



What *Drosophila* Can Teach Us About Radiation Biology of Human Cancers

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Abstract

Ionizing radiation (IR) is used to treat more than half of human cancer patients. The therapeutic effect of IR is due to its ability to induce apoptosis. Success of radiation therapy relies not only on apoptosis induction but also on whether surviving cancer cells proliferate and regenerate a tumor. *Drosophila melanogaster* is a premier genetic model and, relevant to radiation biology of cancer, *Drosophila* larvae display an amazing capacity to regenerate. IR doses that kill more than half of the cells in larval tissues still allow complete regeneration to produce an adult fly of normal size and pattern. It is by understanding not only the initial effects of IR such as DNA damage and cell death but also longer-term regenerative responses that we may manipulate and improve radiation therapy of cancer. In this regard, *Drosophila* offers an unparalleled model to study both types of responses.

Keywords

Drosophila · Cancer · Ionizing radiation · Apoptosis · Regeneration

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Abbreviations

| | |
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| AiP | Apoptosis-induced Proliferation |
| F1 and F2 | Filial 1, Filial 2 |
| IR | Ionizing Radiation |
| JAK | Janus kinase |
| JNK | c-Jun N-terminal Kinase |
| PGE2 | Prostaglandin E2 |
| ROS | Reactive Oxygen Species |
| STAT | Signal Transducer and Activator of Transcription |

13.1 Introduction

Ionization Radiation is radiation with sufficient energy to dislodge electrons from a target atom, to produce ions. Types of IR include γ -rays, x-rays and particle radiation, all of which are used in radiation therapy of cancer. Therapeutic effect of IR relies on its ability to kill cells. The main cell killing mechanisms by IR are apoptosis and clonogenic or reproductive death in which irradiated cells lose their ability to multiply. Paradoxically, IR exposure can also stimulate the proliferation of some surviving cells. Proliferation of surviving cells repopulates the tumor to confer resistance to radiation therapy. Understanding how IR kills cells but also stimulates proliferation and repopulation is key to improving radiation therapy. As discussed in sections below,

Drosophila melanogaster, provides a useful model to study these seemingly opposing effects of IR.

13.2 Basic Understanding of What IR Does; X-Rays Induce Mutations

In the early 1900s, *Drosophila* geneticists had been studying naturally occurring mutations such as those affecting eye color and eye shape. Many wanted to go beyond spontaneous mutations and wanted to instead induce mutations so that more gene functions may be studied. When others failed to induce mutations using chemicals, Hermann Joseph Muller succeeded using X-rays. Radiation had been tried for mutagenesis by Muller's PhD mentor, Thomas Hunt Morgan, and others, but those efforts had been unsuccessful [3]. Muller thought that lack of success was not because radiation lacked activity but because detection methods for mutants were not optimal. He therefore chose recessive lethal mutations as

the read out, as opposed to visible phenotypes such as eye color or wing shape. He designed the original stocks and subsequent genetic crosses such that induced recessive lethal mutations could be detected readily by simply examining the progeny for the absence of certain classes. For example, he used a stock carrying a CIB chromosome which is an X chromosome with three genetic elements: a dominant visible mutation called Bar (B), a recessive lethal mutation (l), and a crossover suppressor (C) [Female parent in Fig. 13.1, [3, 24]]. The properties of these genetic elements are as follows. Bar mutation changes the eye shape so that animals carrying the CIB chromosome could be identified readily simply by inspecting their eyes. A recessive lethal chromosome meant CIB animals that also carried a wild type X chromosome, such as the female parent in Fig. 13.1, were viable whereas males with just the CIB X chromosome were lost. The cross-over suppressor was known genetically to do exactly that, to suppress crossing over in meiosis such that homologous chromosomes were inherited intact from one generation to the next

