was propelled forward by Langenau and co-workers with the development of a transgenic model in which the rag2 promoter (expressed in T cells but also a subset of muscle cells) was used to drive c-myc, resulting in lymphoma and leukemia (Langenau et al., 2003). Many other interesting transgenic models of cancer have been developed since then, including a melanoma model (Figure 1C, in which the melanocyte-specific *mitf* promoter drives $BRAF^{V600E}$), rhabdomyosarcoma (in which the rag2 promoter drives KRAS), pancreatic models (in which the ptf1 promoter drives KRAS), hepatocellular cancer (in which the fabp10 promoter drives KRAS), and a neuroblastoma model involving the loss of NF1 (Patton et al., 2005; Langenau et al., 2007; Park et al., 2008; Nguyen et al., 2011; He et al., 2016). This list is not meant to be comprehensive but instead meant to demonstrate that nearly any cancer can be generated in the zebrafish with the proper combination of oncogenes, tumor suppressors, and tissue-specific promoters. These models closely resemble the corresponding human diseases at histological and transcriptomic levels. Alternative models involve stable transgenics that develop cancer over time and F0 transient transgenics, which can be used to test whether an oncogene can initiate cancer development.

Sophisticated vector systems enable interesting genetic experiments in the fish. In one example, Ceol et al. (2011) developed a model for testing the effects of different oncogenes on melanoma formation. In this model, $BRAF^{V600E}$ expression is driven by the *mitf* promoter, in the background of *mitf* and p53 mutations. These fish do not get melanoma because *mitf* is required for the generation of melanocyte progenitors (called melanoblasts) as well as mature melanocytes. However, a vector called miniCoopR (Ceol et al., 2011; Iyengar et al., 2012) carries an *mitf* minigene that rescues melanocytes in a mosaic fashion in the F0 generation. The vector also contains a cassette, in which

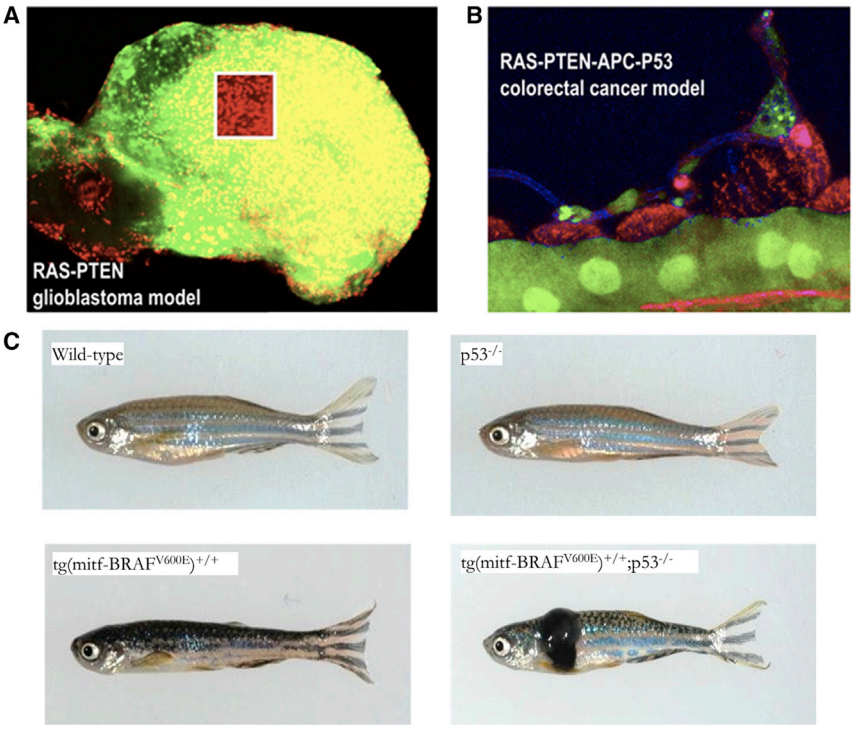

Figure 1. Examples of Tumors in Flies and Fish

(A) RAS^{G12V}; $PTEN^{-/-}$ double mutant clones formed large glial-derived tumors in the fly brain. Red, *repo* (all glia); green, GFP (transformed glia). Insert shows the abnormally high density of glial-cell nuclei. From Read et al., 2009.

