Pathobiology of Aging & Age-related Diseases

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ANIMAL MODEL BRIEF

Planarians as a model of aging to study the interaction between stem cells and senescent cells *in vivo*

Patrick M. Perrigue[1]*, Joseph Najbauer[2], Agnieszka A. Jozwiak[1], Jan Barciszewski[1], Karen S. Aboody[3,4] and Michael E. Barish[3]

[1]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland; [2]Department of Immunology and Biotechnology, University of Pécs, Pécs, Hungary; [3]Department of Neurosciences, City of Hope National Medical Center and Beckman Research Institute, Duarte, CA, USA; [4]Division of Neurosurgery, City of Hope National Medical Center and Beckman Research Institute, Duarte, CA, USA

The depletion of stem cell pools and the accumulation of senescent cells in animal tissues are linked to aging. Planarians are invertebrate flatworms and are unusual in that their stem cells, called neoblasts, are constantly replacing old and dying cells. By eliminating neoblasts in worms via irradiation, the biological principles of aging are exposed in the absence of wound healing and regeneration, making planaria a powerful tool for aging research.

Keywords: *aging*; *epigenetics*; *cellular senescence*; *stem cells*; *planarian*; *histone H3K27*; *invertebrate*

*Correspondence to: Patrick M. Perrigue, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Z. Noskowskiego 12/14, PL-61-704 Poznan, Poland, Email: pperrigue@ibch.poznan.pl

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Planarians have inherent regenerative properties (1,2). Adult somatic stem cells called neoblasts allow for amputated fragments of the organism to generate an entirely new worm (3,4). Breakthroughs in genome sequencing (5) and development of RNAi technology for knocking down gene expression (6) have opened up the possibility of dissecting gene function in planarians. Because the human aging process is very complex, simple invertebrate organisms are needed to model the pathobiology of aging in order to advance understanding of it. The planarian model is advantageous for the study of aging due to its size, tractable genetics, easy maintenance, and ability to recapitulate the aging process. The regenerative capacity of planarians, along with shared senescence-associated genes with humans, provided the rationale to study interaction between stem cells and senescent cells *in vivo* (7).

The normal function of cellular senescence is tumor suppression (8,9). This is achieved through cellular reprogramming, which inhibits the proliferation of damaged cells. It is postulated that cellular aging, which overlaps with cellular senescence, results in a loss of cell identity (10). If so, a pattern of histone modifications that determine cell fate also determine cellular aging and could be studied using irradiated planarians. Histone H3 lysine 27 trimethylation (H3K27me3) is a repressive epigenetic mark known to control stem cell differentiation (11,12). Notably, the depletion of H3K27me3 has been reported in senescent cells and implicated in many diseases of aging (13). This epigenetic mechanism appears to drive the progression of cellular senescence and 'inflammaging' (increased inflammation with aging). We hypothesized that aging is accompanied by a global decrease in H3K27me3 levels, which will be reflected in accumulation of senescent cells in irradiated planarians.

The planarian, *Dugesia tigrina*, was purchased from Ward's Natural Sciences (Rochester, NY) as a mixed population of unknown genetic background. A standard maintenance and care protocol was followed (14). Worms were housed in plastic food storage containers with purified spring water at room temperature and fed fresh beef liver every 3–4 days. Worms between 10 and 12 mm long were selected and starved for 1 week before all experiments. All procedures with animals were followed in accordance with the Helsinki Declaration of 1975, as revised in 2008.

