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Effects of Photon Hormesis on Cells Irradiated by Alpha Particles

Y.K. Fung¹, K.N. Yu^{1,2,*}

¹ Department of Physics and Materials Science, City University of Hong Kong, Kowloon Tong, Hong Kong; E-Mails: yikifung2-c@my.cityu.edu.hk

² State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong

* Author to whom correspondence should be addressed; E-Mail: peter.yu@cityu.edu.hk
Tel.: +852-34427812; Fax: +852-34420538.

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Abstract: Hormetic responses generally refer to biphasic dose-response relationships showing low-dose stimulation and high-dose inhibition. In relation, “photon hormesis” refers to the phenomenon in which a low-dose photon irradiation mitigates the cellular damages induced by other ionizing radiations. “Photon hormesis” can mean gamma-ray hormesis or X-ray hormesis depending on the origin of the photons. In the present study, photon hormesis was studied using wild type (WT) and p53^{-/-} HCT116 colon cancer cells by comparing the number of p53 binding-protein 1 (53BP1) foci per cell (fpc) in the cells which were (1) irradiated by a toxic dose of alpha particles from an ²⁴¹Am source, and (2) irradiated by the same alpha-particle exposure with an additional small X-ray dose. With the additional X-ray dose, the numbers of 53BP1 fpc were significantly reduced at 24 h post-irradiation for the WT HCT116 cells, which confirmed the presence of photon hormesis, but not for the p53^{-/-} HCT116 cells. This showed that photon hormesis was induced through a p53-dependent pathway. The data for WT HCT116 cells showed that photon hormesis were observable only after ~ 7 h post irradiation, which was in agreement with the role played by p53 in the rapid response in repairing DNA double strand breaks. Comparisons between the responses (in terms of numbers of 53BP1 fpc) of WT and p53^{-/-}-HCT116 cells showed different trends in the drop of responses with time for the two types of cells, and suggested a delay in the DNA repair and/or apoptosis induction in the p53^{-/-}-HCT116 cells.

Keywords: hormesis; hormetic effect; alpha particle; X-ray; p53; 53BP1

1. Introduction

Hormetic responses generally refer to biphasic dose-response relationships showing low-dose stimulation and high-dose inhibition [1-3]. In relation, “photon hormesis” refers to the phenomenon in which a low-dose photon irradiation mitigates the cellular damages induced by other ionizing radiations [4]. “Photon hormesis” can mean gamma-ray hormesis or X-ray hormesis depending on the origin of the photons.

Photon hormesis has been suggested as the underlying reasons for mitigation of alpha-particle-induced lung cancers [5,6], decreased micronucleus formation in neutron-irradiated human lymphocytes [4], reduced response of zebrafish embryos to neutrons [7], presence of an upper limit of neutron dose for inducing bystander effect in zebrafish embryos [8], and non-induction of radioadaptive response in zebrafish embryos by neutrons [9]. Although photon hormesis has been successfully employed to explain these interesting phenomena, there have been few attempts to study photon hormesis such as its temporal development or the underlying pathways.

Mechanisms that have been proposed to explain photon hormesis include removal of aberrant cells through early apoptosis and induction of high-fidelity DNA repair [10-12]. Since p53 can activate DNA repair proteins, induce cell-cycle arrest and can also initiate apoptosis, it is interesting and pertinent to examine whether photon hormesis is developed in cells through a p53-dependent pathway. In the present study, we will study photon hormesis in wide type (WT) HCT116 colon carcinoma cells and the clonal p53-deficient (p53^{-/-}) HCT116 cells by comparing the number of p53 binding-protein 1 (53BP1) foci per cell (fpc) formed in these cells which have been (1) irradiated by a toxic dose of alpha particles from an ²⁴¹Am source (hereafter referred to as the IA condition), and (2) irradiated by the same exposure of alpha particles with an additional small X-ray dose (hereafter referred to as the IAX condition). By studying the similarity or difference in the responses of the WT and p53^{-/-}-HCT116 cells, we will illustrate whether photon hormesis is developed through a p53-dependent pathway. We will also study the development of photon hormesis in both cell lines at different time points after irradiation with alpha particles or (alpha particles + X-ray photons).