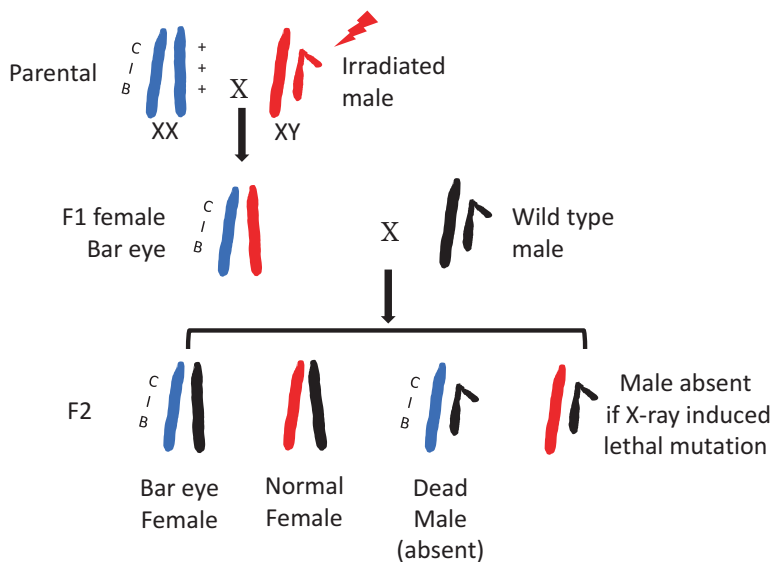


Fig. 13.1 One of the crossing schemes used by Muller to determine whether X-rays induce mutations. Bar eye females are crossed to irradiated males in the parental generation. In the F1 progeny, only the Bar eye females among all possible classes is shown. Crossing these females to

wild type males produce four possible progeny classes in the F2. Males with the CIB chromosome (blue) are absent because of the recessive lethal on this chromosome. If the irradiated X chromosome (red) carries a recessive lethal mutation, non-Bar males would also be absent in F2

without recombination and exchange of alleles. We now know chromosomes with a crossover suppressor as Balancer Chromosomes. Balancer Chromosomes contain multiple inversions such that crossing over produces severely rearranged chromosome products that do not support viable gametes or off-springs. Thus, it is not that crossing over is suppressed, rather, any product of crossing over is not represented in the progeny.

Muller irradiated males and crossed them to a female carrying one CIB chromosome [Fig. 13.1 ‘Parental’ cross, [24]]. F1 female progeny that inherited the irradiated X chromosome (red) from their father and the CIB X chromosome (blue) from their mother were recognized by their Bar eyes. When these F1 females were crossed to wild type males, the progeny in F2 included males with the irradiated X chromosome (red). If X-rays induced recessive lethal mutations, such males would be absent among the viable F2 population. Alternatively, If X-rays induced viable but visible recessive mutations, the phenotype will be manifested in these F2 males. Muller observed both of these outcomes, concluding that X-rays induced mutations, an important and fundamental insight into how IR works [39–41].

In his earlier work with X-rays, Muller used them as a tool to understand what exactly genes were and how they behaved. He discovered the phenomenon of dosage compensation; a gene on the X chromosome when present in two copies in an XX female produced the same phenotype as when it is present in one copy in XY males. Thus, he concluded, there must be mechanisms to compensate for the different gene dose in males and females for genes on the X-chromosomes [3, 42]. He discovered ‘position effect’; a gene from the X chromosome that translocated to another chromosome (e.g. after X-ray induced chromosome breakage and repair) and remained intact could be functional or not depending on the new location [48]. He studied the location of X-ray-induced breakpoints cytologically and correlated their effects on the resulting phenotype, reaching the conclusion that there are regions of chromosome between genes that are not functional [48]. These are fundamental insights into what genes

are, how they are organized and how they function.

It was in later work that he used genes/mutations to understand radiation. Muller’s PhD student S. P. Ray-Chaudhuri found that the a given dose of IR was equally mutagenic whether the dose was administered acutely (in 30 min) or split into smaller doses delivered over a longer period of time (a month) [49]. The conclusion that even low, diagnostic doses of radiation could be harmful remains controversial now as it was when Muller first disclosed it [66], but has led to the current regulations concerning exposure monitoring of radiation workers; we now monitor total exposed dose over time.