To induce aging, worms were treated with one dose of radiation (100 Gy) and subsequently imaged for 10 days (Fig. 1). Irradiated worms appeared phenotypically healthy at 1 and 3 days post irradiation (dpi), followed by a gradual decrease in body size and appearance of lesions at 5 and 7 dpi. All of the irradiated worms lysed by 10 dpi due to inability to maintain tissue homeostasis and eventually all died at 14 dpi ($n = 10$). Similar to previous reports, the physical appearance, body mass, and size of the 10 dpi worm compared to the untreated control in

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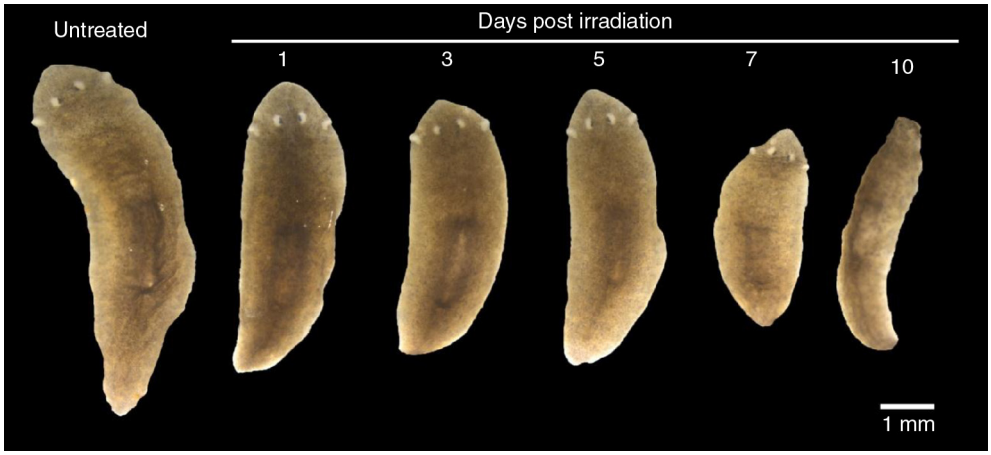

Fig. 1. Images of planaria that were γ-irradiated using a ^{137}Cs source (100 Gy).

Fig. 1 suggest that radiation treatment triggered large amounts of apoptotic cell death (15). In planarians, apoptosis of differentiated cells has been reported during regeneration and remodeling of preexisting tissues in planarians (16). It is unknown if the status of some of the remaining cell types that evade apoptosis reprogram to cellular senescence and contribute to the process of radiation-induced aging in planarians.

We next investigated histone modifications related to stem cells and aging in a similar time-course experiment. Neoblasts are the only dividing cells in planarians and can be tracked using the proliferation marker phosphohistone H3 serine 10 (H3S10P) (17). Western blot analysis indicated the complete elimination of neoblasts by 3 and 5 dpi, followed by a decrease in H3K27me3 levels by 5 and 7 dpi (Fig. 2A). These data suggest that depletion of neoblasts results in accumulation of senescent cells in irradiated worms.

Cellular senescence is related to the limited proliferation capacity of stem cells observed during the biological processes of normal and pathological aging (18,19). To directly detect the presence of senescent cells

