Ionising Radiation Induces Autophagy in the Adult *Drosophila* Brain and Intestine.

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Abstract

Exposure to various genotoxic and oxidative stresses can induce changes in the internal state of a cell leading to cell death. Ionizing radiation is routinely used in clinics to treat various types of cancers, with secondary effects sometimes damaging internal organs particularly the gut. *Drosophila melanogaster* has become one of the most trusted model organisms to study human disease and various biological pathways. Here we have used gamma radiation to determine for the first time whether they have an effect on autophagy in two different tissues in *Drosophila*; the gut and the brain. We find that in both tissues exposure to radiation leads to an increase in autophagy.

Keywords: Ionising radiations • Autophagy • *Drosophila* • Cancer • Gamma radiation

Introduction

Maintaining a healthy cellular homeostasis is crucial for the survival of animals. This requires the ability to protect cells and cellular components from damage from toxins and various sources of mutagens including chemicals and radiation since we cannot avoid them completely as we sometimes use them to our advantage, e.g. we use chemicals to treat diseases and radiations to treat cancers. The effect of these mutagens and toxins vary according to their targets, all leading to some level of cellular stress. While the genotoxic effects of radiation are well known, their effects on other vital cellular processes such as autophagy are much less known.

Radiation has been used in medicine for over a century with varied application [1,2]. In modern medicine, radiation is used mostly for the treatment of cancers, and it is estimated it should be administered to approximately 52% of all cancer patients [3]. Yet the effects of radiation on the surrounding tissues is unavoidable and can lead to a range of short- and long-term side effects including hematopoietic and gastrointestinal syndrome, cardiovascular and nervous system syndromes and a range of other bystander effects. [4-7]. Once a cell has been exposed to high doses of radiation, which are required for treatment of cancers, they respond by activation of a signalling cascade either leading to cell cycle arrest or apoptosis.

Radiation-induced free radical generation can lead to oxidative stress in the cell which predisposes it to apoptosis. Unless the stress molecules are quenched (such as ROS quenching by antioxidants), they may lead to protein oxidation and subcellular organelle damage. If unchecked, these damages can lead the cell to undergo apoptosis. Other responses to radiation exposure may include inflammation of the surrounding tissues, signalling macrophages and other immune responsive cells to the site of injury further escalating the damage response [8,9].

Once a cell has incurred some organelle or protein damage, it may respond by activation of autophagy, degrading and removing the damaged particles. This however requires functional lysosomes that contain the hydrolases on which autophagy depends. The damaged substrates are recognised by the cell and tagged for degradation. Failure to do so can result in the cell building up damaged materials such as oxidised proteins and lipids which can then interfere with the normal functioning of the cell [10].

Radiation cause hydrolysis of water, producing ROS, which interact with macromolecules and induce oxidative damage and stress [11]. Radiation-induced oxidative damage has been extensively studied in humans, and ROS imbalances have been shown to persist after treatment [12]. From looking at the *Drosophila* research, it is clear that radiation induces oxidative stress, at least short-term. Genes such *GstT4* and *GstD1* involved in oxidative metabolism have been shown to have increased expression post irradiation [13,14]. ROS imbalance is capable of inducing autophagy through the mTOR-AMPK pathway [15].

While it is well known that exposure to radiation leads to activation of the apoptotic pathway [16], nothing is known about how radiations affect autophagy, one of the cells most common response to toxins and damage [17,18]. Here we used *Drosophila melanogaster* as a model system to evaluate the effect of radiation exposure on autophagy. We find that radiation exposure leads to an increase in the levels of autophagy in two distinct cell types in *Drosophila* indicating that the initial response to radiation might be modulated through autophagy.

Materials and Methods

Drosophila stocks and husbandry

Drosophila were raised at 25°C on standard cornmeal/molasses/agar media. The following *Drosophila* stocks were used: *uas-mCherry::Atg8* (gift from T. Neufeld), *GMR61G12-Gal4* (Flylight collection, Jenelia Farm), *Myo1A-Gal4* (gift from J. de Navascués) and *uas-mCD8::GFP* (Bloomington Stock Centre).