2. Results and Discussion

Figure 1 shows the numbers of 53BP1 fpc for the two studied cell lines (WT and p53^{-/-} HCT116 cells) at different time points (i.e., 3, 7, 12 and 24 h post-irradiation) under IA and IAX conditions. From the data for WT HCT116 cells shown in Figure 2(a), we observe that the numbers of 53BP1 fpc for IAX groups are larger than those for the IA groups at 3 and 7 h, which means that photon hormesis is not observable for 7 h post irradiation or earlier. At 12 and 24 h post irradiation, the numbers of 53BP1 fpc for IAX groups have become smaller than those for the IA groups. In particular, the difference is statistically significant at 24 h ($p < 0.05$), which demonstrates the presence of photon hormesis. From the data for p53^{-/-} HCT116 cells shown in Figure 2(b), we observe that the numbers of 53BP1 fpc for IAX groups are invariably larger than those for the IA groups at all studied time points. In particular, the difference is statistically significant at 24 h ($p < 0.05$). These demonstrate that photon hormesis has not been developed in p53^{-/-} HCT116 cells. The different responses of the WT and p53^{-/-} HCT116 cells particularly at 24 h post irradiation illustrate that photon hormesis is developed through

a p53-dependent pathway. The significant differences only obtained at the time point of 24 h post irradiation might seem a bit unexpected at first sight since 53BP1 foci appear shortly after irradiation. However, it is well established that the 53BP1 foci will remain for some time after irradiation, and more significant differences can be obtained when the numbers of fpc become smaller [13-18].

