Nitric oxide protects the quiescent human normal lung fibroblast cells after γ-irradiation

Normal tissue radiation response involves morphological and functional changes and is of great importance to populations subjected to medical, accidental or intentional exposure. Furthermore, Chernobyl and Fukushima Daiichi disasters have demonstrated that the first responders to such emergencies show high risk of radiation exposure[1,2]. The kinetics of these responses appears to vary with radiation dose, quality (low and high linear energy transfer), phases in the cell cycle and cell type. It is known that radiation alters the genome of the proliferating cell(s). However, in addition to the four conventional phases of the cell cycle (G_1, S, G_2 and M), G_0, a fifth phase, which denotes the quiescent state (non-proliferating) of cells that have withdrawn from the active cell cycle[3] is poorly understood. As most organs are composed of both proliferating and quiescent cells, understanding the effects of radiation on the quiescent cells and their damage response may represent a key aspect in tissue response to radiation exposure. Quiescent cells are considered to be dormant with reduced metabolic activity[4]. However, recent reports have challenged this notion, suggesting that they retain the capacity to re-enter the cell cycle and divide again[5]. Therefore, quiescence is critical for cell survival and tissue homeostasis.

Since radiation sensitivity varies within the cell cycle phases, we have used both proliferating and quiescent normal human foetal lung fibroblast cells (MRC-5) to study their sensitivity from low to moderate doses (0.10–1.5 Gy) of γ-irradiation. The radiation doses used are likely to occur in populations during medical interventions, as well as accidental or intentional radiation exposure. Since the lung is one of the most radiosensitive organs and frequently irradiated as part of various treatment programmes[6], we have studied the key proteins involved in DNA damage response and cell cycle regulations in total and sub-cellular (cytoplasm and nucleus) fractions. Furthermore, the effect of nitric oxide (NO), a primary regulator of various physiological processes which also protects the cells and tissues against radiation[7,8], is used to study the radioprotective effect, if any, of the quiescent (G_0) cells.

Cells (MRC-5) were obtained from the Coriell Cell Repository (Camden, NJ, USA) and maintained as described previously[9]. For the study of quiescent cells, they were synchronized by growing them to confluence at high cell density by contact inhibition and maintained in that state for about two weeks with a change of medium every three days[9]. Cells were irradiated with different doses (0–1.5 Gy) of γ-radiation using ^{137}Cs source at a dose rate of 0.85 Gy/min. At least three independent experiments were performed for each treatment. For protein analysis, cells were harvested 2 h after irradiation, a time interval selected from our earlier study[10]. Sodium nitroprusside (SNP; Sigma, St Louis, MO, USA), which acts as a NO donor molecule was used at 50 and 100 μM concentrations to treat the cells 1 h before radiation exposure and the cells were harvested 2 h after irradiation. SNP concentrations were selected from the dose response study. Total cellular proteins, and cytosolic and nuclear fraction proteins were isolated following the method described in Baskar *et al.*[10,11]. Western blot analyses of p53, p21 and actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were performed using specific antibodies.

In the present study, as shown in Figure 1 *a* and *b*, a dose-dependent increase in p53 is observed in both the proliferating and quiescent cells. Along with p53, downstream protein p21 is also induced in both phases of the cells (Figure 1 *a* and *b*). Figure 1 *c* shows the expression of p53 in cytoplasm and nucleus of both the proliferating and quiescent cells. p53 is found only in the nucleosolic fraction. A dose-dependent increase in p53 is evident in the proliferating cells. However, interestingly p53 also shows an increase in the nuclear fraction of the quiescent cells, which are in G_0 phase.

NO is a known radiation sensitizer[12]; however, its clinical use is limited by systemic side effects. To examine the cellular responses of NO treatment, we first examined the dose-dependent induction of p53 in the proliferating cells (Figure 2 *a*). From the preliminary study (Figure 2 *a*), 50 and 100 μM of NO were selected and checked for their effect on radiation in both the proliferating and quiescent cells (Figure 2 *b* and *c*). Proliferating cells exposed to both NO and radiation show a slight increase in the expression of p53. Whereas p21 does not show any increase compared to cells which are exposed to radiation alone. In

