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Low Dose-Rate Irradiation of Gamma-Rays-Induced Cytotoxic and Genotoxic Alterations in Peripheral Erythrocytes of p53-Deficient Medaka (*Oryzias latipes*)

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Morphological alterations and nuclear abnormalities in fish erythrocytes have been used in many studies as bioindicators of environmental mutagens including ionizing radiation. In this study, adult Japanese medaka (*Oryzias latipes*) were irradiated with gamma rays at a low dose rate (9.92 μGy/min) for 7 days, giving a total dose of 100 mGy; and morphological alterations, nuclear abnormalities, and apoptotic cell death induced in peripheral erythrocytes were investigated 8 h and 7 days after the end of the irradiation. A variety of abnormalities, such as tear-drop cell, crenated cell, acanthocyte, sickled cell, micronucleated cell, eccentric nucleus, notched nucleus, and schistocyte, were induced in the peripheral erythrocytes of the wild-type fish, and a less number of abnormalities and apoptotic cell death were induced in the p53-deficient fish. These results indicate that low dose-rate chronic irradiation of gamma rays can induce cytotoxic and genotoxic effects in the peripheral erythrocytes of medaka, and p53-deficient medaka are tolerant to the gamma-ray irradiation than the wild type on the surface.

Keywords: gamma-rays, low dose-rate irradiation, erythrocyte, apoptosis, medaka

INTRODUCTION

Gamma irradiation induces ionization and cellular damage, including morphological alterations, nuclear abnormalities, DNA damage, and apoptosis (Muslimovic et al., 2012; Sayed et al., 2014, 2016b; Mohamed et al., 2016). In medaka fish blood cells, Sayed et al. (2014) reported that gamma radiation induces DNA aberrations such as nuclear abnormalities. The tumor protein p53 plays a critical role in determining whether DNA will be repaired or the damaged cell will undergo apoptosis. P53-deficient medaka fish showed less degree of damage after exposure to γ-irradiation,

UVA, and 4-non-ylphenol (Sayed et al., 2014, 2016a, 2017, 2018a,b, 2020) confirming the resistance of p53 mutant to apoptosis and morphological alterations after gamma-ray irradiation.

Ionizing radiation (IR) directly and/or indirectly damages DNA double strands, and the accumulated DNA lesions would lead to the transformation of the cell. In the case where the dose of radiation is low and the induced DNA lesions are repaired by the cells, it has been considered that the risk of carcinogenesis can be negligible (International Commission on Radiological Protection [ICRP], 2007). When cells are exposed to the same dose of IR, the lower the dose rate is, the less the risk of carcinogenesis per dose is, since induced DNA lesions would be repaired by the cell during the irradiation period and genomic damage can be reduced ("dose-rate effect"; Steel et al., 1987; Nakamura et al., 2005, 2006). Since 100 mGy is the dose for the lower limit of epidemiologically evaluated carcinogenic risk (International Commission on Radiological Protection [ICRP], 2007), IR exposure at doses lower than 100 mGy is considered not to increase carcinogenic risk, and its biological effects have not been emphasized.

After the accident of the Chernobyl nuclear power plant in 1986, researchers recognized the need to closely examine the impacts of low dose and low dose-rate irradiation on living organisms and ecosystems, and numerous studies have been started and are still ongoing (International Atomic Energy Agency [IAEA], 2006; Braga-Tanaka et al., 2018; Kamstra et al., 2018). In 2011, the Fukushima Daiichi nuclear power plant caused the accident to contaminate the wide area of east Japan (International Atomic Energy Agency [IAEA], 2015; Johansen et al., 2015; Maruyama et al., 2021). It has made the researchers to recognize again the need to elucidate the impacts of low dose and low dose-rate irradiation and has accelerated the research, especially in Japan (Hiyama et al., 2012; Braga-Tanaka et al., 2018; Shimura et al., 2018). As a result of these studies, many biological effects of low dose and low dose-rate chronic irradiation other than potential carcinogenic promotion have been revealed or confirmed, and the effects on human health and ecosystems are attracting researchers and social attention.

Even though DNA lesions by low dose and low dose-rate irradiation would be less than to induce carcinogenic risk epidemiologically, it is obvious that low dose and low dose-rate irradiation, no matter how small it is, induces DNA lesions at some degree and instabilizes genomes (Rothkamm and Löbrich, 2003; Nakamura et al., 2005, 2006). Based on this fact, International Commission on Radiological Protection (ICRP) recommends to use the linear no-threshold model (LNT model) in radiation protection (International Commission on Radiological Protection [ICRP], 2007), which assumes the absence of a lower threshold for stochastic effects by radiation and assumes a linear relationship between dose and the health risk by irradiation.

