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Effects of alpha particles on zebrafish embryos

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ABSTRACT

Dechorionated zebrafish embryos were irradiated at 1.5 h post fertilization (hpf) to low-doses of alpha particles, viz., 1.4, 2.8, 5.6, 11.2 mGy (determined using Monte Carlo simulations). At 24 hpf, these embryos were then examined for apoptotic cells through acridine orange staining. The mean number of apoptotic cells was found to decrease significantly from controls to 1.4-mGy irradiation, and then to increase almost linearly to 2.8, 5.6 and 11.2-mGy irradiation. This trend is a typical characteristic of a hormetic effect.

1. Introduction

Radiation hormesis describes the phenomenon that low doses of ionizing radiation have beneficial effects (Calabrese and Linda, 2003; Kaiser, 2003; Feinendegen, 2005). According to this effect, ionizing radiation at natural environmental levels may promote health by stimulating defense and repair mechanisms. Nevertheless, the radiation hormesis model does not dispute the detrimental effects associated with irradiations much higher than the natural environmental levels. The 2005 report of The Académie des Sciences-Académie nationale de Médecine (French Academy of Sciences-National Academy of Medicine) remarked that 40% of laboratory studies on cell cultures and animals have observed radiation hormesis (Duport, 2003; Calabrese, 2004), but at the same time also cautioned that occurrence of radiation hormesis in humans was not yet well established (Aurengo et al., 2005).

The most studied ionizing radiation at natural environmental levels is the alpha particles emitted from progeny of ^{222}Rn gas, exposures to which are shown to lead to lung cancers. It is well established that elevated levels of radon gas and its progeny in underground mines have posed significant lung-cancer risks for the miners. However, miner exposures were typically 30 times larger than residential exposures, so extrapolation of risk over such a substantial range of exposures remains dubious.

There have been more than twenty case-control studies of the dependence of lung-cancer risk on radon levels in homes. A meta-analysis of 17 studies suggested a linear dependence (Pavia et al., 2003). The pooled analysis of seven North American studies (Krewski et al., 2005, 2006) found agreement with the "linear, no threshold" (LNT) model but the 95% CI included the possibility of a threshold. The pooled analysis of 13 European studies (Darby et al., 2005) produced data which also fitted the LNT model but the 95% CI excluded a threshold. Furthermore, the two poolings of Chinese data (Lubin, 2003; Lubin et al., 2004) excluded a threshold. Conversely, in the most recent case-control study, Thompson et al. (2008) found evidence supporting a hormetic dose-response for radon exposures $< 150\,\mathrm{Bqm}^{-3}$, which deviated significantly from the LNT scenario. Thompson et al. (2008) attributed the differences in the outcomes to the design in their studies, such as locations of the radon monitors, weighting of measurements according to in-house mobility, and corrections according to changing lifestyle.

Ecological studies of the dependence lacked the individual matching of case-control studies. The studies by Cohen (1995, 1997) were by far the largest and most fully analyzed, also having been criticized and defended. Interestingly, they also found a hormetic response. However, the BEIR VI report (National Research Council, 1999) reviewed the ecologic studies and judged that they were not informative because of inherent limitations of the ecologic method.

On the cellular level, evidence is accumulating that exposures to low doses of oxidants may have a stimulatory effect on cellular processes (Macklis and Beresford, 1991; Miyachi, 2000), in contrast to cytotoxic effects of exposures to high doses. In this connection, Miyachi et al. (2003) attempted to study the effect of low doses of X-ray on zebrafish development. They found a significant decrease in time to hatching following exposures of the zebrafish embryos to 0.025-Gy X-ray irradiation during the cleavage period (1.5 h after fertilization), and also observed that the greatest decrease in this interval after exposures during the blastula period (3.5 h). On the other hand, they also noticed that this radiation-induced effect was eliminated when the dose was increased to 0.15 Gy. They concluded that exposures to low-dose

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X-rays might induce positive effects on physiological functioning (Miyachi et al., 2003).