13.3 Cytological Responses to IR

Muller was the sole recipient of the 1946 Nobel Prize in Physiology or Medicine ‘for the discovery of the production of mutations by means of X-ray irradiation’. After his seminal findings, there followed many decades of deeper studies of *Drosophila* and IR, including studies that analyzed how environmental factors, dose rate, and organism age influence X-ray mutagenesis [for example, [4, 56]], how IR affects aging and fertility [for example, [58]], the effect of IR on developmental patterning [for example, [46, 64]], and X-ray-induced somatic crossing over [for example, [18]]. The results of many of these studies laid the ground for the next level of investigation in the 1970’s in which *Drosophila* geneticists added cell biological tools to phenotypic observations at the organism level. Peter Bryant and colleagues carefully quantified cell death and mitoses in irradiated larval imaginal discs, and measured the size of cytologically marked clones of cells that formed as irradiated discs regenerated [17, 22]. Clonal analysis revealed cells that died by apoptosis as well as cells that were alive but suffered clonogenic death in that these cells did not proliferate during regeneration [17]. X-rays first inhibited mitosis, which we now know to be due to cell cycle checkpoints [22]. But mitosis recovered eventually and surviving cells were even more proliferative than un-irradiated cells [22].

The data led Bryant and others to suggest that extra proliferation served to compensate for cells killed by IR [17, 22], a phenomenon we now call compensatory proliferation [6, 7, 38, 51]. In short, collective work from this era defined cell biological phenomena that are conserved in mammals. As summarized in the next sections, *Drosophila* has been an extremely useful model to dissect the molecular mechanisms responsible for these phenomena.

13.4 Apoptosis-Induced Proliferation and Accelerated Proliferation

Many tissues such as the skin and gut epithelia regenerate using dedicated stem cells. But tissues and organs without dedicated stem cells also regenerate. *Drosophila* larval imaginal discs are one such example. Imaginal discs are precursors of adult organs. Each imaginal disc is composed of a single layer of columnar epithelium juxtaposed with a single layer of squamous epithelium. Exposure to IR doses that kill half of the columnar epithelial cells [17, 22] or surgical

removal of up to 25% of the disc [23, 52, 69] is still compatible with complete regeneration to produce a viable adult fly of normal size and patterning. Regeneration of damaged discs occur by proliferation of the surviving cells as opposed to the use of dedicated stem cells (Fig. 13.2a). This model of regeneration resembles, for example, how the mammalian liver regenerates after surgery, by proliferation of remaining hepatocytes [14, 36, 37].

Wing discs in 3rd instar larvae exposed to 25 or 40 Gy (2500 or 4000 R) of γ -rays show reduced mitotic index as early as 1 h after irradiation [22, 68]. We have found a similarly rapid block of M and S phases using 20–40 Gy (2000–4000 R) of X-rays, with these responses requiring conserved check point proteins encoded by *mei-41* (*Drosophila* ATR) and *grapes* (*Drosophila* Chk1) [21, 31]. Mitotic index recovers to pre-irradiation levels at 6–8 h after irradiation [22, 68], and at 48 h after irradiation, mitotic index in the wing disc exceeds the levels found in unirradiated controls [22]. Higher than normal frequency of mitoses was observed also in the larval eye discs at 24 h after exposure to 20 Gy of X-rays [21]. In other words, at longer time during recovery, irradiated cells proliferate faster than unirradiated