The level of DNA damage and repair capacity can be measured directly after irradiation by rapid formation of γ-H2AX foci (Gwozdzinski, 1991; Rogakou et al., 1998). Since teleosts erythrocytes are nucleated and have limited ability to repair DNA damages, genome instability induced by environmental mutagens can be evaluated with high sensitivity, monitoring the nuclear abnormalities like micronucleus (MN) in teleosts erythrocytes (Al-Sabti and Metcalfe, 1995; Mekkawy et al., 2011; Anbumani and Mohankumar, 2012; Praveen Kumar et al., 2015; Sayed et al., 2016a). Japanese medaka (*Oryzias latipes*) has been a useful animal model in radiation biology, and a large body of knowledge of the biological impacts of irradiation have been accumulated (Shima and Shimada, 1991; Shimada and Shima, 2001; Kubota et al., 1992; Kuwahara et al., 2003; Tsyusko et al., 2011; Yasuda et al., 2012, 2016; Sayed et al., 2014, 2016b, 2017; Perez-Gelvez et al., 2021a,b). To estimate the genome instability induced by low dose and low dose-rate irradiation, we irradiated adult medaka with low dose and low dose-rate gamma rays and examined the nuclear abnormalities induced in erythrocytes in this study.

MATERIALS AND METHODS

Medaka

Adult (about 6 months old) wild-type (Hd-rR strain) female medaka fish (*O. latipes*) and homozygous p53-deficient female (Yasuda et al., 2012, 2016) fish were used. They were kept at 26–28°C with a 14 h light: 10 h dark cycle and fed brine shrimp (*Artemia franciscana*) once a day and powder diet (Tetra-fin, Spectrum Brands Japan Inc., Tokyo, Japan) twice a day, in a fish facility in The University of Tokyo.

Irradiation

Low dose-rate and long-term irradiation of gamma rays was conducted with the Low Dose and Low Dose-rate Irradiation System (^{137}Cs, Radiation Biology Center, Kyoto University) with a dose rate of 9.92 μGy/min for 7 days to give a total dose of 100 mGy. During the irradiation, the fish were put in a water tank (width 30 cm × depth 10 cm × height 20 cm) with free swimming and kept at 26–28°C with a 14 h light: 10 h dark cycle and fed powder diet (Tetra-fin) once a day. Irradiation was stopped temporarily (for 15–30 min) once a day to feed the fish. The concentrations of NH_4^+, NO_2^-, and NO_3^- of the water in the tanks were measured daily with Packtest^{TM} ammonium, nitrite, and nitrate (Kyoritsu Chemical-check Lab., Corp., Yokohama, Japan), respectively, according to the instructions of the manufacturer, and the water was replaced if necessary.

Blood Sample Collection From Fish and Cell Viability Assay

Blood samples were collected from the caudal veins of the control and the irradiated fish after they were deeply anesthetized with 0.02% (*w/v*) MS-222 solution. Each sample was immediately placed on ice to prevent endogenous DNA destruction and to inhibit DNA repair before the chemical fixation of the cells during preparation. Blood smears were prepared on clean glass slides (triplicate slides from each fish). The numbers of blood cells in the blood samples were counted, and their viability was checked with a hemocytometer and trypan blue dye exclusion test before fixation. Only blood samples showing >90% viability and with more than 10^6 cells/100 μl cells were used.

Observation of Morphological Alterations and Nuclear Abnormalities in Blood Cells

Blood smears were fixed by dipping the slides in absolute methanol, air-dried, and stained with May-Grünwald solution for 15 min following 6% Giemsa staining for 30 min as reported previously (Tavares-Dias and Moraes, 2003). Slides with high staining quality were selected and coded, randomized, and scored in a blinded manner. In each group, 10,000 cells (minimum of 1,000 cells per slide) were examined as previously reported (Al-Sabti and Metcalfe, 1995) under a 40x objective with a 10x eyepiece to identify morphologically altered erythrocytes. Established criteria for identifying micronuclei (Schmidt, 1975) were strictly followed to ensure authentic scoring.