Based on the finding of a hormetic dose-response for low-level radon exposures (Thompson et al., 2008) and the positive effects on physiological functioning of zebrafish embryos induced by low-dose X-rays (Miyachi et al., 2003), it becomes pertinent to study the radiation hormesis for low-dose alpha particles in zebrafish embryos, which forms the objective of the present work. In the present work, the zebrafish, *Danio rerio*, a small vertebrate from Southeast Asia, will be employed. It is remarked here that, in recent years, *Danio rerio* has become a preferred model for studying human disease, including carcinogenesis. The most important advantage is that the human and zebrafish genomes share considerable homology, including conservation of most DNA repair-related genes (Barbazuk et al., 2000). Rapid embryonic development is another advantage in that major organ systems become evident within 24 h post fertilization (hpf).

2. Materials and methods

2.1. Dechorionation of zebrafish embryos

A number of previous research works have been carried out using the zebrafish embryo as an *in vivo* model to study the DNA damage response to ionizing radiation. However, energetic photons (X-rays and gamma rays) were normally used (e.g., Bladen et al., 2005; McAleer et al., 2005, 2006; Daroczi et al., 2006; Geiger et al., 2006). As mentioned in the introduction, the objective of the present work was to study the radiation hormesis for low-dose alpha particles in zebrafish embryos. Recently, we described an experimental setup for studying the effects of alpha particles on zebrafish embryos (Yum et al., 2007). One of the extra steps in dealing with the zebrafish embryos (when compared to those in experiments using X-rays and gamma rays) was the dechorionation of zebrafish embryos before alpha-particle irradiation, since the chorions would absorb a significant fraction of the alpha-particle energies. In the present experiments, the embryos were placed in a petri-dish lined with a layer of agarose, where their chorions were removed by hand with forceps.

2.2. Alpha-particle irradiation of zebrafish embryos

About 40 dechorionated embryos were prepared for alpha-particle irradiation each time. They were first transferred into a custom-made PADC-film based holder as shown in Fig. 1 (see Chan et al., 2006). Polyallyldiglycol carbonate (PADC) films were used as the substrate because they were biocompatible (e.g., Li et al., 2006) and their thickness could be controlled precisely through chemical etching to control the residual alpha-particle energy incident onto the dechorionated embryos (Yum et al., 2007). For alpha-particle irradiation of zebrafish embryos, it is only feasible to quantify the alpha energies incident on the embryos if the alpha particles pass through the substrate to strike the embryo cells (Fig. 2), because there is always a fluid layer with variable thickness above the embryos. PADC films are one of the most commonly used solid-state nuclear track detectors, on which a recent review on SSNTDs has been given by Nikezic and Yu (2004). In the present work, PADC films with a thickness of 16 μm were prepared from commercially available PADC films with a thickness of 100 μm (from Page Mouldings (Pershore) Limited, Worcestershire) by chemical etching in NaOH/Ethanol (Chan et al., 2007), which were then glued by an epoxy (Araldite® Rapid, England) to the bottom of a custom-made holder.

In the present work, alpha-particle irradiation of the dechorionated zebrafish embryos were made with a planar ^{241}Am source (with an alpha-particle energy of 5.49 MeV under vacuum and an activity of 4.259 kBq (0.1151 μCi) from the side of the PADC film at 1.5 hpf. At this developmental stage, the cells have not assumed differentiated cell fates. This time point (1.5 hpf) is also within the cleavage period of embryogenesis (0.7–2.2 h) (Kimmel and Law, 1985; Kimmel et al., 1995). Walker and Streisinger (1983) found that embryos older than 3 h were considerably more resistant to γ-rays, which suggested a possible repair mechanism after the cleavage stages.

