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The threshold number of protons to induce an adaptive response in zebrafish embryos

V W Y Choi[1], Teruaki Konishi[2], Masakazu Oikawa[2], S H Cheng[3,4] and K N Yu[1,4]

[1] Department of Physics and Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Hong Kong

[2] Department of Technical Support and Development, National Institute of Radiological Sciences, Chiba 263-8555, Japan

[3] Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Hong Kong

[4] State Key Laboratory in Marine Pollution, City University of Hong Kong, Hong Kong

E-mail: peter.yu@cityu.edu.hk

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Abstract

In this study, microbeam protons were used to provide the priming dose to induce an *in vivo* radioadaptive response (RAR) in the embryos of zebrafish, *Danio rerio*, against subsequent challenging doses provided by x-ray photons. The microbeam irradiation system (Single-Particle Irradiation System to Cell, acronym SPICE) at the National Institute of Radiological Sciences (NIRS), Japan, was employed. The embryos were dechorionated at 4 h post fertilisation (hpf) and irradiated at 5 hpf by microbeam protons. For each embryo, one irradiation point was chosen, to which 5, 10, 20, 30, 40, 50, 100, 200, 300 and 500 protons each with an energy of 3.4 MeV were delivered. The embryos were returned to the incubator until 10 hpf to further receive the challenging exposure, which was achieved using 2 Gy of x-ray irradiation, and then again returned to the incubator until 24 hpf for analyses. The levels of apoptosis in zebrafish embryos at 25 hpf were quantified through terminal dUTP transferase-mediated nick end-labelling (TUNEL) assay. The results revealed that at least 200 protons (with average radiation doses of about 300 and 650 mGy absorbed by an irradiated epithelial and deep cell, respectively) would be required to induce RAR in the zebrafish embryos *in vivo*. Our previous investigation showed that 5 protons delivered at 10 points on an embryo would already be sufficient to induce RAR in the zebrafish embryos. The difference was explained in terms of the radiation-induced bystander effect as well as the rescue effect.

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1. Introduction

Radioadaptive response (RAR) refers to the biological response where an exposure of cells or animals to a low dose of radiation (priming dose) induces mechanisms that protect the cells or animals against the detrimental effects of a subsequent larger radiation exposure (challenging dose). The phenomenon was first reported by Olivieri *et al* (1984). There has been extensive research since then to study RAR *in vitro* using various biological endpoints such as the survival of cells (Ryan *et al* 2008), neoplastic transformation (Ko *et al* 2006), chromosomal aberrations and micronucleus formation (Kurihara *et al* 1992, De Toledo and Azzam 2006), as well as mutation frequency (Yatagai *et al* 2008).

On the other hand, RAR has also been shown in *in vivo* studies. Mice have been used to examine RAR induction, e.g., using their survival rate as a biological endpoint (Yonezawa *et al* 1996, Tiku and Kale 2004, Ito *et al* 2007). Our group has recently studied RAR in embryos of the zebrafish (*Danio rerio*) *in vivo* induced by broad-beam alpha particles (Choi *et al* 2010c, 2011) as well as microbeam protons (Choi *et al* 2010b), and demonstrated that irradiation of zebrafish embryos at 5 h post fertilisation (hpf) and 10 hpf with 5 h interval time is feasible in inducing RAR. It is remarked that the human and zebrafish genomes share considerable homology, including conservation of most DNA repair-related genes (Barbazuk *et al* 2000). The advantage of using zebrafish embryos is that they have larger proportions of dividing cells which are radiosensitive, and cellular damage is more likely to translate into damage at the organismic level due to active morphogenesis. As such, our group has also employed zebrafish embryos to study the effect of low-dose radiation (Yum *et al* 2007, 2009a, 2009b, 2010).

