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Photon hormesis deactivates alpha-particle induced bystander effects between zebrafish embryos

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ABSTRACT

In the present work, we studied the effects of low-dose X-ray photons on the alpha-particle induced bystander effects between embryos of the zebrafish, *Danio rerio*. The effects on the naive whole embryos were studied through quantification of apoptotic signals (amounts of cells undergoing apoptosis) at 24 h post fertilization (hpf) using vital dye acridine orange staining, followed by counting the stained cells under a fluorescent microscope. We report data showing that embryos at 5 hpf subjected to a 4.4 mGy alpha-particle irradiation could release a stress signal into the medium, which could induce bystander effect in partnered naive embryos sharing the same medium. We also report that the bystander effect was deactivated when the irradiated embryos were subjected to a concomitant irradiation of 10 or 14 mGy of X-rays, but no such deactivation was achieved if the concomitant X-ray dose dropped to 2.5 or 5 mGy. In the present study, the significant drop in the amount of apoptotic signals on the embryos having received 4.4 mGy alpha particles together X-rays irradiation from 2.5 or 5 mGy to 10 or 14 mGy, together with the deactivation of RIBE with concomitant irradiation of 10 or 14 mGy of X-rays supported the participation of photon hormesis with an onset dose between 5 and 10 mGy, which might lead to removal of aberrant cells through early apoptosis or induction of high-fidelity DNA repair. As we found that photons and alpha particles could have opposite biological effects when these were simultaneously irradiated onto living organisms, these ionizing radiations could be viewed as two different environmental stressors, and the resultant effects could be regarded as multiple stressor effects. The present work presented the first study on a multiple stressor effect which occurred on bystander organisms. In other words, this was a non-targeted multiple stressor effect. The photon hormesis could also explain some failed attempts to observe neutron-induced bystander effects in previous studies, as neutron sources invariably emit neutrons with concomitant gamma-ray photons, which is often referred to as gamma-ray contamination.

1. Introduction

The recent Fukushima reactor accident has rekindled immense concerns and interests on radioecological effects of nuclear fallouts. One of the most intriguing phenomena in radioecology is the allelopathy that coordinates a species-level survival response (Mothersill et al., 2007) towards ionizing radiation, which appears to be present at least in aquatic species living in close proximity and sharing the same media.

One essential element contributing to allelopathy was the radiation-induced bystander effect (RIBE) between living organisms (Choi et al., 2015; Mothersill et al., 2006, 2007, 2009; Smith et al., 2011, 2013; Surinov et al., 2005). RIBE was first discovered by Nagasawa and Little (1992) in an *in vitro* study, and the term RIBE was originally used to describe the non-targeted effects where non-irradiated cells responded as if they had themselves been irradiated upon receiving signals from irradiated cells through either partnering or medium transfer (Blyth and Sykes, 2011; Goldberg and Lehnert, 2002). It had been generally accepted that RIBE signals could affect distant or neighboring cells through diffusing soluble molecules from the irradiated cells into the medium conditioning the non-irradiated cells or through cellular gap-junction intercellular communication (Goldberg and Lehnert, 2002; Little, 2006; Morgan and Sowa, 2007; Mothersill and Kadhim, 2012; Mothersill and Seymour, 2001, 2004; Prise and O'Sullivan, 2009; Wang et al., 2015). RIBE was later also confirmed to occur between individuals of mice (Surinov et al., 2005), freshwater rainbow trout

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(Mothersill et al., 2006), zebrafish (*Danio rerio*) and Medaka (*Oryzias latipes*) (Mothersill et al., 2007, 2009), bullfrog tadpoles (*Rana catesbeiana*) (Audette-Stuart and Yankovicha, 2011), and zebrafish (*Danio rerio*) embryos (Choi et al., 2010a, 2012a, 2013; Yum et al., 2009).

Besides ionizing radiations, Asur et al. (2009) suggested chemicals could also induce bystander signaling. Various studies were carried out to investigate the capability of chemicals to induce bystander effect. For instance, Rugo et al. (2005) reported that the progeny of cells exposed to mitomycin C (MMC) could induce genomic instability in unexposed neighboring cells. The ability of chloroethyl nitrosourea, a chemotherapeutic DNA-alkylating agent, to induce the bystander effect through soluble factors in primary melanomas was also demonstrated by Demidem et al. (2006). Moreover, Cogan et al. (2010) showed that a short low dose of Cr (VI) could induce bystander signaling similar to those generated upon exposures to low doses of radiation. More recently, exposures of rat pheochromocytoma (PC12) cells to 10 mM of lead acetate were also found to induce bystander effects in neighboring cells (Guo et al., 2014).