Apoptosis Detection

Apoptotic erythrocytes were stained by acridine orange (AO; A1031, Life Technologies, Carlsbad, CA, United States) in a modified protocol by Sayed et al. (2016b). In brief, blood samples were smeared on clean glass slides and rinsed in phosphate-buffered saline (PBS; pH = 7.2), and AO solution (17 μg/L AO in PBS buffer) was added to the slides. The slides were incubated for 30 min in the dark and then washed with PBS four times every 30 min. Decolorized blood cells were fixed in 4% paraformaldehyde in PBS for 5 min and observed under a fluorescence microscope (BX50; Olympus, Tokyo, Japan) equipped with a digital color still camera (DP-70; Olympus, Tokyo, Japan) (Abrams et al., 1993; Furutani-Seiki et al., 1996).

Immunostaining of Cleaved Caspase 3

Immunostaining was performed as previously described with minor modifications (Sayed et al., 2016b, 2018b). Blood samples (2 μl) were collected from the fish and mixed with 5 μl of PBS, further diluted to 10^6 cells/ml in PBS, and then 100 μl of cell suspension (10^6 cells/ml) was mixed with 100 μl of 2x fixation buffer [50 mM Tris (pH 7.5), 2% Triton-X100, 2 mM EDTA, 0.02% BSA, and 0.4% paraformaldehyde]. The mixture was immediately vortexed and incubated for 10 min at room temperature in the dark. Then, the fixed erythrocytes were centrifuged at 2,500 rpm for 5 min at 4°C, washed twice with 200 μl PBS, and further incubated for 30 min with blocking buffer (0.5% Triton X-100, 0.2% normal goat serum). The cells were again centrifuged at 2,500 rpm for 5 min at 4°C, resuspended in 0.1% BSA/PBS (−), and incubated with the primary antibody [1:250, #9661, Cleaved Caspase-3 (Asp175), Cell Signaling Technology, Danvers, MA, United States] for 3 h at room temperature. The cells were collected by centrifugation and kept in 0.1% BSA/PBS (−) overnight at 4°C. After that, the cells were further incubated with Alexa Fluor 488-conjugated secondary antibody [A-11008, Goat anti-Rabbit IgG (H + L), Thermo Fisher Scientific, Waltham, MA, United States] in 3% BSA/PBS (−) for 3 h at room temperature. Then, the cells were washed three times with 0.1% BSA/PBS (−), counterstained with 4-,6- diamino-2-phenylindole (DAPI, Fujifilm Wako Pure Chemical, Osaka, Japan) for 5 min at room temperature, washed with PBS, re-suspended in 4 μl of PBS, and mounted on a new glass slide. Fluorescence images were captured using an inverted fluorescence microscope (IX-81, Olympus, Tokyo, Japan) equipped with a digital still camera (DS Ri1, Nikon, Tokyo, Japan).

Statistical Analysis

The data are presented as mean ± standard deviation (SD). Multiple comparisons were performed using one-way ANOVA using the SPSS package and the Tukey's test at 0.05 significance level.

Ethics Statement

All experiments were performed in accordance with the Japanese laws and guidelines for the care of experimental animals according to the University of Tokyo Animal Experiment Enforcement Rule. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Tokyo (Permit Number: C-19-05). All surgery was performed under MS-222, and all efforts were made to minimize suffering.

RESULTS

Morphological Alterations and Nuclear Abnormalities in Erythrocytes of Irradiated Wild-Type and p53-Deficient Medaka

Morphologically altered erythrocytes or those with nuclear abnormalities were seldom observed in the non-irradiated wild-type fish. Normal erythrocytes in the non-irradiated wild-type and p53-deficient fish were shown in **Figures 1A,D,G,J**, respectively. After the low dose-rate irradiation (100 mGy in total at 9.92 μGy/min) of gamma rays, morphological alterations and nuclear abnormalities were induced in the erythrocytes of the wild-type and p53-deficient medaka, such as micronucleated cell (Mn; **Figure 1B**), acanthocyte (Ac; **Figures 1C,H,I**), crenated cell (Cr; **Figures 1C,H,I**), sickled cell (Sk; **Figures 1C,E,I,K**), cell with notched nucleus (Nn; **Figures 1E,K**), tear-drop cell (Tr; **Figures 1F,I**), cell with eccentric nucleus (Ecn; **Figure 1H**), and schistocyte (Sch; **Figure 1L**). These altered cells were observed 8 h and 7 days after the end of the irradiation, whereas the micronuclei cells were observed only in the irradiated wild-type fish 8 h after the end of the irradiation (**Figure 1B**). The erythrocytes of the irradiated wild-type fish showed more serious morphological alterations than the erythrocytes of the irradiated p53-deficient fish. These results clearly demonstrated that the gamma-ray irradiation of low dose (100 mGy in total) at low dose-rate (9.92 μGy/min) for 7 days can induce various types of morphological abnormalities in peripheral erythrocytes of medaka fish.