Considering the physical size of the cells in the embryos and the large number of alpha particles involved, the absorbed dose received by the embryos can be surrogated by the irradiation

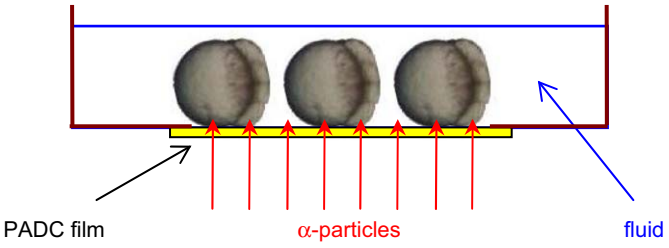

Fig. 2. The irradiation of the zebrafish embryos through the PADC-film based holder.

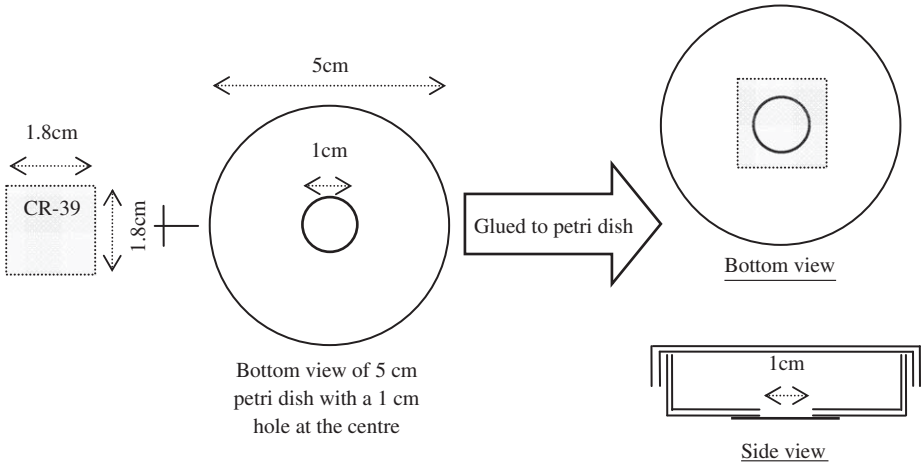

Fig. 1. Preparation of a custom-made PADC-film based holder by gluing a thin PADC film onto the bottom of a petri dish with 5 cm diameter and with a 1 cm hole drilled at the center of the bottom (Chan et al., 2006).

duration and can be estimated through Monte Carlo simulations. Absorbed dose is the amount of energy absorbed by a unit mass of the material, being the embryo cells in the present case. Due to the geometry of the embryos, the alpha particles will have to travel different distances in the water column to reach the embryos after traversing the PADC film. As a result, the alpha particles reach the embryos with different energies, and some may even be stopped in the water column and cannot reach the embryos at all. The energy distribution of alpha particles reaching the embryos is best taken care of using Monte Carlo simulations. Nevertheless, if it is necessary, the absorbed dose on each individual embryo can be determined more precisely through counting the number of alpha particles incident on each embryo (Yum et al., 2007).

In the present work, the average mass of a zebrafish embryo at 1.5 hpf was determined using the average mass determined from 300 dechorionated embryos weighted (see Hagedorn et al., 1997). The average mass ($\pm$ one standard error) was found to be 222 ± 6 μg. From the Monte Carlo simulations, the average alpha-particle dose rate on an embryo was 1.4 mGy/min. Using the doses employed by Miyachi et al. (2003), viz., 0.025, 0.05, 0.15, 0.25 or 0.5 Gy, as guidelines, and taking into account the relative biologic effectiveness by adopting the radiation weighting factor of 20 for alpha particles, we irradiated the embryos for 1, 2, 4 and 8 min, and 0 min (as controls), thereby providing alpha-particle doses of 1.4, 2.8, 5.6 and 11.2 mGy. After alpha-particle irradiation at 1.5 hpf, the embryos were placed into petri-dishes lined with layers of agarose and returned to the 37 °C incubator until they developed into 24 hpf, which is our chosen endpoint for more detailed analyses of apoptosis, a highly regulated biological process during embryonic development. Before 24 hpf, the untreated zebrafish embryos undergo high apoptotic activities as part of the organogenesis processes (Chan and Cheng, 2003). The 24 hpf endpoint was also used by Bladen et al. (2005) who commented that increasing pigmentation after 24 hpf might obscure the signals from the apoptotic cells.