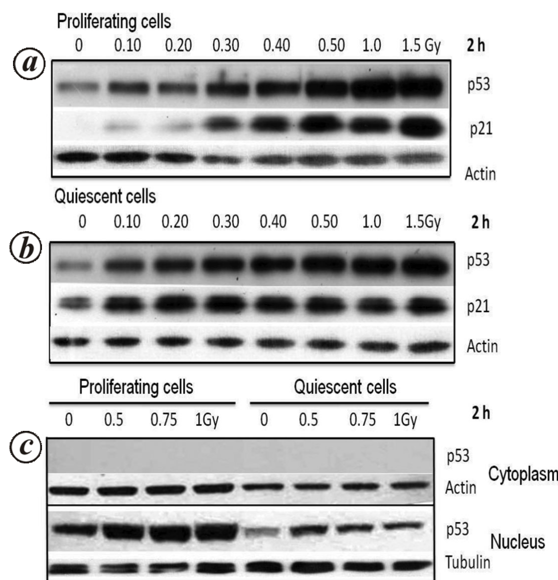

Figure 1. Effect of γ-irradiation (0.10–1.5 Gy) on stress (p53) and cell cycle (p21) response proteins in (***a***) total cell, (***b***) cytoplasm and (***c***) nucleus of the proliferating and quiescence cells. Western blots shown are representative of three independent experiments.

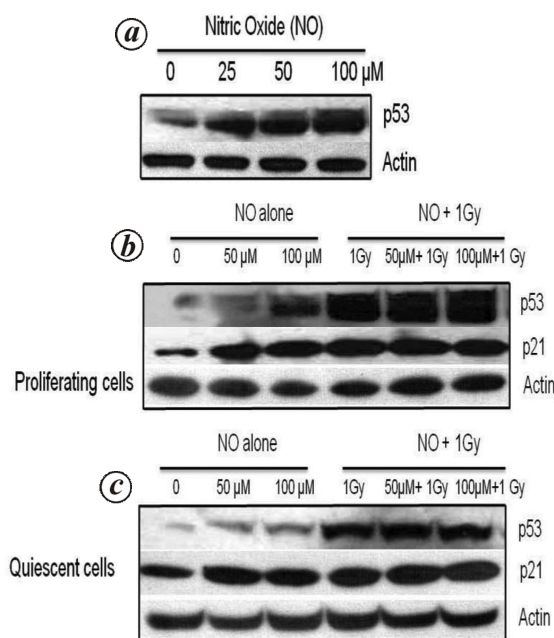

Figure 2. Sodium nitroprusside (SNP), which acts as a nitric oxide (NO) donor molecule was incubated for 1 h and then the cells were irradiated with 1 Gy of γ-radiation and harvested 2 h after incubation. ***a***, Selection of optimum SNP concentration using the proliferating cells. ***b***, ***c***, Levels of p53 and p21 were studied in both (***b***) proliferating and (***c***) quiescent cells. Western blots shown are representative of three independent experiments.

the quiescent cells, expression of p53 and p21 treated with combined NO and radiation is additive and does not show any higher level of p53 or p21, indicating radioprotection of the cells resting in G_0 (quiescent) state, similar to that of the proliferating cells.

p53 is a tumour suppressor protein which is present in low concentrations in the normal cells and plays a pivotal role in DNA damage response[13]. In the present study, no detectable p53 protein was observed in cytosolic fraction of both the proliferating and quiescent cells. Whereas a dose-dependent increase in p53 was observed in both the proliferating and quiescent cells of nuclear fractions. In total cell lysates followed by p53, p21 an inhibitor of cyclin-dependent kinases[14] also showed an increase in both the proliferating and quiescent cells. In an earlier study, we have reported a specific activation of protein kinase C isoforms, which regulate numerous cellular responses, in both the proliferating and quiescent cells[10]. DNA damage rapidly activates the checkpoint machinery to delay or stop the cell cycle process, thereby preventing duplication and segregation of damaged DNA[15]. This is evident in the present study, whereby proliferating cells show a higher level of p53, followed by its downstream protein p21. Whereas cells that are in the G_0 state also show similar response to that of proliferating cells, indicating the response of quiescent cells to low doses of radiation.

In the present study, we demonstrate that the pretreatment of SNP (NO donor) results in the abolition of enhanced p53 and p21 expression in both proliferating and quiescent cells. It was shown earlier that NO plays an important role in mediating the radiation-induced cellular responses in the proliferating cells[7,16]. In the present study, we have shown a radioprotective effect of NO in the quiescent cells. Furthermore, protection of 'sleep-like' quiescent cells (e.g. fibroblasts, lymphocytes and stem cells) is crucial for tissue repair, regeneration and growth of higher multicellular organisms, such as mammals[17]. The results of this study may provide a better understanding on the health effects associated with low doses of radiation exposure for the modulation and evaluation of protocols to improve the efficacy of radiation treatments.

Conflict of interest: The authors declare no conflict of interest.

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