We found that 4.28 ± 1.33% of the erythrocytes of the irradiated wild-type fish altered morphologically 8 h after the end of the irradiation, and the morphologically altered cells decreased to 3.10 ± 1.00% 7 days after the end of the irradiation. The background level of the morphologically altered erythrocytes in the non-irradiated wild-type fish was less than 2% (**Table 1**). These results strongly suggest that the damages in the

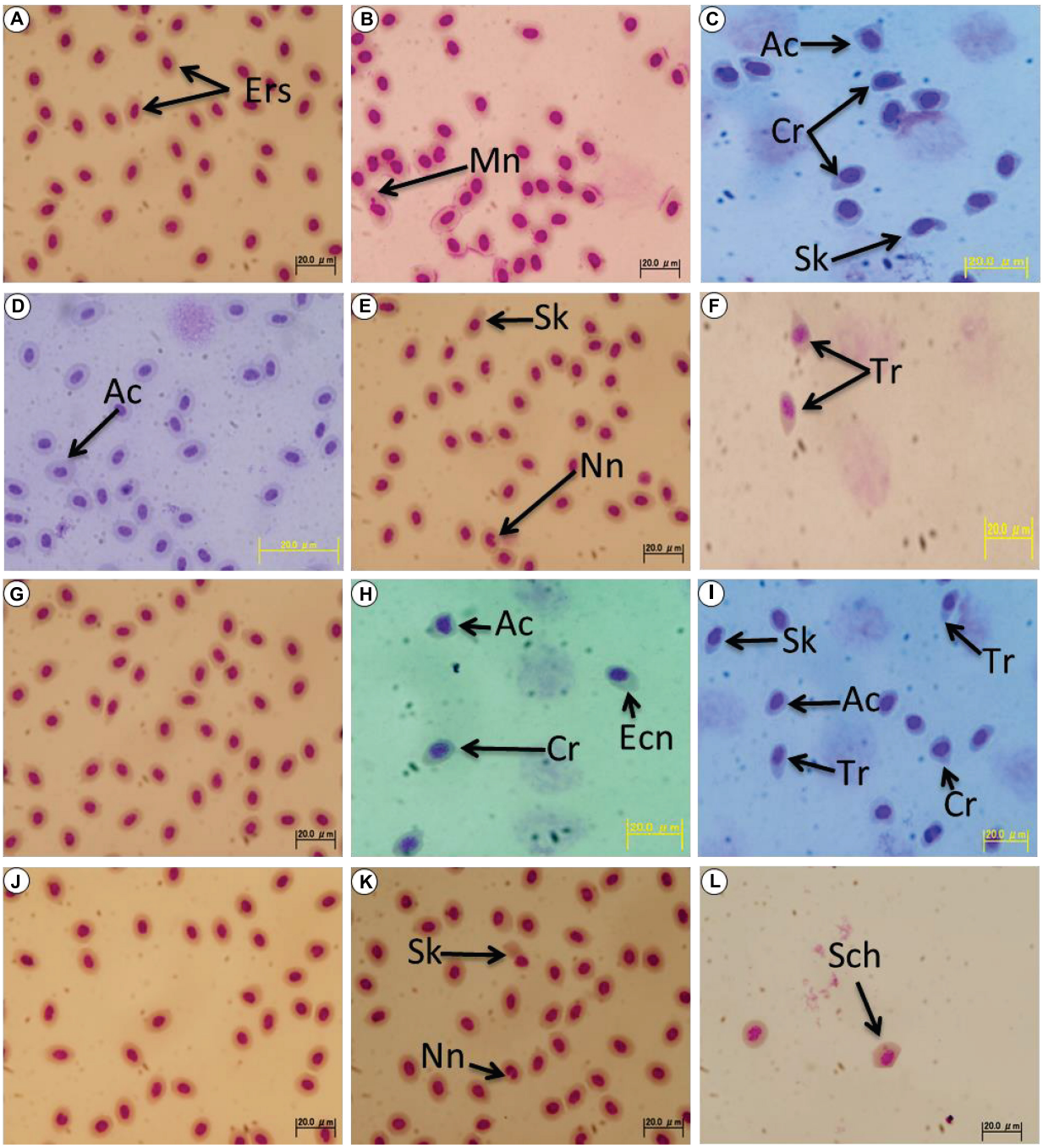

FIGURE 1 | Wright-Giemsa stained blood smears showing morphological and nuclear abnormal erythrocytes found in the irradiated medaka. **(A,D)** Normal erythrocytes collected from the non-irradiated wild-type female (Hd-rR strain) are presented. Morphologically altered erythrocytes or those with nuclear abnormalities were seldom observed in the non-irradiated wild-type fish, and an acanthocyte (Ac) is shown in d. **(B,C)** The erythrocytes of the wild-type fish 8 h after the end of the irradiation. **(E,F)** The erythrocytes of the wild-type fish 7 days after the end of the irradiation. **(G,J)** The erythrocytes of the non-irradiated p53-deficient fish. Abnormal erythrocytes were seldom observed. **(H,I)** The erythrocytes of the p53-deficient fish 8 h after the end of the irradiation. **(K,L)** The erythrocytes of the p53-deficient fish 7 days after the end of the irradiation. Ers, erythrocytes (normal erythrocytes); Tr, tear-drop cell; Cr, crenated cell; Ac, acanthocyte; Sk, sickled cell; Mn, micronucleated cell; Ecn, cell with eccentric nucleus; Nn, notched nucleus; and Sch, schistocyte.

erythrocytes and erythropoietic cells induced by the irradiation were removed via apoptosis or repaired over 7 days after the irradiation in the wild-type fish. In contrast, in the p53-deficient fish, 2.60 ± 1.05% erythrocytes had altered morphology 8 h after the end of the irradiation, and the percentage of the altered erythrocytes decreased to the control level (1.68 ± 0.98%) within

TABLE 1 | Percentage of altered, micronucleated, apoptotic erythrocytes (mean ± SD) per 100 erythrocytes in the irradiated medaka (*O. latipes*) after the low dose (100 mGy in total) and low dose-rate irradiation (at 9.92 μGy/min for 7 days) of gamma-rays.

	Time after Irradiation	8 h		1 week	