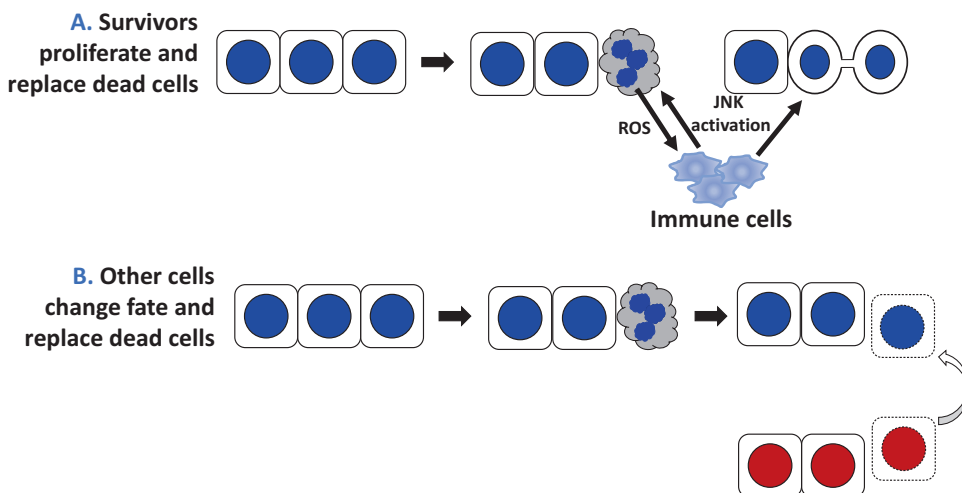


Fig. 13.2 Two sources of regenerative cells in systems that lack dedicated stem cells. (a) In response to cell death (grey cells), survivors proliferate to regenerate the tissue. Dying cells produce Reactive Oxygen Species (ROS) to recruit immune cells. Immune cells stimulate

JNK signaling in the dying cells (for a positive feedback loop) and JNK signaling in surviving cells (to stimulate proliferation). (b) Unrelated cells (red) change fate to replace dying cells

cells. Irradiated wing discs contain 30% fewer cells than unirradiated discs even at 48 h after irradiation [22]. Therefore, extra proliferation observed may be stimulated by the need to replace cells lost to IR-induced apoptosis, which can be detected for as long as 48 h after irradiation in these experiments. The phenomenon in which surviving cells in irradiated tissues proliferate faster than unirradiated cells is conserved in mammalian tumors and is called ‘accelerated proliferation’ [page 384 of [15]]. Accelerated proliferation provides one explanation for the greater success of fractionated radiation therapy in multiple small doses given at regular intervals than delivery as a single large dose; each fractionated dose could kill proliferative cells stimulated by the preceding dose.

What molecular mechanisms stimulate surviving cells to proliferate when their compatriots have been killed by IR? The signals that instruct survivors to proliferate, we now know, come from the dying cells themselves in a process called Apoptosis induced Proliferation or AiP, a phenomenon seen also in human cancer models [reviewed in [6, 7, 9, 38, 51]]. In *Drosophila* where AiP is best understood, the required components in the dying cells include death regulators p53, JNK and apical caspase Dronc (see

Fig. 13.3 for apoptosis signaling in *Drosophila*). AiP in some contexts also requires mitogens Wg and Dpp (for AiP from dying epithelial cells) or Hh (for AiP from dying photoreceptors in the eye disc). These mitogens are thought to be produced in the dying cells, with their production being dependent on p53, Dronc and JNK.

Most experiments in *Drosophila* that addressed AiP employed apoptosis induction with genetic means rather than IR. In these experiments, expression of pro-apoptotic genes such as *hid* and *reaper* are targeted to a subset of cells in imaginal discs. Regulation of their expression temporally with the Gal80-Gal4 system allows a burst of apoptosis followed by a period of regeneration. In a variation of this protocol, co-expression of caspase inhibitor p35 generates ‘undead cells’ (see Fig. 13.3). In these cells, apoptosis program has been initiated and apical caspase Dronc is active because it is refractory to inhibition by p35. But effector caspase activity is inhibited so that the cell does not die but persists in a sustained apoptotic state. Both cells that complete genuine apoptosis and undead cells elicit AiP. When AiP occurs in response to cells that complete apoptosis, the product of induced proliferation serves to replace the dead cells and is considered to be ‘compensatory pro-