A critical parameter in inducing RAR is the magnitude of the applied priming dose. Iyer and Lehnert (2002) discovered that supernatants conditioning normal human lung fibroblasts (HFL-1) irradiated with 10 mGy of α-particles could increase the radioresistance of bystander unirradiated HFL-1 cells. Broome *et al* (2002) found an apparent limit or threshold dose between 0.1 and 1 mGy for low-LET radiation such as γ-rays from ^{60}Co for inducing adaptation. Sorensen *et al* (2002) and Sasaki *et al* (2002) suggested the priming dose limit to be less than 100 mGy in mammalian cells, while Matsumoto *et al* (2007) reported that the priming dose range should be around 10–200 mGy using low-LET radiation. On the other hand, a priming dose range of 50–100 mGy for whole-body x-ray exposure on mice was employed by Yonezawa *et al* (1996).

Interestingly, however, our previous results were not able to identify a lower threshold dose for induction of RAR. For broad-beam alpha particles, RAR was successfully induced by the smallest number of alpha particles, i.e., 4, incident on the zebrafish embryo (Choi *et al* 2011). For microbeam protons, RAR was successfully induced by the smallest number of protons per position, i.e., 5 protons per position, incident onto 10 different positions on the zebrafish embryo (Choi *et al* 2010b). In the present work, the induction of RAR using microbeam protons was further studied, but using one single irradiation point only. The main objectives were threefold, namely (1) to determine the threshold number of protons irradiated onto one single position to induce RAR in zebrafish embryos, (2) to explain the dependence of the threshold number of protons, if any, on the number of irradiation positions, and (3) to determine the dimensions of the epithelial cells and deep cells in a 5 hpf zebrafish embryo to enable determination of the proton dose absorbed by the cells.

2. Materials and methods

2.1. Zebrafish embryos

Adult zebrafish were kindly provided by the RIKEN Brain Science Institute, Japan (courtesy of Professor Hitoshi Okamoto) and were kept in water tanks in an indoor environment with

an ambient temperature of 28 °C and a 14/10 h light–dark cycle. Once the 14 h photoperiod began, a special plastic embryo collector (Choi *et al* 2010c) was lowered to the bottom of each tank to collect the embryos for a short period lasting only 15–30 min to ensure more-or-less synchronisation of the developmental stages of the collected embryos. The collected embryos were then incubated in a 28 °C incubator until 4 h post fertilisation (hpf) for dechorionation (Choi *et al* 2010c).

2.2. Preparation of the embryo dish for irradiation

A specially designed dish consisting of a Si_3N_4 plate (7.5 × 7.5 mm^2 frame with a thickness of 200 μm, and with a 3 mm × 3 mm hole area at the centre, Silson Ltd, Northwood, UK) and a steel ring of 33 mm diameter was used to hold the embryos for microbeam irradiation (Choi *et al* 2010b). A Mylar film with a thickness of 2.5 μm (Chemplex Industries, Inc., FL, USA) was stretched across the steel rings and formed a substrate for the embryos. The Si_3N_4 plate was attached to the centre of the Mylar film by Vaseline (Wako Pure Chemical Industries Ltd, Osaka, Japan) to restrict the movement of the embryos.

2.3. Irradiation conditions

In the present study, the microbeam irradiation system (Single-Particle Irradiation System to Cell, acronym SPICE) (Konishi *et al* 2009) at the National Institute of Radiological Sciences (NIRS), Japan, was employed, which was capable of delivering a desired number of 3.4 MeV protons within a beam diameter of 2 μm. Protons with an initial energy of 3.4 MeV first travelled through a Si_3N_4 exit window with a thickness of 100 nm, and then through a 2.5 μm Mylar film with less than 50 μm air gap between the exit window and the Mylar film before the protons finally reached the target. The energy of the protons would drop to 3.37 MeV when they arrived at the target.