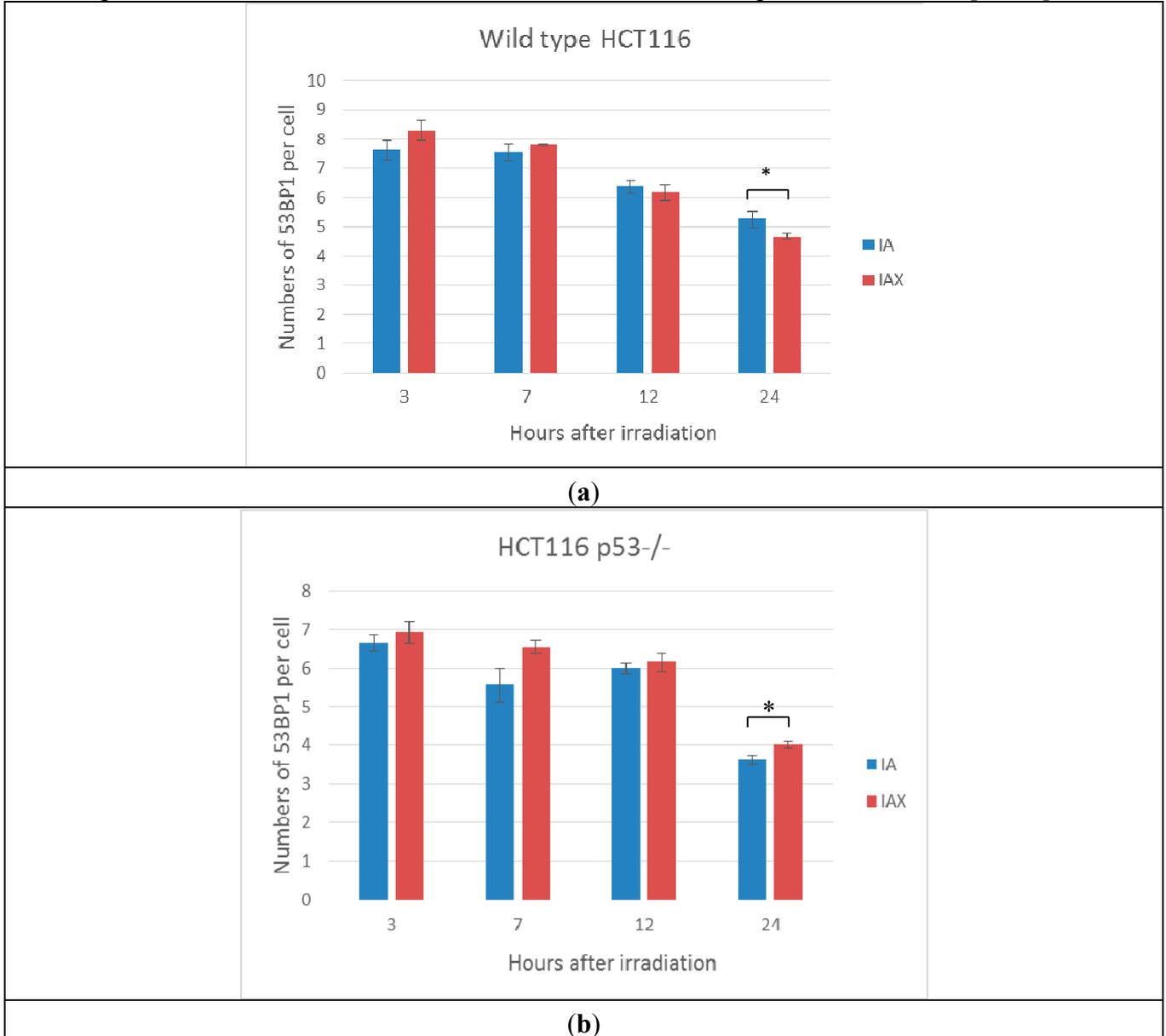


Figure 1. The numbers of 53BP1 foci per cell in (a) wild type HCT116 and (b) p53^{-/-} HCT116 cell lines, after being exposed to alpha-particle radiation (IA) and alpha-particle irradiation together with a small dose of X-ray (IAX) at different time points. (* $p < 0.05$)

The data for WT HCT116 cells in Figure 2(a) show that the effects of photon hormesis are observable only after about 7 h post irradiation. This finding is in agreement with the observation that p53 played an important role in the rapid response in repairing DNA double strand breaks (DSBs) between around 1 to 6 h [19].

