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p53 binding protein 1 foci as a biomarker of DNA double strand breaks induced by ionizing radiation

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ABSTRACT

Foci of p53 binding protein 1 (53 BP1) have been used as a biomarker of DNA double-strand breaks (DSBs) in cells induced by ionizing radiations. 53 BP1 was shown to relocalize into foci shortly after irradiation, with the number of foci closely paralleling the number of DNA DSBs. However, consensus on criteria in terms of the numbers of 53 BP1 foci to define cells damaged by direct irradiation or by bystander signals has not been reached, which is partly due to the presence of 53 BP1 also in normal cells. The objective of the present work was to study the changes in the distribution of cells with different numbers of 53 BP1 foci in a cell population after low-dose ionizing irradiation (<0.1 Gy) provided by alpha particles, with a view to propose feasible criteria for defining cells damaged by direct irradiation or by bystander signals. It was proposed that the change in the percentage of cells with 1–3 foci should be used for such purposes. The underlying reasons were discussed.

1. Introduction

The p53 binding protein 1 (53 BP1) is a member of the BRCT (BRCAI C-terminal) repeat family, which consists of many members, including the DNA damage response proteins NBS1 and BRCA1. Ionizing radiation produces a broad spectrum of DNA damages, including base lesions and strand breaks [1]. 53 BP1 is involved in repair and checkpoint signal transduction, and is required for the phosphorylation of numerous ataxia-telangiectasia-mutated substrates during the double-strand break (DSB) response [2,3]. Shortly after an exposure to ionizing radiation, 53 BP1 was shown to relocalize into foci, with the number of foci closely paralleling the number of DNA DSBs [4]. As such, fluorescent detection of these foci of 53 BP1 protein was used as a marker of DNA damages [5–8].

However, consensus on criteria in terms of the numbers of 53 BP1 foci to define cells damaged by direct irradiation or by bystander signals has not been reached, which is partly due to the presence of 53 BP1 also in normal cells. Different criteria have been proposed in previous works, including the use of a "critical" number of foci per cell (fpc; namely, a positive expression when the fpc is larger than the "critical" number and a negative expression when the fpc is smaller than or equal to that "critical" number) [9–11]; the use of an average number of fpc in a population of cells [12] and the use of different kinds of distributional grouping of fpc (such as examining the percentage of cells in the groups with 0–5 fpc, 6–30 fpc, … <90 fpc) [13].

In the present work, we studied the effects of low-dose (<0.1 Gy) alpha-particle irradiation as well as the associated radiation-induced bystander effect (RIBE) on HeLa cells in terms of changes in the distribution of HeLa cells with different numbers of 53 BP1 foci, with a view to propose feasible criteria for defining cells damaged by direct irradiation or by bystander signals. Immunostaining of 53 BP1 protein was used to characterize the DNA DSBs. RIBE has been widely studied since its revelation by Ref. [14], and refers to the phenomena that irradiated cells might release some stress signal factor(s) to affect the cells nearby or to affect the cells that have received the medium conditioned by the irradiated cells.

2. Methodology

2.1. Fabrication of substrates

In the present work, thin polyallyldiglycol carbonate (PADC) films with a thickness of 17 μm were fabricated using the method devised by Chan et al. [15], and were used as our cell culture substrates. PADC substrates can record positions of alpha-particle hits and give information on the energy of the alpha particles

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through the optical appearance of the etched alpha-particle tracks [16,17]. Thick PADC films (with thickness > 100 μm) are commercially available as CR-39 detectors and are commonly used solid-state nuclear track detectors (SSNTDs) [18]. Thin PADC films (with thickness < ~20 μm) were successfully employed as PADC substrates in alpha-particle radiobiological experiments involving cell cultures [19–22] and those involving zebrafish embryos [23–27]. In the present work, we prepared thin PADC films with a size of 1.5×1.5 cm^2 from 100 μm thick CR-39 detectors (Page Moldings) by chemical etching in 1 N NaOH/ethanol at 40 °C until the desired thickness of 17 μm was reached.

2.2. Experimental setup and cell culture

Handling of HeLa cells was described in detail by Ng et al. [28]. The cells were trypsinized, adjusted to 1.2×10^5 cell/ml cells for a total of 120 μl medium and plated out on PADC substrates for 1 d. The cells were then irradiated for 20 min with 5 MeV alpha particles having traveled a distance of 5 mm in air from an ^{241}Am alpha-particle source (main alpha particle energy of 5.48 MeV) with incident angles between 70.8° and 90° (normal incidence) controlled by a collimator. As irradiation was performed from the bottom of the substrates using the SRIM program [29], the residual energy of the 5 MeV alpha particles after traversing the 17 μm PADC film was found to be ~2.5 MeV [30].