For the adaptive group of embryos, the priming exposure was delivered by microbeam protons to the dechorionated zebrafish embryos at the 5 hpf stage. The hit positions of the beam onto the embryo cells were chosen by the user. Proton irradiation was effectively normal to the cells, as the irradiation point was very close to where the zebrafish embryo touched the substrate. Ten sets of experiments were conducted by delivering 5, 10, 20, 30, 40, 50, 100, 200, 300 and 500 protons to the zebrafish embryos. The embryos were then returned to the incubator until 10 hpf to further receive the challenging exposure, which was achieved using 2 Gy of x-ray irradiation from an x-ray generator (TITAN, Shimazu Corporation, Kyoto, Japan) set at 200 kVp and 20 mA. The x-ray irradiation was made through a copper and aluminum filter with a thickness of 0.5 mm producing an effective energy of approximately 83 keV. The dishes holding the embryos were set 580 mm away from the x-ray target, which received x-ray doses at a dose rate of about 1 Gy min^{-1}. After the x-ray irradiation, the embryos were returned to the incubator until 24 hpf for analysis. The adaptive control group of embryos was sham irradiated with protons at 5 hpf followed by a 2 Gy x-ray irradiation when they had developed for 10 hpf. Finally, the control group of embryos was sham irradiated with protons at 5 hpf and with x-rays at 10 hpf.

The application of priming and challenging doses at 5 and 10 hpf, respectively, followed the practice of Choi *et al* (2010c), who succeeded in inducing RAR in zebrafish embryos when the priming dose was applied at the blastula stage (2.2–5.2 hpf), at which the DNA repair mechanism should have started, and when there was a 5 h time interval between the priming and challenging doses.

2.4. TUNEL assay

The number of apoptotic signals in the embryos at 24 hpf was chosen as the biological endpoint in this study. Terminal dUTP transferase-mediated nick end-labelling (TUNEL) assay was performed to reveal the apoptotic cells in the embryos. The 25 hpf embryos were fixed in 4%

paraformaldehyde in phosphate buffered saline (PBS) with 0.1% Tween 20 at room temperature for 5 h. The fixed embryos were then dehydrated, and were then rehydrated and treated with 60 μg ml^{-1} protease kinase (Wako Pure Chemical Industries Ltd, Osaka, Japan) for 10 min. After the protease kinase treatment, the embryos were fixed in 4% paraformaldehyde in PBS with 0.1% Tween 20 again. The TUNEL staining was achieved by using an *in situ* apoptosis detection kit (MK500, Takara Bio. Inc., Japan). The fixed embryos were submerged in the permeabilisation buffer for 30 min on ice. The apoptotic cells were labelled by immersing the embryos in the mixture of terminal deoxynucleotidyl transferase (TdT) enzyme and labelling safe buffer containing fluorescein labelled-2′-deoxyuridine, 5′-triphosphate and FITC-dUTP in the ratio of 1:9. The embryos were then incubated in a 37 °C humidified chamber for 120 min. The embryos were finally washed thoroughly by PBS in 0.1% Tween 20. The apoptotic signals were recorded in images captured by a confocal laser microscope (FV-1000, Olympus Corporation, Tokyo, Japan) with a 4× objective lens (NA: 0.16, UPLSAPO 4×, Olympus Corporation, Tokyo, Japan). For each embryo, a total of 15–20 sliced images (2.12 mm × 2.12 mm, 2.06 μm/pixel) were captured with 25 μm intervals from top to bottom of the embryo. An apoptotic cell would appear as a bright dot in the image (Choi *et al* 2010b).

2.5. Statistical analysis

The number of apoptotic signals on each 25 hpf embryo after TUNEL assay was quantified with the help of ImageJ software freely obtainable from the website http://rsb.info.nih.gov/ij/. The images obtained from the confocal microscope were first converted into binary images. The number of apoptotic signals was then determined using the 'Analyze particle' function in ImageJ. Possible outliers were identified and removed before a *t*-test was used to ascertain the statistical significance for differences between samples. A p value smaller than 0.05 was considered to correspond to a statistically significant difference.

2.6. Absorbed dose

The numbers of delivered protons were used to determine the absorbed doses in the irradiated cells of the zebrafish embryos. The dose absorbed by a cell was calculated by E/M, where E is the energy of the protons deposited in the cell and M is the average mass of that cell. The target cells in a zebrafish embryo included the epithelial and deep cells (Campana *et al* 2010).