2.3. Apoptotic cell staining using acridine orange

At 24 hpf, the embryos were collected and examined for cell death by vital dye staining according to the method previously described by Chan and Cheng (2003). Briefly, the embryos were transferred to a culture medium containing 5 μg/ml of acridine orange. Embryos were stained for 50 min followed by thorough washing in the culture medium for three times. Embryos were then anaesthetized with 0.016 M tricaine (Sigma, St. Louis, MO, USA). Images of the stained embryos were captured under a florescent microscope. The number of apoptotic cells for each embryo was counted with the help of the software MetaMorph version 7.0r0 (1992–2006 Molecular devices).

3. Results and discussion

The mean numbers of apoptotic cells for zebrafish embryos irradiated for different periods of time are represented in Table 1 as well as in Fig. 3. It is very interesting to observe that the mean number decreases significantly from 0-min irradiation (i.e., the controls) to 1-min irradiation, and then increases almost linearly to 2-, 4- and 8-min irradiation. This trend is a typical characteristic of a hormetic effect. The statistical significances of differences among the mean numbers of apoptotic cells were determined using two-tailed t-tests and assuming different variances. The p values for the comparisons are presented in Table 2, and the p values smaller than 0.05 are considered statistically significant. As can be observed in Table 2, the number of apoptotic cells in zebrafish embryos for 1-min irradiation was significantly smaller than that corresponding to 0-min irradiation (controls), which translates to a statistically significant hormetic effect.

Moreover, the number of apoptotic cells in zebrafish embryos for 4-min irradiation was significantly larger than those corresponding to 1- and 2-min irradiation, and that for 8-min irradiation was significantly larger than those corresponding to 0-, 1-, 2- and 4-min irradiation. These results showed that DNA damages during zebrafish embryogenesis can be induced by alpha-particle irradiation, which suggested that zebrafish is a potential model for assessing the effects of alpha-particle radiation.

From Table 2 and Fig. 3, we observed that alpha-particle irradiation of the zebrafish embryos for 1 min (or equivalently 1.4 mGy) produced a hormetic effect, that for 2 min (or equivalently 0.0028 Gy) might also produce a hormetic effect but without a statistical significance. Those for 4 and 8 min (or equivalently 5.6 and 11.2 mGy, respectively) did not lead to

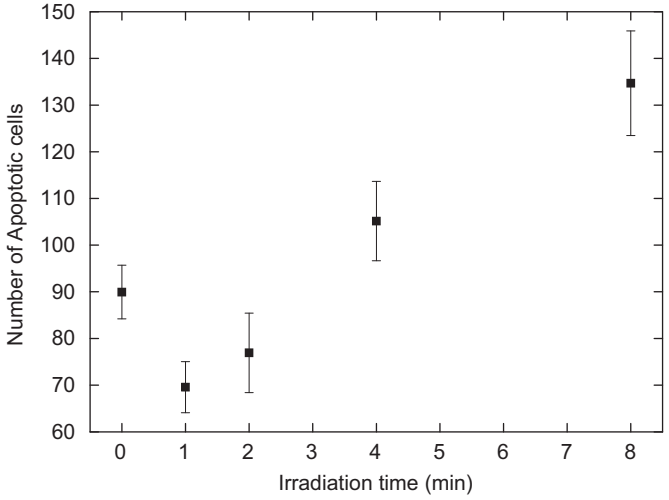

Fig. 3. The relationship between the mean number of apoptotic cells (error bars showing one standard errors) obtained in zebrafish embryos irradiated for different durations.

Table 1
The mean number of apoptotic cells ($\pm$ standard error) for zebrafish embryos irradiated for different periods of time.

	0 min	1 min	2 min	4 min	8 min
Mean $\pm$ SE	90 ± 6	70 ± 5	77 ± 9	105 ± 9	135 ± 11
N	51	45	39	34	35

(N is the sample size).