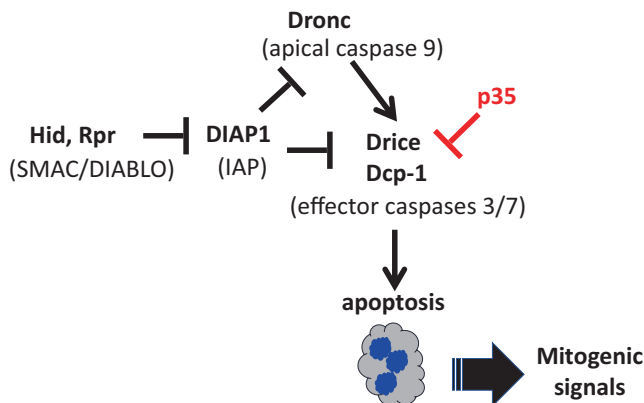


Fig. 13.3 Basic components of apoptotic signaling in *Drosophila*. Mammalian homologs are shown in brackets. Apoptosis requires caspase activity, which is normally kept in check by Inhibitor of Apoptosis Proteins (IAPs). Upon apoptosis induction, for example by X-rays, pro-apoptotic proteins Hid and Rpr neutralize IAPs to result in caspase activation. Apoptotic cells produce mitogenic sig-

nals to maintain tissue homeostasis. Viral caspase inhibitor p35 inhibits effector caspases but not apical caspases. A cell exposed to both death stimuli and p35 activates apical caspases and initiates the apoptotic program, but cannot complete it. Such an ‘undead’ cell remains alive and shows sustained mitogenic signaling

liferation' that restores normal structures. Genetic screens for mutations that fail to restore normal structures have identified many components of AiP as well as regulators that ensure precise growth control and tissue repatterning during regeneration [for example, [2, 27, 53–55]]. When AiP occurs in response to undead cells, the product of induced proliferation creates supernumerary cells. Because undead cells produce sustained mitogenic signaling, AiP from undead cells results in tissue overgrowth and hyperplasia. Genetic screens for mutations that suppress such overgrowth have identified new components of AiP [for example, [7, 8, 10]].

Caspase-driven mitogenic signaling by dying cells is conserved in mammals in a phenomenon called Phoenix-Rising which has proved to be highly relevant to radiation therapy [19, 33]. Here, mitogenic signaling by lethally irradiated cancer cells or fibroblasts stimulate other cells to proliferate, both in culture and in mice. This effect requires effector caspase 3, which cleaves calcium-independent Phospholipase A2, ultimately leading to the production of Prostaglandin E2 (PGE2), a signaling molecule known to stimulate stem cell proliferation, tissue regeneration and wound healing [33]. Caspase 3^{-/-} mutant mice show attenuated skin wound-healing and liver regeneration [33], and fail to repopulate the tumors after radiation treatment [19]. This is as expected if caspase-mediated mitogenic signaling is important for regeneration after IR damage. In human head and neck or breast cancer patients, activated caspase 3 staining in the tumor correlates with recurrence and reduced survival [19], suggesting that findings from *Drosophila* and mice are likely relevant to human cancers. PGE2 is not the only mitogen from dying cells. Another study identified WNT16B as the mitogen released by dying fibroblasts that promote survival and proliferation of prostate cancer cells [59]. Yet another study identified Shh signaling as a component of mitogenic signaling from irradiated cancer cells to unirradiated cancer cells [34]. PGE2 or similar molecules have not been implicated in AiP in *Drosophila* but Wg (*Drosophila* Wnt1) and Hh (founding member of the conserved family that includes Shh) are both

known mediators of AiP and compensatory proliferation as described in a preceding section. Thus, *Drosophila* models can predict not only conserved phenomena but also conserved molecular mechanisms.