In the present work, the dimensions for these cells were first obtained from the cross-sectional images of the zebrafish embryos. The following describes the procedures for paraffin embedding and sectioning of the zebrafish embryos. 5 hpf zebrafish embryos were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) with 0.1% Tween 20 at 4 °C overnight. The fixed embryos were then dehydrated. The embryos were soaked in 50% ethanol, 70% ethanol and 90% ethanol, each for 10 min, and were then soaked in 95% ethanol and 100% ethanol twice for 10 min during the dehydration process. The dehydrated embryos were transferred to 3 ml 50% xylene (Sigma Aldrich, USA) in ethanol for 3 min and immediately transferred to 3 ml 100% xylene twice for 3 min. Paraffin wax (Kendall, USA) was melted at 60 °C. The embryos previously treated with xylene were then transferred into the melted paraffin wax. Renewal of the paraffin wax was required five times, once every hour. The embryos were then allowed to remain in the paraffin wax overnight. The embryos were then carefully embedded into a paraffin block under an optical microscope to ensure that horizontal or vertical sections of the embryos could be obtained. The paraffin block was cut into slices each 7 μm thick. The paraffin slices were then placed into a 37 °C water bath and were recovered using glass slides. The samples were dried overnight at 37 °C. The cross sections of the embryos were examined and the images were captured under the phase-contrast mode of a compound microscope (Nikon) with 200× magnification.

Table 1. Mean net apoptotic signals D (±SE) for the adaptive group of embryos and their p values when compared with the adaptive control group. N = sample size of the irradiated group, while the size of the adaptive control group was 89. D = (mean of apoptotic signal for adaptive control group − mean of apoptotic signal for adaptive group) (±SE); p represents the p value determined from the t-distribution (cases with $p < 0.05$ are asterisked).

Number of protons	N	D	p
5	23	-29.3 ± 13.8	0.0283*
10	24	-46.2 ± 21.1	0.0217*
20	20	-17.9 ± 17.0	0.162
30	7	-0.922 ± 3.650	0.443
40	27	-87.5 ± 15.9	0.000 005 35*
50	27	-94.4 ± 18.3	0.000 0124*
100	25	-28.2 ± 13.7	0.0322*
200	20	21.5 ± 8.9	0.0233*
300	26	12.2 ± 5.1	0.0510*
500	30	17.4 ± 7.1	0.0241*

3. Results

In the present study, the TUNEL assay was employed to reveal and quantify the apoptotic cells present in the 25 hpf zebrafish embryos. Typical images for apoptotic signals on 25 hpf embryos obtained by TUNEL assay and recorded using a confocal microscope can be found in our previous paper (Choi *et al* 2010b). Each green spot corresponds to an apoptotic signal. Apoptotic signals were observed throughout the body in both irradiated and non-irradiated embryos.

We considered the number of apoptotic signals from the control group of embryos in each set of experiments as the background signals. Both the number of apoptotic signals in the adaptive group and the number in the adaptive control group therefore first had the background signals subtracted from them. After this, all the adaptive control groups (with a survival rate of 96.4%) were merged to form a single adaptive control group, and the mean of the apoptotic signal for this adaptive control group was calculated. Moreover, for each irradiation condition to provide the priming dose, the mean of the apoptotic signal for the adaptive group was calculated. The mean apoptotic signals for the adaptive groups of embryos when compared with the adaptive control groups, D, obtained for the 25 hpf embryos, were calculated as D = (mean of apoptotic signal for adaptive control group−mean of apoptotic signal for adaptive group). A positive value of D shows that the number of apoptotic signals on the embryo has decreased after receiving both the priming and challenging doses, when compared to receiving the challenging dose only, and indicates the presence of RAR.

Table 1 summarises the values of D for different irradiation conditions to provide the priming dose, together with the sample size for each irradiation condition. The size of the adaptive control group was 89. The significance of the D values was determined by the t-distribution after outliers, if any, were removed, and those with $p < 0.05$ were considered statistically significant. From table 1, all three sets of experiments (namely, with irradiations with 200, 300 and 500 protons) showing a positive D value, i.e., those showing the RAR, had a $p \leq 0.05$. All other irradiation conditions (with <200 protons) showed negative D values, i.e., absence of RAR, with $p \leq 0.05$ except for irradiations with 20 and 30 protons. These clearly showed that the number of protons for inducing RAR in zebrafish embryos was between 100 and 200.