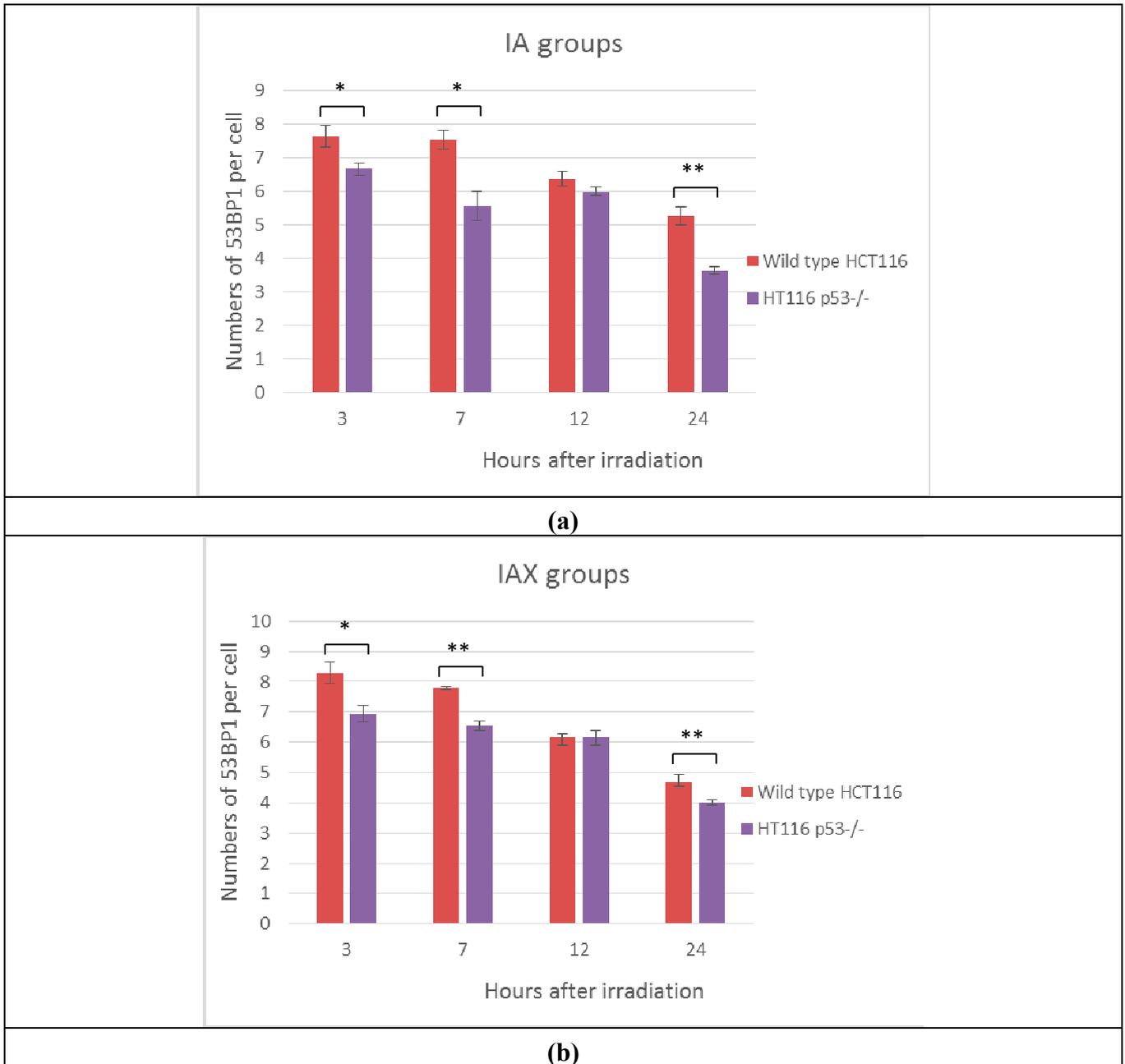


Figure 2. The numbers of 53BP1 foci per cell in (a) IA group of cells exposed to alpha-particle radiation and (b) IAX group of cells exposed to alpha-particle irradiation together with a small dose of X-ray at different time points. (* $p < 0.05$; ** $p < 0.01$)

The alpha-particle source we have employed in our experiments has an activity of 4260 Bq. Considering 2π geometry of the source and the radius of the active area as 2.5 mm, and an irradiation time of 4 min, the number density of alpha particles striking the targets will be $2.6 \times 10^4 \text{ mm}^{-2}$. If we adopt that the average area of an HCT116 cell is $266 \mu\text{m}^2$ [20], each cell will be on average hit by about 7 alpha particles. As such, we do not need to consider the complications brought about by the radiation-induced bystander effect [21-23] or the rescue effect [17,24].

Figure 2 shows the responses (in terms of numbers of 53BP1 fpc) of WT and p53^{-/-} HCT116 cells in both IA and IAX groups at different time points. Except for the time point of 12 h post irradiation for both IA and IAX groups, all responses of WT HCT116 cells were larger than those of p53^{-/-}

HCT116 cells. Both Figures 2(a) and 2(b) show that the responses start to drop more significantly at earlier time points for WT HCT116 cells (after 7 and 3 h post irradiation for IA and IAX groups, respectively) when compared to p53^{-/-} HCT116 cells (after ~ 12 h post irradiation for both IA and IAX groups). However, when the responses of the p53^{-/-} HCT116 cells start to drop after 12 h post irradiation, the magnitudes of the drop are more significant than those of the WT HCT116 cells. The similar responses of WT and p53^{-/-} HCT116 cells at 12 h post irradiation and the significantly lower responses of p53^{-/-} HCT116 cells at 24 h post irradiation for both IA and IAX groups suggest a delay in the DNA repair and/or apoptosis induction in the p53^{-/-} HCT116 cells.

3. Experimental Section

3.1. Cell Culture

WT and p53^{-/-} HCT116 cells are cultured in McCoy's 5A Medium (Gibco, 16600) which is mixed with 10% fetal bovine serum (Gibco, 10270) and 1% 10,000 U/mL penicillin-streptomycin (Gibco, 15140-122). The cells are incubated under 5% carbon dioxide at 37°C.