Table 2
The p values for comparisons between mean numbers of apoptotic cells for zebrafish embryos irradiated for different periods of time, determined using two-tailed t-test and assuming different variances.

	1 min	2 min	4 min	8 min
0 min	**0.0117**	0.209	0.143	$\mathbf{8.16 \times 10^{-4}}$
1 min		0.469	$\mathbf{8.40 \times 10^{-4}}$	$\mathbf{3.39 \times 10^{-6}}$
2 min			**0.0218**	$\mathbf{1.14 \times 10^{-4}}$
4 min				$\mathbf{3.97 \times 10^{-2}}$

The p values smaller than 0.05 are considered statistically significant and are in bold.

observable hormetic effects. These observations were in line with the results obtained by Miyachi et al. (2003) who attempted to study the effect of low doses of X-ray on zebrafish development. They found a significant decrease in time to hatching following exposures of the zebrafish embryos to 0.025-Gy X-ray irradiation during the cleavage period (1.5 hpf), but this radiation-induced effect was eliminated when the dose was increased to 0.15 Gy. It is also interesting to note that Bladen et al. (2005) found an absence of ectopic apoptotic cell death in zebrafish embryos irradiated to 0.15 Gy gamma rays at 6 hpf, but a large amount at a 3-fold higher dose, and the authors suggested the existence of a threshold below which radiation-induced cell death did not occur.

Incidentally, as mentioned in the introduction, Thompson et al. (2008) found evidence supporting a hormetic dose-response for radon exposures $< 150\,\mathrm{Bqm}^{-3}$. The annual absorbed dose in the lungs is estimated as 1.2 mGy from the radon gas concentration of $150\,\mathrm{Bqm}^{-3}$ using nominal values for the equilibrium factor of 0.4, an indoor occupancy factor of 0.7 and an effective dose conversion coefficient of 5 mSv/WLM, also considering the tissue weighting factor of 0.12 for the lungs and radiation weighting factor of 20 for alpha particles.

4. Conclusions

The present work studied the radiation hormesis for low-dose alpha particles in zebrafish embryos. In these alpha-particle experiments, dechorionaed zebrafish embryos were used for alpha-particle irradiation, and thin (16 μm) polyallyldiglycol carbonate (PADC) films were used as the substrate and irradiation from the side of the PADC films were required. Irradiation of 1.5 hpf dechorionated zebrafish embryos was made with an ^{241}Am source with an activity of 4.259 kBq (0.1151 μCi) for 1, 2, 4 and 8 min, and 0 min (as controls), corresponding to alpha-particle dose of 1.4, 2.8, 5.6, 11.2 and 0 mGy, respectively, determined using Monte Carlo simulations. Subsequently, the embryos were placed into petri-dishes lined with layers of agarose and returned to the 37 °C incubator until they developed into 24 hpf. At 24 hpf, the embryos were collected and examined for cell death through staining using acridine orange.

The mean number of apoptotic cells was found to decrease significantly from 0-min irradiation (i.e., the controls) to 1-min irradiation, and then to increase almost linearly to 2-min, 4-min and 8-min irradiation. This trend is a typical characteristic of a hormetic effect. The differences were statistically significant ($p < 0.05$) between 0- and 1-min irradiation, between 4-min and 1- or 2-min irradiation, and between 8-min irradiation and all other irradiations.

References

Aurengo, A., Averbeck, D., Bonnin, A., Le Guen, B., Masse, R., Monier, R., Tubiana, M., Valleron, A.-J., 2005. Dose-effect relationships and estimation of the carcinogenic effects of low doses of ionizing radiation. Académie des Sciences & Académie nationale de Médecine.

Barbazuk, W.B., Korf, I., Kadavi, C., Heyen, J., Tate, S., Wun, E., Bedell, J.A., McPherson, J.D., Johnson, S.L., 2000. The syntenic relationship of the zebrafish and human genomes. Genome Res. 10, 1351–1358.