Figures 1 and 2 show the horizontal and vertical cross-sectional images, respectively, of a 5 hpf zebrafish embryo after paraffin embedding and sectioning. Only the outermost layer

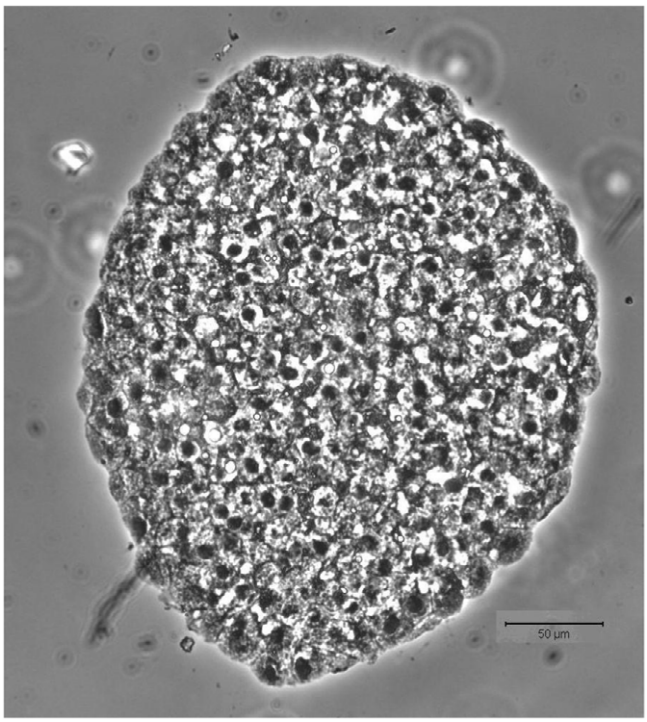

Figure 1. Horizontal cross-sectional view of a 5 hpf zebrafish embryo after paraffin embedding and sectioning, captured under the phase-contrast mode of a compound microscope (Nikon) with 200× magnification.

contained the epithelial cells while all other cells are deep cells (Campana *et al* 2010), which enabled identification of these cells directly from figures 1 and 2. Concha and Adams (1998) observed morphological changes of zebrafish cells during the gastrula stage under an inverted microscope equipped with DIC optics. The results suggested that deep cells of the late blastula period as well as the early gastrula period (50% epiboly) were spherical, loosely organised and separated by regions of intercellular space. From the images in figures 1 and 2, the mean deep cell diameter and thickness were determined as 11.0 ± 0.3 and 11.6 ± 0.2 μm, respectively (sample size $= 20$), while the mean epithelial cell diameter and thickness were determined as 15.1 ± 0.6 and 9.4 ± 0.7 μm, respectively (sample size $= 6$). As such, the average masses of a deep cell and an epithelial cell were calculated as 0.735 and 1.122 ng, respectively (assuming the density of water). The dimensions implied that the proton microbeam size of 2 μm was much smaller than the diameters of the epithelial cells and the deep cells. Figure 2 shows that the normally incident microbeam protons would pass through one epithelial cell and six layers of deep cells. Using this information, the energies and doses deposited by a 3.37 MeV proton in an epithelial cell and the deep cells were estimated as follows:

(1) epithelial cell: 0.104 MeV and 14.8 mGy;
(2) first layer deep cell: 0.136 MeV and 29.6 mGy;
(3) second layer deep cell: 0.137 MeV and 29.8 mGy;
(4) third layer deep cell: 0.146 MeV and 31.8 mGy;
(5) fourth layer deep cell: 0.149 MeV and 32.4 mGy;

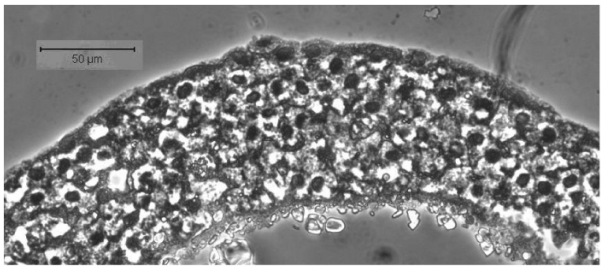

Figure 2. Vertical cross-sectional view of a 5 hpf zebrafish embryo after paraffin embedding and sectioning, captured under the phase-contrast mode of a compound microscope (Nikon) with 200× magnification.