(B) High magnification view of a multi-target (involving four mutations) cancer model achieved by overexpressing RAS^{G12V} and using RNAi-mediated knockdown of PTEN, APC, and P53. Green, transformed hindgut cells; red, muscle cells; and blue, trachea. See Bangi et al., 2016.

(C) A transgenic zebrafish melanoma model. Expression of $BRAF^{V600E}$ under the melanocyte-specific *mitfa* promoter resulted in stripe disruption and nevi. When crossed with *p53*-mutant fish, the resultant mitfa-$BRAF^{V600E}$;$p53^{-/-}$ fish developed melanomas with 100% penetrance.

an *mitf* promoter can drive the expression of any gene of interest. Thus, rescued melanocytes promote melanoma formation and any gene of interest can be tested for its effect on melanoma formation. This system was first used to find that SETDB1 accelerates melanoma (Ceol et al., 2011). More recently, a MAZERATI vector system has been adapted from miniCoopR. This newer system uses the *mitf* minigene rescue, expresses Cas9 from the *mitf* promoter, and also has the U6 promoter driving a gRNA for gene targeting. This tissue-specific vector system allows testing of tumor suppressor activity. Combining miniCoopR with the tissue-specific targeting vector allows modeling of many genotypes for cancer. For example, Ablain et al., who recently studied all genotypes of mucosal melanoma using this system, found that SPRED1 was mutated in human mucosal melanoma and showed a specific genetic interaction with kit mutations (Ablain et al., 2018). Given the ability to easily overexpress and knock out genes in a tissue-specific manner in the zebrafish, the model should allow modeling of tumors of many different tissues.

One drawback of the above approaches is that they were not optimized for the study of metastasis. These initial models rely on embryo injections, making it impossible to control where or when the tumor arises. A more recent transgenic system, called transgene electroporation in adult zebrafish (TEAZ) addresses this limitation by directly electroporating the above DNA constructs into adult fish, at a specific location and time of life (Callahan et al., 2018). Using this system in the BRAF/p53/*mitf* background, Callahan et al. (2018) demonstrated that simultaneous electroporation of MiniCoopR plus Cas9 knockout of RB1 led to a 100% penetrant melanoma right at the site of electroporation. This system works not only for melanoma but also for numerous other tumors, including sarcoma. One key feature of the TEAZ approach is that it can precisely monitor for metastases, given that the initial tumor site is known.

Imaging

An important adjunct to the powerful transgenic tools in flies and fish is imaging. Developing flies have discreet "islands" of emerging epithelia known as imaginal discs; in live whole mounts, imaginal disc cells can be imaged with high resolution while, for example, cells are removed by cell competition or by TNF signals from circulating hemocytes (fly blood cells) (Cordero et al., 2010; Ohsawa et al., 2011). However, imaging is especially powerful for zebrafish. Zebrafish have long been heralded for their optical transparency, which allows for highly detailed imaging of their developmental processes. To extend this to cancer required a transparent adult, which was generated by combining pigment mutants to produce a semi-transparent strain named casper (White et al., 2008). This model is powerful in that it allows for single-cell imaging *in vivo*, at a scale difficult to achieve in any other vertebrate system. For example, Kim et al. used casper to image the fate of individual metastatic cancer cells as they exit blood vessels and engraft into new tissues and identified the genes EDN3/ECE2 as drivers of metastasis (Kim et al., 2017). The fish can be kept alive for many hours, and one unique feature is that with commonly used stereo or confocal microscopes, imaging that scales from the entire animal all the way down to single cells can be achieved. Other groups have begun using the fish for subcellular localization studies, such as trafficking of fluorescent exosomes from one site to another (Hyenne et al., 2019; Verweij et al., 2019).

Cell Transplantation in Flies

A core technology of many cancer studies is the ability to transplant transformed cells between flies, which allows one to assess the tumorigenic potential of a given cell population (i.e., stemness). Cell transplantation has long been used to examine developmental questions such as tissue induction and transdetermination but, more recently, has also been used in the cancer context to extend the "age" of tumors in *Drosophila*, for example, to examine chromosome variations such as aneuploidy that may arise over time (e.g., Bowman