Ionizing radiation exposure

3 to 6-day old flies were collected in vials containing cornmeal medium. A group of these was exposed to gamma irradiation using a Cesium-137 γ-ray irradiator for a total of 150 Gy, administered at 0.43 Gy/min. Control group was kept in the same room for the duration of gamma ray exposure.

Dissections, imaging and analysis

Flies expressing *UAS-mCherry::Atg8* and *UAS-mCD8::GFP* driven by *GMR61G12 Gal4* were dissected as described previously [19]. Briefly, fly brains were dissected from 3 to 6-day old flies in cold PBS and fixed in 4% paraformaldehyde for 30 min. The tissues were then washed three times with PBST (10 min each), mounted in vectashield and stored at 4oC until imaged. Images were captured using Zeiss Spinning Disc Confocal (Cell Observer) microscope and a 63X oil immersion objective (numerical aperture 1.3).

Midguts were dissected from 3 to 6-day old flies directly into ice-cold PBS, fixed in a formalin (4%) solution under a heptane phase for 15 min. Tissue was permeabilised using methanol (100%) for 15 min. Permeabilised tissue was then blocked 3X for 15 min each using PBS containing 0.1% Triton-X 100 (PBT) and 2% bovine serum albumin (BSA). Tissue was stained with the primary antibody Rabbit anti-RFP (Takara 632496) overnight (~16 hrs) at 4°C with mild rocking, followed by washing in PBT (3X rinses and 3X washes). Tissue was stained with the secondary antibody Donkey anti-Rabbit-A594 (Thermo Scientific A21207) for 2 hr at room temperature with mild rocking. DNA was stained with Hoescht at 1:5,000 (Sigma Aldrich B2261, stock solution at 10 mg/ml) which was added alongside secondary antibodies. Tissue was washed with PBT and mounted in home-made mounting medium (Glycerol:PBS 80:20 with 4% propyl gallate). Confocal stacks were obtained in a Zeiss LSM 710 with an EC Plan 16 Neofluar 40X and 63X oil immersion objectives (numerical aperture 1.3). All stack positions were acquired in the posterior midgut with same laser intensities and exposure time. Images were processed using FIJI [20], and statistical analysis was performed using Graphpad (PRISM 9.0).

Results

Effect of radiation on gut

To assess the effect of gamma irradiation on the gut, we used UAS-mCherry *Atg8* as a marker. We exposed a group of flies expressing mCherry-tagged

Atg8 in the gut (*Myo1A-Gal4>UAS-mCherry::Atg8*). Irradiation did not cause any lethality nor any other gross defects in flies until they were dissected. In comparison to controls, the gamma irradiated flies showed an increase in *Atg8* levels in the gut one day after irradiation (t-test, p<0.008) (Figures 1A-1D).

Effect of radiation on the brain

To determine the effect of irradiation on adult fly brain we generated flies expressing mCherry-tagged *Atg8* in a small subset of neurons in the central brain which normally express in the *Pigment dispersing factor (pdf)* using *GMR61G12-Gal4*. These neurons are much bigger than the rest of brain neurons and could be easily imaged. This way we were able to focus on a small subset of neurons to look for changes in the mCherry signal within individual neurons. In comparison to the controls, we observed a significant increase in the *Atg8* levels indicating an increase in autophagy levels (Figures 2A-2D).