13.5 Cross Talk Between Radiation Responses and the Immune System

Tissue damage in multicellular organisms stimulates the immune system. A study of AiP that results from undead cells in the *Drosophila* larval eye imaginal discs found that innate immune system is activated upon tissue damage and plays a role in AiP [10]. The study was designed to investigate how caspase activity leads to JNK activation. The data identified an extra-cellular signaling loop that involves Reactive Oxygen Species (ROS). Specifically, apical caspase Dronc is required cell-autonomously to activate a membrane-associated NADH oxidase Duox. Duox activity results in the production of extra-cellular ROS. Indeed, mis-expression of enzymes that reduce cytoplasmic ROS had little effect on AiP while mis-expression of enzymes that reduce extracellular ROS reduced JNK activation and AiP [10]. In agreement with these results, an independent study in regenerating larval wing discs found that up-regulation of a co-factor for Duox was required to sustain ROS production and regenerative signaling [27].

Duox was required for the recruitment of hemocytes to undead cells and for the induction of a JNK activity reporter [10], suggesting that extra-cellular ROS was required to recruit circulating hemocytes and activate JNK. An allele of transcription factor Srp that specifically inhibits hemocyte differentiation also reduced JNK activation and AiP. Ectopic JNK activation, however, did not recruit hemocytes, suggesting that hemocyte recruitment is upstream of JNK activation. These data led to the model in which hemocytes activate JNK in the dying cells for mitogen production, triggering a positive feedback loop, and hemocytes activate JNK in surviving neighbors, to stimulate

proliferation (Fig. 13.2a). *Drosophila* TNF- α homolog Eiger and its receptor Grnd were identified as possible mediators of hemocyte-to-epithelial cell signaling [10]. Thus immune cell presence and activity at the site of damage promotes regenerative proliferation. In *Drosophila* neoplastic tumors, where oncogenic RAS activity maintains tumor cells in an undead state, caspase activity like-wise produces both intracellular and extracellular ROS, hemocyte recruitment, and further proliferation of tumor cells [43].

The above-described studies employed cells dying or undead because of genetic ablation. In the context of cell killing by IR in *Drosophila*, there is very little known about immune cell involvement. In a study using UV radiation instead of IR, damage to the retina results in the production of Pvf1 (a *Drosophila* PDGF/VEGF-like ligand) production, which in turn activates its receptor Pvr in hemocytes and induces a macrophage-like morphology [25]. Components of this paracrine signaling is required to prevent tissue loss after UV exposure, suggesting that stimulation of the immune cells by signals from the dying cells somehow contribute to regeneration. We have shown that exposure of larval discs to ionizing radiation (IR) also results in transcriptional up-regulation of Pvf1 and Pvf2 [60]. Pvf1, we found, is likewise needed to limit IR-induced apoptosis [1]. It remains to be seen if Pvf1 from IR-damaged cells also stimulates immune cells.

IR is known to induce intracellular Reactive Oxygen Species [50]. Whether IR also induces extracellular ROS and whether such induction has similar consequences as AiP in genetic ablation models remain to be investigated. But IR activates both apical and effector caspases, as well as JNK. IR also induces AiP [28, 44]. Thus all indications are that IR exposure also engages in immune-cell-mediated paracrine signaling described in preceding paragraphs for experiments using genetic ablation, but this possibility has not been tested experimentally. But if such an interaction exists, then it would parallel the cross talk between IR responses and the immune system seen in mammalian tumors [for example, [67]].

13.6 Cell Fate Changes Induced by IR

In studying the effect of X-rays on larval wing discs, we identified a second mode of regeneration in addition to AiP [61–63]. We found that cells of the future wing hinge region are protected from IR-induced apoptosis by the actions of Wg (*Drosophila* Wnt1) and JAK/STAT activity acting cell-autonomously within these cells [61]. Lineage tracing shows that as the disc regenerates during a 3 day period after IR, some hinge cells lose the hinge fate, translocate to the future pouch area that suffers more cell death, and express pouch markers [61, 63]. This represents a mode of regeneration in which one cell type changes into another to help replace the lost tissue (Fig. 13.2b). IR-induced cell plasticity here acts to restore the organ but parallels IR-induced cell plasticity that produces tumor-initiating cells after radiation therapy as explained below.