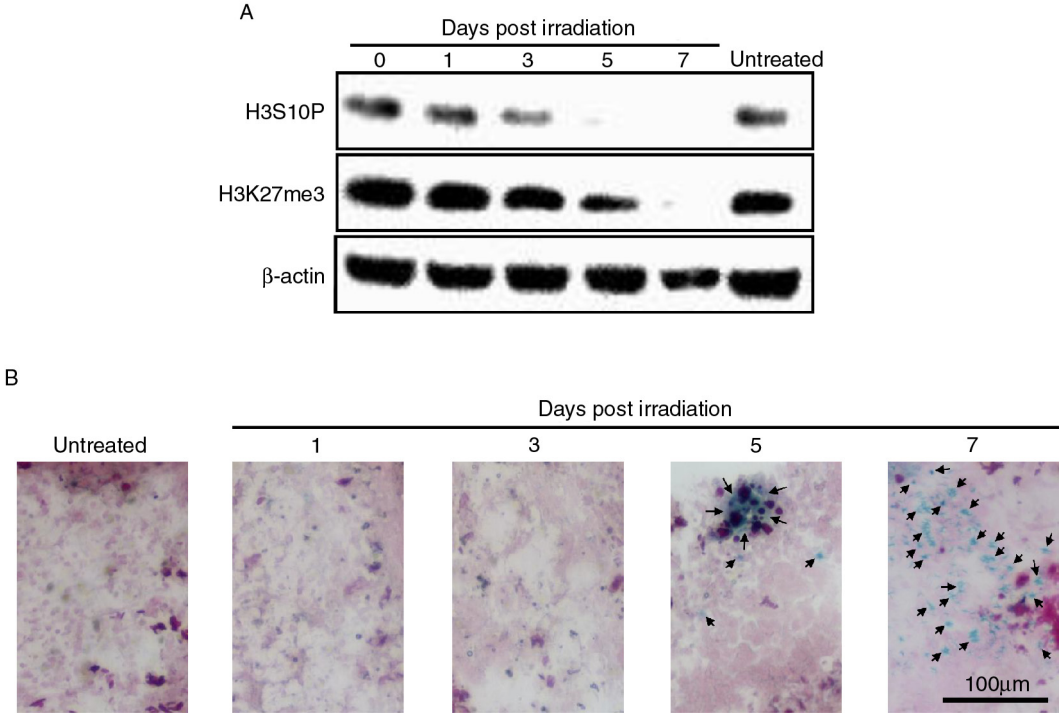

Fig. 2. (A) Western blot of H3S10P, H3K27me3, and β-actin. The following commercially available antibodies were used at the indicated concentrations: H3S10P (05-806, Millipore, 1:50,000), H3K27me3 (GTX12184, Genetex, 1:1,000), and β-actin (GTX109639, Genetex, 1:12,500). Lysates for western blotting were made from five worms that were collected at each time point. (B) SA-β-gal activity staining in irradiated worm tissues. Black arrows point to positive staining for SA-β-gal activity.

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in the tissues of irradiated planarians, we stained for senescence-associated beta-galactosidase (SA-β-gal) activity, which is a known biomarker of cellular senescence in human tissues, as described previously (20,21). SA-β-gal activity was detected at 5 and 7 dpi, but rarely in untreated and 1 and 3 dpi worm tissues (Fig. 2B). Altogether, these findings provide a basis and rationale for further studies in planarians of the basic principles of aging.

Stem cells are known to build and repair the body, but the signals that stem cells use to navigate through normal tissue towards an injury site or to replace old and dying cells are poorly understood. Accompanying the process of cellular senescence is the upregulation of select genes encoding cytokines, proteases and growth factors, collectively known as the senescence-associated secretory phenotype (SASP) (22). Our previous work has shown that the SASP is activated by a specific histone H3K27 demethylase, jumonji domain-containing protein 3 (JMJD3) (23). In cancer, the removal of methyl groups from H3K27me3 by JMJD3 activates a gene expression signature which overlaps with inflammaging. This same mechanism is linked to the migration of stem and other cell types to tumors (23). Notably, nucleosome loss and a drop in total histone protein levels are also linked to cellular aging (24). Future studies are needed to determine whether the global loss of H3K27me3 observed during radiation-induced aging in planarians is a mechanism of histone loss/exchange or of active demethylation.

In planarian worms, senescent cells that accumulate in healing wounds and aged tissues may secrete chemokines to recruit neoblasts needed for healing and immortality. The conservation of senescence-related genes overlapping with the SASP in planarians may also provide a model to study the process of inflammaging. In addition, we speculate that in planarians senescent cells accumulate in healing wounds and function to curb fibrosis during tissue repair. RNAi screens in this relatively simple organism (25,26) can be used to identify additional genes involved in wound healing and aging. Expression products of these genes could then be evaluated further for their role in senescence and stem cell migration. Identification of mechanisms that control cell proliferation and migration may provide the basis for designing therapeutic interventions against dysfunctional senescent cells to inhibit aging as well as tumor progression.

Authors' contributions

Conception and design: PMP and JN. Development of methodology: PMP and AAJ. Acquisition of data: PMP and AAJ. Analysis and interpretation of data: PMP, JN, AAJ, JB, KSA and MEB. Writing, review, and/or revision of the manuscript: PMP, JN, AAJ, JB, KSA and MEB. Administrative, technical, or material support: PMP, JN, AAJ, JB, KSA and MEB.

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