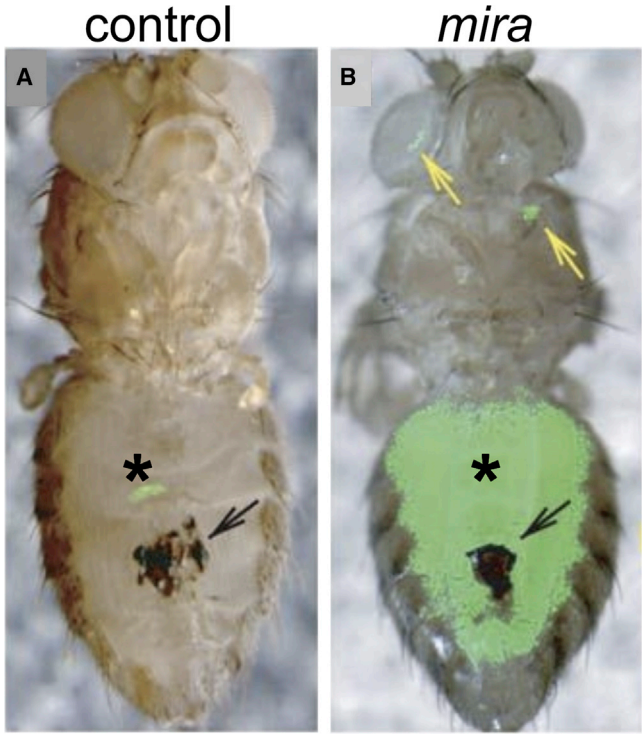

Figure 2. Examples of Tumor Transplantation in Drosophila
Black arrows indicate site of injection and yellow arrows highlight distant secondary tumors.
(A) GFP-labeled wild-type larval brain tissue (green, asterisk) exhibited minimal growth 2 weeks after implantation.
(B) Fragments containing *mira* mutant clones—which transform cells by altering cell polarity—dramatically expanded and yielded distant clones. From Caussinus and Gonzalez, 2005.

et al., 2008; Caussinus and Gonzalez, 2005; Dekanty et al., 2012; Gonzalez, 2013; Eroglu et al., 2014). These tumors can spread widely (Figure 2), impacting the host in interesting ways that mirror disease progression in humans. For example, pairing oncogenic RAS^{G12V} with loss of the tumor suppressor SCRIB led to transformed tissue; transplanting RAS-SCRIB epithelial tissue into wild-type hosts demonstrated that signals from the transformed cells are sufficient to provoke a cachexia-like wasting syndrome (Figueroa-Clarevega and Bilder, 2015).

Cell Transplantation in Fish

Transplantation technology has been widely adopted by the zebrafish community as well, at a scale unimaginable in murine systems. Transplantation may involve either zebrafish or human cancer cells. Heilmann et al. developed a zebrafish-specific cell line called ZMEL1, which was derived from a GFP + MiniCoopR melanoma (Heilmann et al., 2015). This is a highly stable cell line and, when transplanted back into casper recipients, reliably gives rise to widespread metastases (Figure 3). It can also be easily genetically manipulated and, depending on the age of the recipient, can be transplanted into immunocompetent hosts (i.e., when transplanted into larval fish that have not yet developed adaptive immunity, the cells are rapidly engrafted without immunosuppression). In another approach, cells from freshly isolated tumors were separated by fluorescence-activated cell sorting (FACS) into different populations based on gene expression and then transplanted into recipient animals to determine which population was most stem like in embryonic rhabdomyosarcoma (Hayes et al., 2018).

Several groups have also been exploring whether human cells can be transplanted into the fish (Liu et al., 2018; Stoletov et al., 2007; Wertman et al., 2016). This entails many technical challenges, including the vast temperature differential between fish (28°C) to humans (37°C) and the obvious immune barriers. Despite these challenges, some of these tumors proliferate and even enter the caudal hematopoietic tissue, the site of embryonic hematopoiesis, similar to the natural migratory path of blood stem cells, which may have similarity to the way in which tumors often disseminate to the bone marrow. Tumor spreading has been evaluated with genetics and chemical biology using this zebrafish model. Several drugs are effective at killing tumor cells in this model, which may serve as a preclinical model for drug testing. Another method of studying human tumors in fish is xenografts. In this method, immunodeficient zebrafish are used as the recipient of human tumor cells, which prevents immune rejection of those cells (Moore et al., 2016). This would be an ideal system to test genes and drugs for cancer biology. It is still unclear if a drug tested in fish would be allowed to be directly tested in patients in a clinical trial or whether mouse xenografts would be required. Nevertheless, the throughput and cost of this transplant system have significant advantages over the mouse system.