	Dose of Irradiation	**Control (*n* = 4)**	**100 mGy (*n* = 4)**	**Control (*n* = 6)**	**100 mGy (*n* = 6)**
Wild Type	Total of altered cells (%)	$1.85 \pm 1.00^{c*}$	4.28 ± 1.33^{a}	1.33 ± 1.33^{c}	3.10 ± 1.00^{b}
	Micronucleated cells (%)	0*	0.22 ± 0.05^{a}	0	0
	Apoptotic cells (%)	$4.05 \pm 1.03^{c*}$	9.68 ± 1.36^{a}	3.13 ± 1.36^{d}	5.58 ± 1.56^{b}
	Number of foci of cleaved CAP3	1.83 ± 1.39^{b}	2.85 ± 1.74^{a}	1.45 ± 1.17^{b}	2.00 ± 1.13^{b}
p53-deficient	Total of altered cells (%)	$1.82 \pm 1.00^{c*}$	2.60 ± 1.05^{b}	1.31 ± 0.94^{c}	1.68 ± 0.98^{c}
	Micronucleated cells (%)	0*	0	0	0
	Apoptotic cells (%)	$4.03 \pm 1.07^{c*}$	5.00 ± 0.83^{b}	2.95 ± 0.99^{d}	1.98 ± 0.94^{e}
	Number of foci of cleaved CAP3	1.77 ± 1.24^{b}	2.06 ± 1.28^{b}	1.51 ± 0.92^{b}	1.58 ± 0.90^{b}

Different letters indicate significance at $p < 0.01$.
**Cell count was done for 1,000 cells/sample.*

7 days after the irradiation. These results demonstrated that p53-deficient fish were more tolerant to the low dose and low dose-rate irradiation of gamma rays on the surface.

Apoptosis in Erythrocytes of Irradiated Wild-Type and p53-Deficient Medaka

In the blood smear prepared from the irradiated wild-type fish, 9.68 ± 1.36% and 5.58 ± 1.56% of the peripheral erythrocytes were apoptotic 8 h and 7 days after the end of the irradiation, respectively. Since 4.05 ± 1.03% and 3.13 ± 1.36% of the erythrocytes were apoptotic 8 h and 7 days after the end of the irradiation in the non-irradiated fish, respectively, the low dose (100 mGy in total) and low dose-rate (9.92 μGy/min) irradiation of gamma rays significantly induced apoptosis in peripheral erythrocytes of medaka ($p < 0.01$; **Table 1**). On the other hand, 5.00 ± 0.83% and 1.98 ± 0.94% of the erythrocytes were apoptotic in the irradiated p53-deficient medaka 8 h and 7 days after the end of the irradiation, respectively, demonstrating that significantly less apoptotic cell death was induced in the peripheral erythrocytes of the p53-deficient fish ($p < 0.01$) after the irradiation (**Table 1**). In the non-irradiated p53-deficient fish, 4.03 ± 1.07 and 2.95 ± 0.99% of the erythrocytes were apoptotic 8 h and 7 days after the end of the irradiation, respectively, demonstrating that the background level of the apoptosis in erythrocytes is the same between the wild-type and p53-deficient fish without irradiation.