(6) fifth layer deep cell: 0.156 MeV and 34.0 mGy;
(7) sixth layer deep cell: 0.165 MeV and 35.9 mGy.

For simplicity, we adopted 32.3 mGy as the average dose absorbed by a deep cell.

4. Discussion

The results in table 1 clearly show that the number of protons for inducing RAR in zebrafish embryos is between 100 and 200, if only one single irradiation point is used. When compared to our previous study (Choi *et al* 2010b), we concluded that the capability to induce RAR in the zebrafish embryos depends on the number of irradiated cells.

Until now, the mechanisms involved in the RAR have not been fully understood. However, Youngblom *et al* (1989) studied RAR in human lymphocytes and attributed the adaptation to induction of repair enzymes (proteins), and showed that application of the protein synthesis inhibitor cycloheximide prevented the RAR. Moreover, the need to exceed a threshold level of radiation injury for full induction of repair processes has been established by work on the adaptive response (Shadley *et al* 1987, Raaphorst and Boyden 1999). On the other hand, Ikushima *et al* (1996) studied repair kinetics of DNA damage in Chinese hamster V79 cells and showed that the RAR could be a result of DNA repair mechanisms which led to less residual DNA damage, but not from the induction of protective mechanisms that reduced the initial DNA damage.

In the current experiment with only one irradiation position, 5 protons were too few to inflict a sufficient amount of damage, which would therefore lead to a relatively small amount of repair proteins, and the surplus would be insufficient to deal with the damage inflicted by the subsequent challenging dose. The radiation-induced bystander effect (RIBE) might also need to be considered here. In our previous studies, RAR was successfully induced in zebrafish by a relatively small number of protons (Choi *et al* 2010b) or alpha particles (Choi *et al* 2011). In these cases, it was not possible for all the cells of the embryos to have been hit. As such, it was likely that the RAR in the zebrafish embryos for low-dose proton or alpha-particle irradiation developed through the cell-to-cell bystander effect. In the present work, for irradiation at one position in the embryo, about 1 epithelial cell and 6 deep cells were irradiated. The number is almost negligible when compared with the total of ∼500 epithelial cells and ∼2000 deep cells in an embryo at 5 hpf (Campana *et al* 2010).

The RIBE had been extensively studied since its observation by Nagasawa and Little (1992). Much evidence has been accumulated which supports the idea that irradiated cells might release stress signal factor(s) to affect the neighbouring cells, or affect the cells which

have received the medium conditioned by the irradiated cells (see, for example, the reviews by Little 2006, Mothersill and Seymour 2006, Morgan and Sowa 2007). Recently, our group also found that RAR could be induced in naive zebrafish embryos through the RIBE (Choi *et al* 2010a). Apparently, for irradiation with 5 protons at one position of the zebrafish embryo, the bystander signals sent from the irradiated cells did not inflict sufficient damage (and thus did not trigger sufficient repair proteins in the bystander cells). This is in line with the report of Prise *et al* (2002) that 5 or more protons with 3.2 MeV delivered to a single Chinese hamster V79 cell could cause a reduction in cell survival for the whole population of around 5%, while a single proton through a single cell did not increase the cell killing. This is also commensurate with the data of Schettino *et al* (2003, 2005) showing the decrease in the clonogenic survival of V79 cells after exposure of cell nuclei to an increasing dose of focused C_K ultrasoft x-rays. Gow *et al* (2008) also demonstrated a dose-dependent manner of the bystander effect communicated through the conditioned medium.