3.2. Alpha-particle and x-ray irradiation

About 4×10^3 cells are seeded onto each specially fabricated 35 mm petri dish with a hole drilled on the bottom and covered with a 3.5 μm thick Mylar film. The design minimizes the energy absorption of alpha particles and ensures that the alpha particles can still have sufficient energies to hit the cells after passing through the Mylar film. Figure 3 schematically shows the irradiation protocol for our experiments. Briefly, the cells are irradiated under two conditions: (1) with α particles from a planar ^{241}Am source (average alpha-particle energy in vacuum = 5.49 MeV; activity = 4260 Bq; radius of active area = 2.5 mm; geometry = 2π) for 4 min (IA groups), and (2) with same exposure of α particles followed by a dose of 10 mGy of X-ray (IAX groups). X-ray irradiation is performed using 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, and through a 2.5 mm thick filter made of aluminum, copper and tin. The dose rate of irradiation is ~15 mGy/min.

3.3. Immunofluorescent staining of 53BP1

After irradiation, the cells of each group are fixed at 3, 7, 12 and 24 h post-irradiation using 4% paraformaldehyde under room temperature. The fixed cells are rinsed by phosphate-buffered saline (PBS) and permeabilized with 0.5% Triton-X at 37°C, followed by treating with primary anti-53BP1 antibody (Abcam, ab21083) and 1.5% goat serum blocking solution for 1 h at 37°C. The cells are washed three times with PBS for 5 min each and then exposed to Alexa Fluor 488 goat anti-rabbit IgG (H+L) secondary antibody (Invitrogen, A11008) for another 1 h. At least 500 cells will be counted and analyzed from at least 3 independent sets of experiments.

3.4. Statistical analysis

The data are presented as means \pm SEM. The statistical significance level between the means of two compared groups will be assessed using the Student's t-test. A p value of 0.05 or less will be regarded as corresponding to a significant difference between the means of two compared groups.

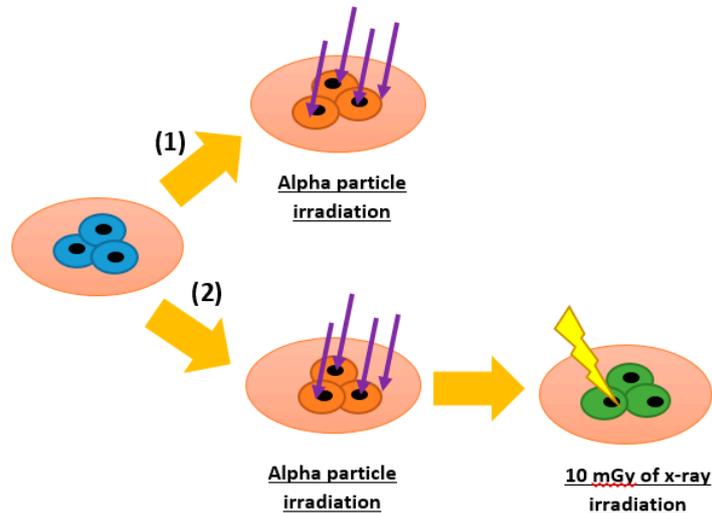


Figure 3. Irradiation protocol of WT and p53^{-/-} HCT116 cells for studying photon hormesis. The cells are irradiated under two conditions: (1) with α particles from a planar ^{241}Am source (IA groups), and (2) with α particles followed by a small dose X-ray (IAX groups).

4. Conclusions

A small X-ray dose (10 mGy) has been found to significantly mitigate the toxic effect of alpha particles (expressed in terms of the number of 53BP1 foci per cell) in WT HCT116 cells at 24 h post irradiation, but not in p53^{-/-} HCT116 cells. These results demonstrate that photon hormesis can be induced in WT HCT116 cells through a p53-dependent pathway. The data for WT HCT116 cells show that photon hormesis are observable only after ~ 7 h post irradiation, which is in agreement with the role played by p53 in the rapid response in repairing DNA DSBs. Comparisons between the responses (in terms of numbers of 53BP1 fpc) of WT and p53^{-/-} HCT116 cells in both IA and IAX groups at different time points show different trends in the drop of responses for the two types of cells, and suggest a delay in the DNA repair and/or apoptosis induction in the p53^{-/-} HCT116 cells.

Author Contributions

Conceived and designed the experiments: YKF, KNY. Performed the experiments: YKF. Analyzed the data: YKF, KNY. Contributed reagents/materials/analysis tools: KNY. Wrote the paper: YKF, KNY.

Conflicts of Interest

The authors declare no conflict of interest.

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