Formation of Cleaved CAP3 Foci in Erythrocytes of Irradiated Wild-Type and p53-Deficient Medaka

Erythrocytes of medaka have a nucleus and can conduct apoptotic cell death (Sayed et al., 2016b, 2017). We found that foci of cleaved CAP3 obviously increased in the erythrocytes of irradiated wild-type fish 8 h after the end of the irradiation (2.85 ± 1.74 foci) and returned to the background level 7 days after the end of the irradiation (**Figure 2** and **Table 1**). In contrast, foci of cleaved CAP3 did not change in the p53-deficient erythrocytes after the irradiation. These results demonstrate that the erythrocytes of wild-type medaka conduct both CAP3-dependent and CAP3 independent apoptosis after the low dose and low dose-rate irradiation of gamma rays, while the erythrocytes of p53-deficient fish only conduct CAP3 independent apoptosis after the irradiation.

DISCUSSION

To date, the effects of gamma-ray irradiation on fish erythrocytes have been studied after acute exposure of high-dose (>1 Gy) gamma rays, and induction of DNA damage has been reported (Anbumani and Mohankumar, 2012; Sayed et al., 2014, 2016b, 2017; Praveen Kumar et al., 2015; Lemos et al., 2017). On the other hand, it was reported that the dose was 130–140 μGy/h in the aquatic environment after the Fukushima accident, which was extensively lower than those used in the previous studies (Johansen et al., 2015). In this study, we conducted low dose and low dose-rate irradiation of gamma rays (100 mGy in total at 9.92 μGy/min for 7 days) on adult medaka fish and found that significant increases of nuclear abnormalities and apoptotic cell death could be induced in the erythrocytes of the irradiated fish after the irradiation. We also found that p53 deficiency suppressed the induction of nuclear abnormalities and apoptotic cell death in peripheral erythrocytes after the low dose and low dose-rate irradiation.

Since erythrocytes are the major site of production of reactive oxygen species (Khoory et al., 2016), as the site of oxygen transport and fish erythrocytes are nucleated, they are highly sensitive to oxidative mutagens and have been studied to evaluate DNA damage induced by mutagens in the environments, including IR irradiation (Al-Sabti and Metcalfe, 1995; Sawhney and Johal, 2000; Sayed et al., 2007, 2014, 2016b, 2017, 2018a,b; Mekkawy et al., 2011; Anbumani and Mohankumar, 2012). There are no studies in the literature on the cytotoxic and genotoxic effects on fish erythrocytes of chronic or long-period irradiation of low dose (<1 Gy) IR, although DNA damage after acute irradiation of x-rays (0.1–1 Gy) or gamma rays (2–15 Gy) is reported in erythrocytes of adult zebrafish (Lemos et al., 2017), carp (Praveen Kumar et al., 2015), and medaka (Sayed et al., 2014, 2017). Anbumani and Mohankumar (2012) reported a significant increase of micronuclei and cellular abnormalities in the erythrocytes of Cyprinidae fish, *Catla catla*, after the