Drug Screening

The past several decades have witnessed remarkable advances in our understanding of the molecular changes driving cancer. The view of cancer as a fundamentally genetic disease led to large-scale DNA sequencing efforts such as the TCGA and ICGC to catalog the genetic alterations across almost all major tumor types. There is little doubt that these efforts have been fruitful. As one example, patients with melanoma driven by $BRAF^{V600E}$ respond strongly to RAF inhibitors. However, while many melanoma patients had dramatic tumor responses to drugs targeted against such genetic mutations, almost all patients relapsed in a very short period of time (~7 months) because of a wide spectrum of resistance mechanisms that all reactivate the mitogen-activated protein kinase (MAPK) pathway. This experience of response then resistance to targeted therapies has been more the rule than the exception. Model systems can help address this challenge by identifying new therapeutic targets.

One underappreciated strength of flies and fish is their use as a whole-animal drug-screening platform. Both animals are readily dosed by either injection or mixing a drug or compound with food (flies) or water (fish); the result is the ability to assess compounds in the context of a whole-animal system. Further, these assays can detect multi-targeting compounds (“polypharmacology”) that act at multiple sites in the animal to reduce tumor progression. Many drugs currently in use are multitargeting, and these “off-targets” can contribute to the drug’s overall efficacy by attacking the tumor plus the micro- and macroenvironments. That is, whole-animal screens can identify novel therapeutic chemical spaces including hits that are effective in whole-body assays; these drugs can prove poorly effective in cell lines and

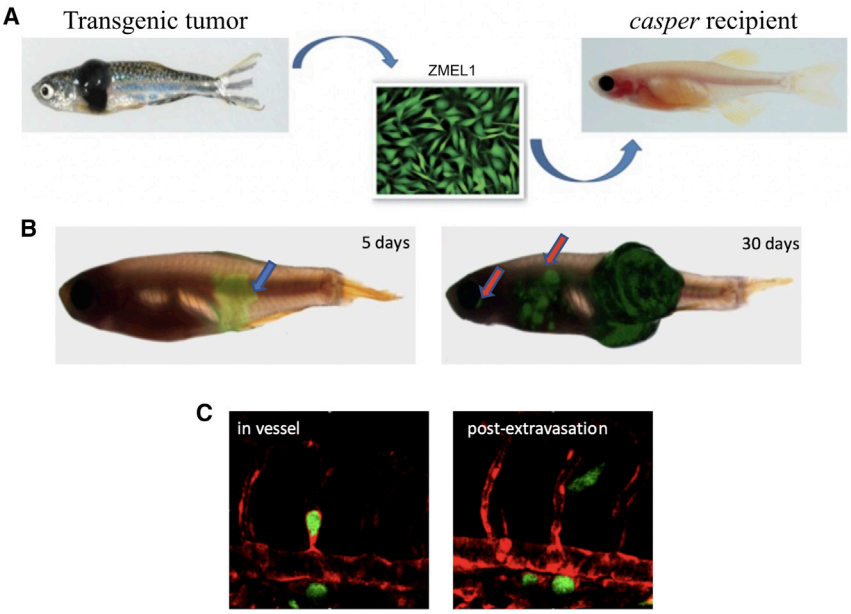

Figure 3. The ZMEL/Casper Transplant Assay Allows for Single-Cell Resolution of the Entire Metastatic Cascade

(A) The ZMEL1 line is derived from a transgenic zebrafish melanoma. It is then transplanted into the skin of the transparent casper recipient.

(B) Tumors initiate at a defined spot where cells are injected (blue arrow) and metastases (red arrows) can then be followed over time.

(C) An example of a single extravasating ZMEL1-GFP cell at a distant site show its appearance while still in the blood vessel (marked by an flk-RFP transgene) and immediately after extravasation out of the blood vessel.

organoids (e.g., Sonoshita et al., 2018) and would be rejected in more traditional screening platforms.

Drug Screening in Flies. Early pioneering efforts using flies for drug screening have led to candidate therapeutics for fragile X syndrome and nervous system-based diseases and for cancer use including targeted therapies and radiosensitizers (e.g., Auluck and Bonini, 2002; Chang et al., 2008; Edwards et al., 2011; Markstein et al., 2014; Stickel et al., 2015). More recent efforts have led to candidate drugs or drug cocktails for lung cancer, colorectal cancer, and—despite their lack of a thyroid—papillary and medullary thyroid cancers (Bangi et al., 2016; Dar et al., 2012; Levine and Cagan, 2016; Levinson and Cagan, 2016; Sonoshita et al., 2018). Regarding the latter, a *Drosophila* medullary thyroid carcinoma (MTC) model was used to help validate the drug vandetanib as useful for MTC (Vidal et al., 2005); subsequent clinical trials led to its FDA approval in 2012, helping to demonstrate that *Drosophila* can be useful as a whole-animal drug platform. Flies do not have a thyroid, so this is an example of helping identify a compound that is effective on the oncogene pathway.