‘Tumor initiating cells’ or ‘Cancer Stem-like Cells’ (CSCs) are defined operationally as cells within a tumor with particularly high ability to regenerate the tumor. Their existence is controversial even with the operational definition, and their numbers in some cancer types appear to depend on experimental conditions. For example, in melanoma, one in a million cancer cells are able to initiate new tumors if implanted into NOD/SCID mice but this number increases to one in three if more immune-compromised NSD (NOD/SCID interleukin 2-receptor gamma chain null) mice were used [47]. What is generally agreed upon is that within a given tumor, cells vary widely in their ability to produce new tumors [35, 71]. In Head and Neck Cancer models where radiation is a major therapy choice, most tumorigenic cells within patient-derived samples show high CD44 expression and the presence of ALDH [26]. Such CSCs represent 0.1% to 4.1% of tumor cell population depending on the patient and can produce tumors nearly 70% of the time when implanted at 1000 cells/mouse. In contrast cells that are ALDH- and show low CD44 expression produced tumors <5% of the time even when 100,000 cells were used per implant. Cancer Stem-like Cells with superior tumor initiating

ability have been identified in multiple types of solid tumors, although associated molecular markers differ for different cancer type, for example CD133 and NPM1 in glioblastoma [70]. Eradication of tumor initiating CSCs is considered necessary for successful therapy and for prevention of metastases to a distant site.

In a hierarchical view of cancer, CSCs produce non-stem cancer cells. In addition, it is now recognized that, non-stem cancer cells are also capable of converting to CSCs. The plasticity that allows non-stem cancer cells and CSCs to interconvert presents a major challenge to any therapy that targets CSCs. Even more concerning, cancer treatments themselves promote the conversion of non-stem cancer cells into CSCs [5, 45]. In particular, IR converts non-stem cancer cells from a variety of cancer types into cells with CSC markers that can initiate new tumors in culture and *in vivo* [30, 32, 65]. An estimated 50% of cancer patients receive IR, alone or as part of their treatment (www.cancer.org). Therefore, it is essential that we understand what aspects of IR exposure induce fate conversion or what factors, cell-internal or external, regulate IR-induced regenerative behavior.

Using the *Drosophila* hinge-to-pouch system to monitor cell fate changes after irradiation, we have been systemically identifying genes needed for cell fate plasticity and cell movement after IR exposure. We have identified signaling molecules [e.g. Wg and STAT, [61]], epigenetic regulators [e.g. Nurf-38, [62]], members of the cell death pathways [e.g. apical and effector caspases, [63]], along with other genes whose exact contribution remains to be dissected. This experimental model has the potential to inform us about IR-induced cell fate plasticity in tumors.

13.7 Drug Screens for Radiation Modulators

IR doses that kill about half of the cells in larval imaginal discs still allow regeneration of these tissues to the extent that viable fertile flies will eclose, albeit after a developmental delay [17,

21]. The extent of delay is IR dose-dependent [17, 21]. IR-induced developmental delay is exacerbated by mutations in DNA Damage Response signaling such as *mei-41* (*Drosophila* ATR) and *grp* (*Drosophila* Chk1) and is dependent on p53 and retinoic acid signaling [16, 68]. The delay in pupariation means that irradiated larvae spent more time feeding than their unirradiated controls, before crawling up the side of the culture vial to initiate the pupa stage. These observations led us to suspect that the delay in pupariation reflects a need to continue food uptake, which in turns allow cellular growth and proliferation needed for regeneration. In support, inhibition of food uptake by switching larvae to poor nutrition after irradiation decreased the survival of larvae into adulthood [20]. Similarly, reduction in the dosage of genes encoding components of growth regulation, using heterozygous mutants in Insulin-like Growth Factor Receptor substrate *chico*, *cdk4* and *Myc*, also reduced the survival of irradiated larvae into adults [20]. *chico*, *cdk4* and *Myc* heterozygotes are viable without irradiation. In other words, (partial) inhibition of growth and regeneration was synthetically lethal with radiation. These findings led us to design a screen for chemical modulators of growth and regeneration that was synthetically lethal with radiation [12, 13, 20]. Such chemicals have the potential for use in combination with radiation therapy.