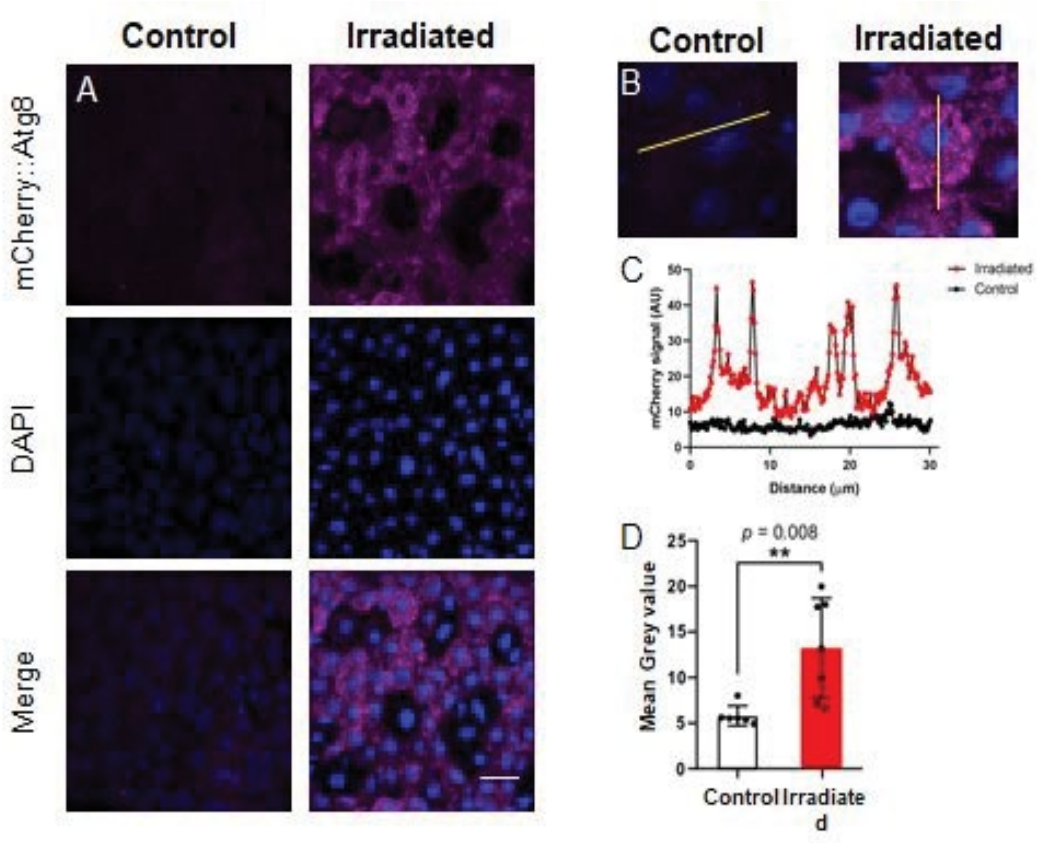

Figure 1. Effects of irradiation on gut autophagy. (A) Representative maximum projections of irradiated and control *Drosophila* gut. Scale bar is 20 microns. (B) Digitally magnified close-ups of cells, yellow line represents sampling path for panel (C). (C) mCherry intensity value per pixel along paths that were drawn in panel. (D) Mean grey value of mCherry in control and experimental tissue. (t-test, n>8) (D).