Bladen, C.L., Lam, W.K., Dynan, W.S., Kozlowski, D.J., 2005. DNA damage response and Ku80 function in the vertebrate embryo. Nucleic Acids Res. 33, 3002–3010.

Calabrese, E.J., 2004. Hormesis: from marginalization to mainstream: a case for hormesis as the default dose-response model in risk assessment. Toxicol. Appl. Pharmacol. 197, 125–136.

Calabrese, E.J., Linda, A.B., 2003. Toxicology rethinks its central belief. Nature 421, 691–692.

Chan, K.F., Tse, A.K.W., Fong, W.F., Yu, K.N., 2006. Feasibility studies of colorless LR 115 SSNTD for alpha-particle radiobiological experiments. Nucl. Instrum. Methods Phys. Res. B 247, 307–312.

Chan, K.F., Lau, B.M.F., Nikezic, D., Tse, A.K.W., Fong, W.F., Yu, K.N., 2007. Simple preparation of thin CR-39 detectors for alpha-particle radiobiological experiments. Nucl. Instrum. Methods Phys. Res. B 263, 290–293.

Chan, P.K., Cheng, S.H., 2003. Cadmium-induced ectopic apoptosis in zebrafish embryos. Archives Toxicol. 77, 69–79.

Cohen, B.L., 1995. Test of the linear-no threshold theory of radiation carcinogenesis for inhaled radon decay products. Health Phys. 68, 157–174.

Cohen, B.L., 1997. Lung cancer rate vs. mean radon level in US countries of various characteristics. Health Phys. 72, 114–119.

Darby, S., Hill, D., Auvinen, A., Barros-Dios, J.M., Baysson, H., Bochicchio, F., Deo, H., Falk, R., Forastiere, F., Hakama, M., Heid, I., Kreienbrock, L., Kreuzer, M., Lagarde, F., Makelainen, I., Muirhead, C., Oberaigner, W., Pershagen, G., Ruano-Ravina, A., Ruosteenoja, E., Schaffrath Rosario, A., Tirmarche, M., Tomasek, L., Whitley, E., Wichmann, H.-E., Doll, R., 2005. Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. Br. Med. J. 330, 223–226.

Daroczi, B., Kari, G., McAleer, M.F., Wolf, J.C., Rodeck, U., Dicker, A.P., 2006. In vivo radioprotection by the fullerene nanoparticle DF-1 as assessed in a zebrafish model. Clin. Cancer Res. 12, 7086–7091.

Duport, P., 2003. A database of cancer induction by low-dose radiation in mammals: overview and initial observations. Int. J. Low Radiat. 1, 120–131.

Feinendegen, L.E., 2005. Evidence for beneficial low level radiation effects and radiation hormesis. Br. J. Radiol. 78, 3–7.

Geiger, G.A., Parker, S.E., Beothy, A.P., Tucker, J.A., Mullins, M.C., Kao, G.D., 2006. Zebrafish as a "Biosensor"? effects of ionizing radiation and amifostine on embryonic viability and development. Cancer Res. 66, 8172–8181.

Hagedorn, M., Kleinhans, F.W., Freitas, R., Liu, J., Hsu, E.W., Wildt, D.E., Rall, W.F., 1997. Water distribution and permeability of zebrafish embryos, brachydanio rerio. J. Exp. Zool. 278, 356–371.

Kaiser, J., 2003. Hormesis: sipping from a poisoned chalice. Science 302, 376–379.

Kimmel, C.B., Law, R.D., 1985. Cell lineage of zebrafish blastomeres. I. cleavage pattern and cytoplasmic bridges between cells. Dev. Biol. 108, 78–85.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zeberafish. Dev. Dyn. 203, 253–310.

Krewski, D., Lubin, J.H., Zielinski, J.M., Alavanja, M., Catalan, V.S., Field, R.W., Klotz, J.B., Letourneau, E.G., Lynch, C.F., Lyon, J.I., Sandler, D.P., Schoenberg, J.B., Steck, D.J., Stolwijk, J.A., Weinberg, C., Wilcox, H.B., 2005. Residential radon and risk of lung cancer: a combined analysis of 7 North American case-control studies. Epidemiology 16, 137–145.