On the other hand, our group discovered in a recent *in vitro* study that irradiated cells could actually derive benefit from the feedback signals sent from the bystander cells (Chen *et al* 2011). By studying human primary fibroblast (NHLF) and cancer cells (HeLa) in a two cell co-culture system, a significant decrease in the numbers of 53BP1 foci, micronucleus formation and extent of apoptosis was observed when the irradiated cells were co-cultured with the bystander cells (Chen *et al* 2011). The effect on the irradiated cells was referred to as the 'rescue effect'. Although the underlying mechanism for the rescue effect is still unknown, it is probable that the feedback signals sent from the bystander cells can help to induce repair proteins in the irradiated cells. Our group also found that rescue signals could be sent by bystander unirradiated zebrafish embryos back to the irradiated zebrafish embryos, and that the strength of the rescue effect depended on the number of rescuing bystander unirradiated embryos (Choi *et al* 2012). Apparently, for irradiation with 5 protons at one position of the zebrafish embryo, the feedback signals sent from the bystander cells surrounding the irradiated cells also did not induce sufficient repair proteins. As such, no RAR was observed.

In contrast, in the previous research of Choi *et al* (2010b) where 10 positions were used with 5 protons irradiated at each position, RAR was successfully detected. Here, the bystander cells obtained accumulated bystander signals from damaged cells at 10 positions. As such the damage inflicted on the bystander cells through the bystander signals would become much larger, and would be able to induce sufficient repair proteins in the bystander cells (Prise *et al* 2002, Schettino *et al* 2003, 2005). Subsequently, the feedback signals sent from these bystander cells back to the irradiated cells were much stronger and could induce sufficient repair proteins in the irradiated cells (cf Choi *et al* 2012). As such, RAR could be induced.

When the number of protons irradiated at a single position to provide the priming dose was increased to 200 or beyond, RAR was successfully induced. Apparently, for such irradiations, the amount of DNA damage was beyond the threshold value, which led to production of repair proteins in a sufficient surplus to deal with the damage inflicted by the subsequent challenging dose.

In the present work, we obtained the dimensions of epithelial and deep cells in the zebrafish embryos at 5 hpf from the optical cross-sectional images obtained after paraffin embedding and sectioning of the zebrafish embryos. The average dose absorbed by an epithelial cell and a deep cell from the traversal of a 3.37 MeV proton were estimated as 14.8 and 32.3 mGy, respectively. As such, irradiation of 200 protons to a single position on a zebrafish embryo would give doses of 296 and 646 mGy. Several previous studies have reported on the range of the priming dose for the induction of RAR *in vitro* (Sorensen *et al* 2002, Sasaki *et al* 2002, Matsumoto *et al* 2007), namely, from 10 to 200 mGy for low-LET radiation. However, we should be cautious of making such direct comparisons. Most previous studies on the induction of RAR have involved

the use of low-LET radiation, in which all the cells were hit, while the current study involved the use of microbeam protons, in which only a few cells were hit. From the previous discussion, the irradiated cells and the bystander cells were not isolated from one another; instead they communicated the bystander signals and rescue signals, which would dictate the damage in the bystander cells, and also the amount of repair proteins formed in the irradiated and bystander cells as a result of the priming dose. In other words, the effects of the irradiation could be spread over to other cells in the zebrafish embryos.

In conclusion, the present paper shows that the number of protons to induce RAR in zebrafish embryos is between 100 and 200, if only one single irradiation point is used, which gives doses of 296 and 646 mGy to the epithelial and deep cells, respectively. The results also reveal that the capability to induce RAR depends on the number of irradiated cells, which can be explained in terms of the radiation-induced bystander effect as well as the rescue effect.

For radiation protection purposes, prediction of risk from an ionising-radiation exposure is enabled by adopting the linear no-threshold (LNT) hypothesis which states that the risk is linearly proportional to the dose normalised by the radiation weighting factor, and which assumes a no-threshold dose value below which no radiation risk is expected. In other words, the LNT model implies that the addition of a dose will lead to an addition in the risk, which, however, does not agree with the induction of radioadaptive response. As such, for accurate and realistic radiation protection considerations, radioadaptive responses should not be neglected. More research on radioadaptive responses, including studies on the underlying mechanisms, is therefore both pertinent and warranted.

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