The ability to carry out large-scale screens in *Drosophila* should not be underestimated. For example, Bangi et al. (2019) recently reported a "personalized fly model" platform in which a genetic model of specific patient tumor mutations was created and then used to screen a near-comprehensive library of FDA-approved drugs in a few weeks. The screen used ~400,000 flies, a scale difficult to imagine in other animal systems. The result was a pair of drugs that showed activity in the fly and, when given to the patient, led to partial regression of a previously treatment-resistant metastatic colorectal tumor, improving the patient's quality of life and likely significantly extending their lifespan (Bangi et al., 2019). An important side benefit of the fly model is the ability to work systematically with chemical biologists, allowing quick testing of drugs at a rate matching that of chemical synthesis: candidate compounds can be tested quickly, informing a new round of chemical synthesis. Through this iterative process, kinase inhibitors have been "evolved" for RET-dependent tumors that demonstrate improved efficacy in mammalian models while retaining drug-like properties (Sonoshita et al., 2018). Flies and chemistry can prove an effective pairing.

Drug Screening in Fish. The zebrafish system is an equally good system for chemical biology. Chemicals can simply be added to the water and evaluated for phenotypes. Chemical libraries of random structures, biologically active chemicals, or drugs that patients receive can be screened. For chemical screening, most drugs work similarly in fish and humans, although the affinity of a drug for the fish protein may be different than its affinity for the human protein, requiring different dosing in humans and fish. For some drugs, the chemical simply does not work in the fish. In one melanoma study, a 'neural crest' signature was found to be important in initiation, and a chemical screen was therefore designed to find suppressors of this neural crest gene pattern (White et al., 2011). This revealed an unexpected target, the metabolic enzyme dihydroorotate dehydrogenase (DHODH), which was found to suppress transcriptional elongation of key genes involved in neural crest specification. This pathway has now been implicated in leukemia as well (Sykes et al., 2016). One DHODH inhibitor, leflunomide, entered clinical trials for the treatment of metastatic disease, and newer trials of improved and less toxic agents will be starting soon. The ability to test drugs *in vivo* through screening approaches is now being extended even further via human cancer cell xenografts into fish. For example, Snaar-Jagalska and co-workers showed that implantation of cells such as MDA-MB-231 breast cancer cells, PC3 prostate cancer cells, and A673 Ewing sarcoma lines can be monitored in real time and drugs tested for their efficacy (Tulotta et al., 2016). These approaches can be also applied to patient-specific approaches, analogous to a PDX model in which fragments of human tumor samples can be implanted into the fish and drugs screened in this manner (Wertman et al., 2016). Such an approach can be used to identify targeted therapies that may have efficacy in the clinical setting, as recently demonstrated for sensitivity to γ-secretase inhibitors in NOTCH1-mutated leukemia (Bentley et al., 2015). These approaches can be extended to the pipeline level, in which patient tumors can be rapidly assessed for drug sensitivities, which can not only influence drug discovery but also inform patient decisions (Veinotte et al., 2014).

Perspective

Flies and fish hold a unique place in cancer biology: they are especially well suited for studying the complex, *in vivo* biology that has made cancer such a difficult disease to treat. Cancer therapies must be targeted from the perspective of not only the tumor cell but also the host-tumor-microenvironment factors. Given the availability of new technologies, several exciting biological areas will likely benefit from these systems.

Identifying New Drug Combinations

Few whole-animal drug screens test combinations of drugs: the number of animals required can be daunting. Flies and fish are small, easy to produce, and inexpensive and can therefore be screened in large numbers, opening the potential for complex combinatorial drug screens designed to hit multiple targets. As the number of new and untested targets continues to grow, we can rapidly identify those that are likely to have *in vivo* bioactivity. As mentioned above, in using flies to screen for a two-drug treatment for a colorectal cancer patient, Bangi et al. (2019) were able to generate a nine-genetic-hit fly in which the key oncogenic mutations found in that patient were all engineered into a single fly. This fly was then used to screen a library of 1,500 compounds through multiple rounds and develop a personalized treatment plan in time to successfully treat a patient with progressive disease (Bangi et al., 2019). Interest in drug combinations is growing, and model organisms can play a useful role in speeding this process while retaining a whole-animal context.