Krewski, D., Lubin, J.H., Zielinski, J.M., Alavanja, M., Catalan, V.S., Field, R.W., Klotz, J.B., Letourneau, E.G., Lynch, C.F., Lyon, J.I., Sandler, D.P., Schoenberg, J.B., Steck, D.J., Stolwijk, J.A., Weinberg, C., Wilcox, H.B., 2006. A combined analysis of North American case-control studies of residential radon and lung cancer. J. Toxicol. Environ. Health A 69, 533–597.

Li, W.Y., Chan, K.F., Tse, A.K.W., Fong, W.F., Yu, K.N., 2006. Studies of biocompatibility of chemically etched CR-39 SSNTDs in view of their applications in alpha-particle radiobiological experiments. Nucl. Instrum. Methods Phys. Res. B 248, 319–323.

Lubin, J.H., 2003. Studies of radon and lung cancer in North America and China. Radiat. Prot. Dosim. 104, 315–319.

Lubin, J.H., Wang, Z.Y., Boice Jr., J.D., Xu, Z.Y., Blot, W.J., De Wang, L., Kleinerman, R.A., 2004. Risk of lung cancer and residential radon in China: pooled results of two studies. Int. J. Cancer 109, 132–137.

Macklis, R.M., Beresford, B., 1991. Radiation hormesis. J. Nucl. Med. 32, 350.

McAleer, M.F., Davidson, C., Davidson, W.R., Yentzer, B., Farber, S.A., Rodeck, U., Dicker, A.P., 2005. Novel use of zebrafish as a vertebrate model to screen radiation protectors and sensitizers. Int. J. Radiat. Oncol. Biol. Phys. 61, 10–13.

McAleer, M.F., Duffy, K.T., Davidson, W.R., Kari, G., Dicker, A.P., Rodeck, U., Wickstrom, E., 2006. Antisense inhibition of cyclin D1 expression is equivalent to flavopiridol for radiosensitization of zebrafish embryos. Int. J. Radiat. Oncol. Biol. Phys. 66, 546–551.

Miyachi, Y., 2000. Acute mild hypothermia caused by a low dose of X-irradiation induces a protective effect against mid-lethal doses of X-rays, and a low level concentration of ozone may act as a radiomimetic. Br. J. Radiol. 73, 298–304.

Miyachi, Y., Kanao, T., Okamoto, T., 2003. Marked depression of time interval between fertilization period and hatching period following exposure to low-dose X-rays in zebrafish. Environmental Research 93, 216–219.

National Research Council, 1999. Committee on Health Risks of Exposure to Radon, Board on Radiation Effects Research, Commission on Life Sciences, Health effects of exposure to radon, BEIR VI, vol. 6. National Academy Press, Washington, DC, p. 49 (App. G).

Nikezic, D., Yu, K.N., 2004. Formation and growth of tracks in nuclear track materials. Mater. Sci. Eng. R 46, 51–123.

Pavia, M., Bianco, A., Pileggi, C., Angelillo, I.F., 2003. Meta-analysis of residential exposure to radon gas and lung cancer. Bull. World Health Org. 81, 732–738.

Thompson, R.E., Nelson, D.F., Popkin, J.H., Popkin, Z., 2008. Case-control study of lung cancer risk from residential radon exposure in Worcester county, Massachusetts. Health Phys. 94, 228–241.

Walker, C., Streisinger, G., 1983. Induction of mutations by gamma rays in pregonial germ cells of zebrafish embryos. Genetics 103, 125–136.

Yum, E.H.W., Ng, C.K.M., Lin, A.C.C., Cheng, S.H., Yu, K.N., 2007. Experimental setup for studying the effects of alpha particles on zebrafish embryos. Nucl. Instrum. Methods Phys. Res. B 264, 171–176.