In the screen, 3rd instar larvae were irradiated with doses that allowed 50% of larvae to reach adulthood. Those that produced viable adults ‘eclosed’ from the pupa case, leaving it empty while those that failed to do so left a ‘full’ pupa case. Thus, counting full vs. empty pupae produced a quantitative measure of radiation sensitivity [12, 13, 20]. Irradiated larvae were placed in culture vials each of which contained a chemical of interest in the screen. Chemicals that reduced survival in a statistically significant manner were identified. Exploiting *Drosophila* genetics, an additional layer was added to the screen. Chemical libraries were screened using p53 or *grp* (*Drosophila* Chk1) mutant larvae and the hits were counter screened against wild type larvae

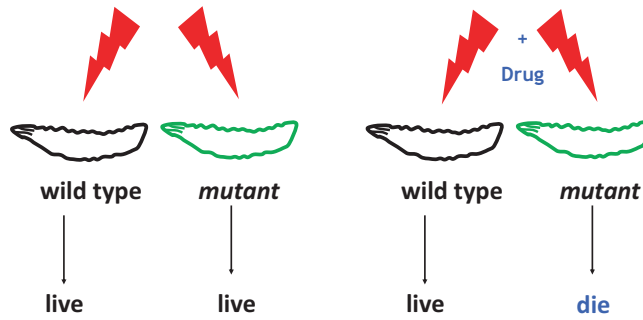


Fig. 13.4 The design of a screen to identify drugs that are synthetic lethal with radiation on mutant larvae. In the absence of the drug, wild type (black) and *grp/Chk1* mutant (green) larvae are equally sensitive to X-rays. The screen is designed to identify drugs, that when present,

allow irradiated wild type larvae to survive but kill irradiated mutant larvae. Thus the drug is synthetically lethal with radiation, with greater effect on *grp* mutants than on wild type

(Fig. 13.4). Those that showed greater effect on larvae with cancer-relevant mutations compared to wild type were further selected for study. Thus, the screen aimed to identify molecules with a potential therapeutic index (greater efficacy on mutant cancer cells over normal tissues).

Screens through chemical libraries identified drugs approved for use in combination with radiation such as camptothecin, a topoisomerase I inhibitor, providing proof of concept data that a *Drosophila* screen can identify drugs that are applicable to human cancer [11, 20]. The screens yielded an interesting group of three chemical scaffolds, all of which to act by inhibiting translation elongation [11]. This is of interest because stimulation of translation elongation, by degradation of the inhibitor EF2 Kinase, has been shown to be critical during recovery from radiation damage in human osteosarcoma cells [29]. Thus, inhibition of translation elongation, with chemical hits found in the *Drosophila* screen, was expected to interfere with recovery after IR damage, thereby increasing the effect of IR. In support of this idea, one of the inhibitors of translation elongation found in the *Drosophila* screen, bouvardin (NSC259968), was subsequently found to enhance the effect of IR in human cancer models [57]. Of more interest, the ability of bouvardin as a radiation enhancer was greater on cancer cells than on non-transformed cells, mirroring how the *Drosophila* screen was

designed to identify chemicals that differentiated between *p53/chk1* mutants and wild type.

13.8 Conclusions

From revealing the mutagenic effect of X-rays to dissecting the molecular basis for Apoptosis-induced Proliferation, *Drosophila melanogaster* has been a proven experimental model to study radiation responses and regenerative mechanisms that are conserved to human. Additional uses of the *Drosophila* model to address other aspects of radiation biology such as the cross-talk with the immune system, IR-induced cell fate plasticity, and identification of chemical radiation-modulators hold promise. With powerful genetic tools, *Drosophila* remains the premier model for gene discovery. It is through innovative use of forward genetic screens, combined with the power of reverse genetics to illuminate mechanism, that we will uncover new mechanisms in *Drosophila* towards improving radiation therapy of human cancers.

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Conflict of Interest The author owns equity in Suvica, Inc.

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