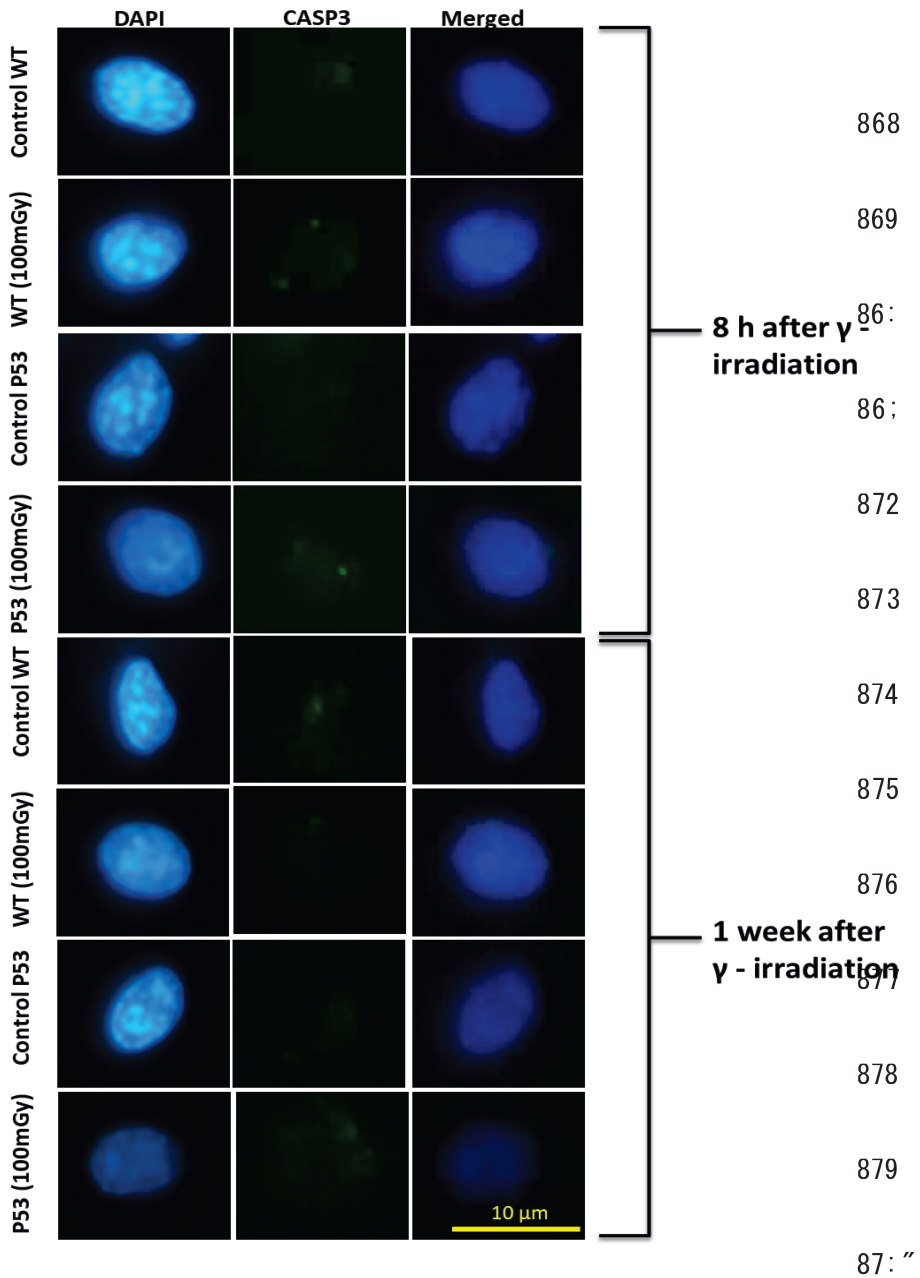

FIGURE 2 | Foci of cleaved caspase 3 (CASP3) in the erythrocytes of the irradiated medaka (*O. latipes*). Left/center/right column shows DAPI-stained (blue), anti-cleaved CASP3 immunostained (green), and merged images of nuclei of the erythrocytes 8 h and 7 days after the end of the irradiation, respectively. Scale bar represents 10 μm.

irradiation of gamma rays (5 Gy) at two dose rates of 3.2 and 0.002 Gy/min, and they suggested that prolonged exposure induced less apoptotic cell death in the erythrocytes than acute exposure. It is widely accepted that IR irradiation at a low dose-rate induces weaker cytotoxicity and genotoxicity presumably because induced DNA lesions are repaired by the cell during irradiation ("dose-rate effect"; Steel et al., 1987; Nakamura et al., 2005, 2006; Tanaka et al., 2013; Rühm et al., 2015). Because of the "dose-rate effect," it was expected that DNA damage induced by low dose and low dose-rate irradiation of IR will be repaired by the cell, and the cytotoxicity and genotoxicity induced would be negligible and hard to be detected. However, the results presented here indicate that the potential cytotoxicity and genotoxicity of low dose and low dose-rate irradiation in the environments can be evaluated on medaka peripheral erythrocytes.