Metastasis and Tumor Dormancy

Most cancer deaths are due to metastasis. Yet, almost no screens have specifically targeted this key event, a major missed opportunity (Anderson et al., 2019). Most cancers are lethal once metastatic, and one promising approach is to interrupt this process before it occurs. Recent work has confirmed that metastasis is not a single-step process, that many tumors disseminate very early in the disease, and that a subset of disseminated cancer cells enters a state of dormancy. For unknown reasons, those dormant cells can eventually 'switch' back on, forming newly aggressive metastatic lesions. What are the molecular mechanisms that mediate this switch, and are these mechanisms druggable? These are questions that can be productively studied in flies and fish and would have a major impact upon clinical care of patients.

The Tumor Microenvironment and the Metabolic Milieu

Finally, we offer some thoughts on the role of model systems in addressing the emerging discipline of the tumor microenvironment. While tumor genetics play a central role in directing cancer, an equally compelling view is that it is a systemic disease that depends on interactions with the tumor microenvironment (Quail and Joyce, 2013). The influence of the tumor microenvironment extends well beyond immune cells and plays a key role in shaping tumor-cell metabolism and epigenetic state. Since cancer cells must take up nutrients from their environment, they actively engage with their surroundings to get the glucose, fatty acids, and amino acids needed to sustain cell proliferation. Zhang et al., for example, identified a role for fatty acids as drivers of metastasis in melanoma, which occurs via a lipid transporter called FATP1 (Zhang et al., 2018). Many metabolic pathways also feed directly into the epigenetic architecture of the cell. For example, in patients with mutations in isocitrate dehydrogenase 1/2 (IDH1/2) the mutant enzyme synthesizes a metabolite called 2-hydroxyglutarate (2-HG), which inhibits other epigenetic enzymes such as histone demethylases and the TET family of 5-methylcytosine (5mC) hydroxylases (Ward et al., 2010). This has recently led to the first FDA approval of IDH inhibitors (DiNardo et al., 2018), but again, the efficacy of this treatment is limited to a relatively small number of patients, and other approaches to targeting metabolic pathways (e.g., metformin) have seen much less success across broad groups of cancer. These observations highlight that sustained success in treating cancers will require interventions that disrupt signals not only in the cancer cell itself but also in the tumor microenvironment.

This is where models such as the fly and the fish can shine. Model systems allow us to study cancers in their native environments, making it possible to assess how interactions between tumor cells and the surrounding microenvironmental cells promote complex tumor phenotypes such as metastasis. We can use *in vivo* imaging, in which different cell populations are labeled with different fluorophores, to observe cellular behaviors. We can also use tissue-specific knockdown or CRISPR approaches to specifically modify either the tumor or the microenvironment—or both—on a large scale, which remains very challenging in cell-culture, organoid, or mouse studies. Outside of simply understanding the biology, many cancer studies also aim to identify new drugs, and for scalability purposes, most of these drug screens are done in 2D cell-culture systems. However, even large-scale cell-culture efforts such as the Cancer Cell Line Encyclopedia (CCLE) or DepMap CRISPR screens (Barretina et al., 2012; McFarland et al., 2018) often fail to reveal relevant *in vivo* targets because of the metabolic and epigenetic flexibility that emerges from interactions between the tumor and the local microenvironment. Model systems such as flies and fish provide the scalability for whole-animal screening, powerful tools for dissecting interactions in real time, and the ability to explore these cancer processes at the level of single cells.

ACKNOWLEDGMENTS

This Perspective was supported by NIH U54OD020353 (R.L.C.); NIH R01CA229215 (R.M.W.); and R35CA220481, R01CA103846, and Howard Hughes Medical Institute (L.I.Z.). Data in Figure 2C was supplied by Nathaniel Campbell (White laboratory).

DECLARATION OF INTERESTS

L.I.Z. is a founder and stockholder of Fate Therapeutics, CAMP4 Therapeutics, and Scholar Rock, which have no direct relation to the submitted work. R.M.W. and R.L.C. declare no competing interests.

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