RIBE could be induced by alpha particles (Azzam et al., 1998; Lorimore et al., 1998), X-ray or gamma-ray photons (Lyng et al., 2000; Mothersill and Seymour, 1997; Prise et al., 1998). Interestingly, however, all previous attempts to demonstrate RIBE induced by neutrons failed, including RIBE between cells (Liu et al., 2006; Seth et al., 2014) and RIBE between zebrafish (Wang et al., 2011). It is well established that neutron sources invariably emit neutrons with concomitant gamma-ray photons, which is often referred to as gamma-ray contamination. It has been proposed that such low-dose photon irradiations can help mitigate cellular damages in living organisms induced by other ionizing radiations. Such mitigation will be hereafter referred to as "photon hormesis" in the present work, which can mean gamma-ray hormesis or X-ray hormesis depending on the origin of the photons. The mechanisms underlying photon hormesis included removal of aberrant cells through early apoptosis and induction of high-fidelity DNA repair (Bauer, 2007; Portess et al., 2007; Scott and Di Palma, 2006). Photon hormesis has been proposed as an explanation for the suppression of alpha-particle-induced lung cancers (Scott, 2008; Scott et al., 2008), reduction in the frequency of micronucleated cells in neutron-irradiated human lymphocytes (Rithidech and Scott, 2008), and mitigation of the dose response of zebrafish embryos to neutrons (Ng et al., 2015a). Despite the fascinating proposal of and predictions for photon hormesis, it is indeed quite surprising that there have been no attempts to the best of our knowledge on proving that low-dose photons do mitigate cellular damages in living organisms induced by other ionizing radiations and deactivate RIBE between living organisms. Investigations on these issues formed the objectives of the present work.

In the present research, zebrafish (*Danio rerio*) embryos were chosen as the model for assessing RIBE between living organisms. Zebrafish embryos had been widely employed for examining biological effects of ionizing radiation (Bladen et al., 2005; Choi et al., 2010b, 2010c; Choi and Yu, 2015; Daroczi et al., 2006; Geiger et al., 2006; McAleer et al., 2005; Yum et al., 2007, 2009, 2010). The advantages of this model included its rapid development, high fecundity, and its genomes sharing considerable homology with human genomes (Barbazuk et al., 2000).

We hypothesize that photon hormesis can mitigate cellular damages in living organisms induced by other ionizing radiations, and can thus deactivate alpha-particle induced bystander effects between zebrafish embryos.

2. Materials and methods

2.1. Experimental animals

Adult zebrafish (*Danio rerio*) with mixed gender were kept in 45 L fish tanks with water maintained at 28.5 °C. A light-dark cycle of 14 h of light and 10 h of dark periods was adopted to maintain a good and stable production of embryos. When the photoperiod began, a specially designed plastic collector was introduced onto the bottom of each fish tank to collect the embryos. All embryos were collected within 30 min to ensure the synchronization of their developmental stages. Embryos were then kept in a 28.5 °C incubator until they reached 4 h post fertilization (hpf). Healthy developed embryos were selected under a stereomicroscope (Nikon, Chiyoda-ku, Tokyo, Japan) at the blastula period (i.e., at 4 hpf). Selected embryos were transferred into a clean Petri dish with a thin layer of agarose gel at the bottom and filled with E3 medium (5 mM NaCl, 0.33 mM $MgSO_4$, 0.33 mM $CaCl_2$, 0.17 mM KCl, and 0.1% methylene blue) for dechorionation using a pair of sharp forceps (Dumont, Hatfield, PA, USA).

2.2. Alpha-particle irradiation

An ^{241}Am source with alpha-particle energy of 5.49 MeV under vacuum and an activity of 4.26 kBq was employed in the present study. The setup for alpha-particle irradiation of zebrafish embryos largely followed that devised by Yum et al. (2007). A thin Mylar film (Dupont, Hong Kong) with a thickness of 3.5 μm was used as a biocompatible substrate during the irradiation. The Mylar film was glued to the bottom of a Petri dish, which had a hole with a diameter of 4 mm at the center, using an epoxy (Araldite Rapid, England). With such a setup, the embryos were irradiated with alpha particles coming from below through the support substrate to minimize energy absorption in the medium before the alpha particles could reach and hit the cells of the embryos.

2.3. X-ray irradiation

A self-contained X-ray irradiation system (X-RAD 320, Precision X-Ray (PXi), Connecticut, USA) with voltage and current set at 200 kVp and 2 mA, respectively, was employed in the present study to irradiate the zebrafish embryos. X-ray irradiation was made through a 2.5 mm thick filter made of aluminum, copper and tin. Under such a setting, the dose rate of irradiation was ~15 mGy/min.

2.4. Experimental protocols

In a previous study, Choi et al. (2012b) successfully demonstrated RIBE between zebrafish embryos after some of the 5 hpf embryos were irradiated with 4.4 mGy of alpha particles. As such, a similar experimental setting with the same alpha-particle dose was adopted to induce RIBE between zebrafish embryos. In the present work, low-dose 200 kVp X-ray photons were employed for the photon hormesis to mitigate cellular damages in the zebrafish embryos induced by the alpha particles. Ng et al. (2015a) demonstrated that gamma-ray hormesis became effective in zebrafish embryos when the gamma-ray dose reached between 7 and 10 mGy. Therefore, four X-ray doses, namely, 2.5 mGy (Condition 1), 5 mGy (Condition 2), 10 mGy (Condition 3) and 14 mGy (Condition 4), were employed, expecting that photon hormesis to be effective under Conditions 3 and 4 but not under Conditions 1 and 2. For simplicity, we represented the four conditions by the symbol "*Y*", where *Y* could take the values of "2.5", "5", "10" or "14", which corresponded to exposures to X-ray doses of 2.5, 5, 10 or 14 mGy, respectively.

For each set of experiment under each condition, dechorionated embryos were divided into 7 groups each having 8–10 embryos. These 7 groups were named as:

(1) ***AX$_Y$-N*** group: embryos which first received ~4.4 mGy **A**lpha-particle irradiation and level Y **X**-ray irradiation at 5 hpf, and which were then partnered with **N**on-irradiated embryos immediately after all irradiations;