Vertebrate embryos, especially early-stage embryos, are highly proliferative and susceptible to IR irradiation (Jacquet, 2004; Streffer, 2004; De Santis et al., 2007; Hurem et al., 2017; Yasuda et al., 2020). Since fish are oviparous and their embryos develop outside the body of the female, the fish embryo has been the major

model to investigate the impacts of IR irradiation on embryonic development, and the researchers reported that the low dose irradiation less than 100 mGy of gamma rays or x-rays could disturb the embryonic development of zebrafish (Miyachi et al., 2003; Bladen et al., 2007; Jaafar et al., 2013; Freeman et al., 2014; Gagnaire et al., 2015). More recently, the chronic or long-term exposure of low dose gamma rays at low dose-rate was conducted, and the adverse effects on zebrafish embryonic development have been revealed (36.8 mGy at 0.4 mGy/h, Hurem et al., 2017; 0.01–0.10 Gy at 55 mGy/min, Zhao et al., 2019). Transcriptome analysis revealed that low dose IR irradiation as low as 1.62 mGy can alter the gene expression profile of zebrafish embryo, inducing abnormalities in embryonic development (Hurem et al., 2017). Kamstra et al. (2018) found that the IR irradiation at low dose-rate (8.7 mGy/day for 27 days) alters DNA methylation in zebrafish. In this study, we conducted low dose-rate irradiation of gamma rays at 9.92 μGy/min (=14.3 mGy/day) for 7 days, and it can be reasonable that the adverse effects on the adult fish erythropoiesis were detected.

Micronucleus test is frequently used in fish erythrocytes to evaluate environmental pollution with mutagens, since MN has a relationship with DNA damage and mutation rate (Lenarczyk and Slowikowska, 1995; Takai A. et al., 2004; Takai T. et al., 2004; Praveen Kumar et al., 2015; Sayed et al., 2017, 2018b). It would be worth noting that the low dose and low dose-rate irradiation of gamma rays in this study induced MN in the peripheral erythrocytes of wild-type medaka; in contrast, high dose (≤15 Gy) of gamma rays was necessary to induce MN in wild-type erythrocytes after the acute irradiation (7.8 Gy/mi) in our previous studies (Sayed et al., 2014, 2016b, 2017). Considering the result of this study that the low dose and low dose-rate irradiation of gamma rays did not induce MN in the erythrocytes of the p53-deficient medaka, the p53 functions to regulate cell cycle checkpoints and/or DNA repair might be crucial to induce MN after irradiation.

We also found that apoptotic cell death in the irradiated erythrocytes was suppressed in the absence of p53 functions, strongly suggesting that p53 has a central role in the cellular responses against the low dose and low dose-rate irradiation as well as against acute high-dose irradiation as previously reported (Yasuda et al., 2012; Sayed et al., 2017). Attenuation of p53 function extends the survival after irradiation with high-dose (10–40 Gy) gamma rays in zebrafish embryos (Duffy and Wickstrom, 2007); of course, p53 plays a crucial role as a tumor suppressor in zebrafish (Guo et al., 2013). Reduction of apoptotic cell death in irradiated medaka erythrocytes may look like an advantage, but it may also have disadvantages.

The current radiation protection is based on the LNT model (Biological Effects of Ionizing Radiation [BEIR], 2006; International Commission on Radiological Protection [ICRP], 2007); however, the argument is still ongoing (Rühm et al., 2015; Cardarelli and Ulsh, 2018; Leblanc and Burtt, 2019). This study provided the point of view that low dose and low dose-rate irradiation induces negligible biological effects on the cell surface, because the induced cytotoxicity and genotoxicity are counteracted and canceled by the protecting mechanisms of the cell. Actually, ataxia telangiectasia (AT)-deficient cultured cells show hypersensitivity to low dose-rate irradiation of x-rays (Nakamura et al., 2006), and knockdown of the 80-kDa subunit of Ku protein, an essential non-homologous end-joining pathway of DNA repair, made the zebrafish embryos hyper-sensitive to gamma-ray irradiation (Bladen et al., 2007). Any low dose and low dose-rate IR irradiation can have an adverse effect on living cells, organisms, and ecosystems, and they might be accepting the risks and paying costs to protect themselves. It is necessary to keep this point of view in mind to deal with radiation protection.

CONCLUSION

This study presented the evidence that low dose (100 mGy) and low dose-rate (9.92 μGy/min) gamma-ray irradiation induces nuclear and cytosolic abnormalities such as MN and apoptotic cell death in peripheral erythrocytes in adult medaka fish and that the potential cytotoxicity and genotoxicity by low dose and low dose-rate IR irradiation in the environments can be evaluated with peripheral erythrocytes of medaka.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This animal study was reviewed and approved by the Committee on the Ethics of Animal Experiments of The University of Tokyo (Permit Number: C-19-05).

AUTHOR CONTRIBUTIONS

AS, KN, HM, JK, and SO designed the experiments. AS, KN, TN, JK, and SO conducted the experiments. AS and SO analyzed the data and drafted the manuscript. AS, KN, HM, JK, and SO interpreted the result of the experiment. KN, HM, and JK edited the manuscript. All authors read and approved the final manuscript.

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