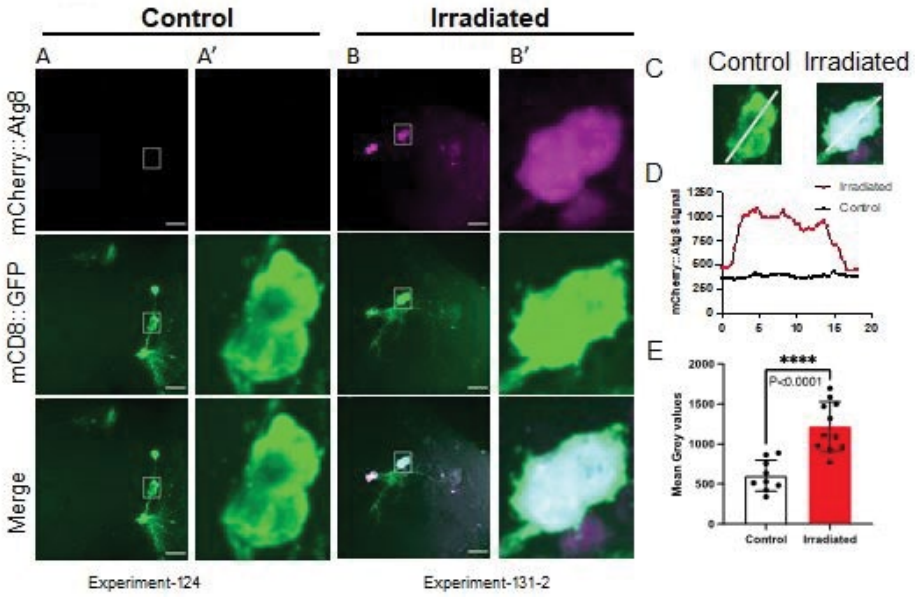

Figure 2. Autophagy is increased in response to gamma irradiation. In comparison to control (A) brains the brains from irradiated flies (B) show increases levels of autophagy marker mCherry::*Atg8* in *pdf*-expressing neurons in the brain. (B). Projections of representative images of the adult brain highlighting the neurons considered for measurement. Scale bar is 20 microns. (A′ and B′) Digitally magnified close-ups of neurons. (C) Representative images of neurons, solid lines indicate sampling path for mCherry signal sampling presented in panel D. (D) mCherry signal profiles along a selected path. (E) Mean grey value of mCherry signal in control and irradiated fly neurons (t-test, n>8).

Discussion and Conclusion

Using *Drosophila* as a model system we have identified the effect of radiation on a biologically relevant pathway. While the exact mechanisms and the genetics involved are yet to be explored our results suggest that an increase in autophagy as a result of irradiation might be a generalised mechanism across different tissues. *Drosophila* provides an opportunity to perform future experiments to explore the genetics and biochemical effects of irradiations because of the repertoire of tools available to do so.

Gamma irradiation is used as a curative measure for treatment of various cancers. The effect of this ionising radiation on different tissues and their radio sensitivity is an important factor which could help determine the optimum dosage for a given patient. The intracellular damage caused by repetitive exposure to such radiation poses a threat to patient's health that is already weakened by the impact of the disease. Knowing the full scale of impact of radiation on cellular mechanisms can help design strategies which can be used to minimise the negative impact of radiations.

Cellular response to oxidative stress includes a waste-clearance mechanism, known as autophagy, which depends on a cascade of events leading to isolation of the damaged subcellular substrates and their degradation by the lysosomal enzymes. The effect of gamma radiations on this mechanism, so crucial for survival from cellular damage, is yet unknown. We show that the cellular autophagy is significantly increased in response to gamma irradiation in two distinct types of cells in young *Drosophila*. While the cellular morphology and the gut intactness in the time scale of our experiment is unaffected, the increase in autophagy might indicate an early response to the gamma radiation. The consequence of increased autophagy can be a determining factor for cellular survival. Significantly high damage to the cellular cytoplasm and DNA could lead to overpowering of the autophagic mechanisms leading to cell death. Since the central nervous system neurons have several supporting tissues, such as microglia and oligodendrocytes, which help clear some of the secreted waste materials produces by stressed neurons, it is pertinent to determine the impact of gamma radiation on these cell types. In addition, to better understand the consequence of increased autophagy and whether the increased levels of autophagy actually represent a blockage of autophagic flux or a stimulation of autophagy, further investigation is required.

Competing Interests

The authors have no competing interests to disclose.

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Author Contributions

BM conceptualised and designed the experiments. BM and TT prepared materials,, collected and analysed data and wrote the manuscript. Both authors read and approved the final manuscript.

Data Availability

The data associated with this manuscript is available from the authors on request.

Ethics Approval

No ethical approvals were needed for the work.

Consent to Participate and Publish

No human subjects were involved in this work and hence there was no need for consent.

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