2.3. Detection of 53 BP1 foci and hit positions

Immunochemical staining of cells was performed largely following the procedures described by Aten et al. [31] with some modifications. Optical and fluorescence images of cells were captured by a fluorescence microscope (Nikon ECLIPES 80i). The cells on PADC substrates were then removed by running water before the substrates were etched in a 14 N KOH solution at 37 °C for 3 h to reveal visible tracks corresponding to alpha particles striking the substrates. The epoxy remained intact at this etching temperature [20]. Optical images of these tracks were captured, which were then superimposed onto images of cells described above, from which the positions of alpha-particle hits on cells could be determined. Due to the short irradiation time, very few cells were hit by more than one alpha particle. In addition, from analyzing the captured fluorescent images with the help of the ImageJ software (http://rsbweb.nih.gov/ij/), the distribution of gray levels of the pixels in the 53 BP1-stained cell nuclei of the normal (unirradiated) cells was determined, in terms of the percentage standard deviations of average gray levels, as described in more details in Section 3 below.

3. Results

The distribution of gray levels of 53 BP1 signals in normal unirradiated HeLa cell nuclei was first determined to provide a basis for determining changes due to radiation effects including RIBE. The percentage standard deviations of average gray levels were required. For example, if scanning the image pixels for one particular cell nucleus gave an average gray level of 18 ± 4.6, where 4.6 was the standard deviation, the percentage standard deviation was calculated as 4.6/18 = 21.1%. By scanning the nuclei of 40 normal unirradiated HeLa cells, we obtained the average percentage standard deviation as 27.4 ± 15.1%. In the present work, a 53 BP1 focus was defined as a particular area in a cell nucleus with a gray level that was 100% larger than the average gray level of that nucleus. Fig. 1 shows representative fluorescence images for 53 BP1 foci in control, as well as irradiated and bystander cells, with superimposed optical images for irradiated and bystander cells.

To take into account the possible variations among different experiments, normalization was also carried out on the percentages in each data set as shown in Figs. 2 and 3. Our experimental results were shown in terms of the percentage of cells (comparing with the corresponding control (Ctrl) in each set) in irradiated (Irr) cells (Fig. 2) and bystander (By) cells (Fig. 3) cultured on

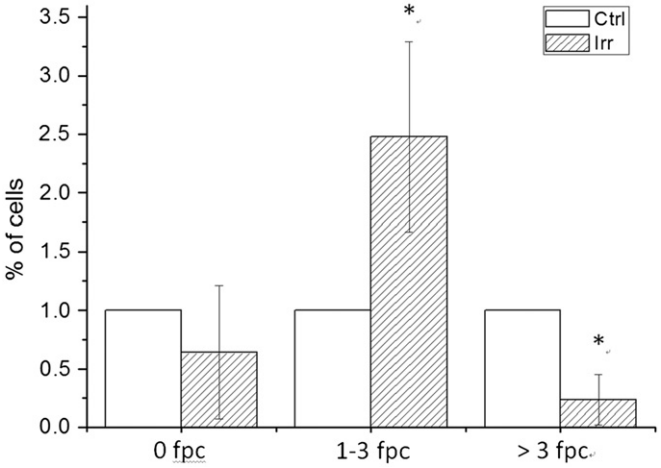

Fig. 2. Normalized percentage of control (Ctrl) and irradiated (Irr) cells cultured on PADC substrates with number of 53 BP1 foci in the nuclei shown in three groups: (1) with 0 fpc, (2) with 1–3 fpc and (3) with > 3 fpc. Error bars represent one standard deviation. The Irr cells had significantly higher and lower normalized percentages in the groups of 1–3 fpc and > 3 fpc, respectively, when compared with the Ctrl cells (* represents $p < 0.05$, $n = 3$).

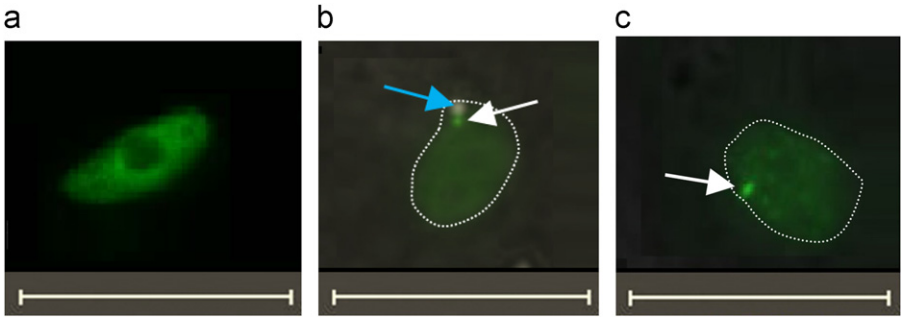

Fig. 1. Representative fluorescent images for 53 BP1 foci in different cells. (a) A control cell. (b) An irradiated cell. The fluorescence image has been superimposed with the optical image. Dashed line: outline for the cell nucleus; White arrow: 53 BP1 focus shown in the fluorescence image; blue arrow: etched alpha-particle track shown in the optical image. (c) A bystander cell. The fluorescent image has been superimposed with the optical image. Dashed line: outline for the cell nucleus; white arrow: 53 BP1 focus shown in the fluorescent image. Bar = 25 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)