Mitochondrial DNA deletion and impairment of mitochondrial biogenesis by reactive oxygen species in ionizing radiation-induced premature senescence

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1. Introduction

The aim of this study was to determine whether an increase of ROS level in cellular senescence induced by IR could mediate mtDNA deletion via impairment of mitochondria biogenesis in IMR-90 human lung fibroblast cells. Our results showed that IR induced cellular senescence, intracellular ROS, and mtDNA deletion, and in particular, suppressed the expression of mitochondrial biogenesis genes (NRF-1, TFAM). Furthermore, these IR-induced events were abolished using a potent antioxidant, NAC, which suggests that ROS is a key cause of mtDNA deletion in IR-induced cellular senescence, and that the alteration of mitochondrial biogenesis may mediate these processes.

2. Methods and Results

For irradiation, cells were exposed to 137Cs γ-rays at 4Gy. Intracellular ROS and SA-β-gal activity were assessed using 2′,7′-dichlorofluorescein diacetate and di-β-D-galactopyranoside. The presence of mtDNA deletion and expression of mitochondrial biogenesis genes were detected by nested-PCR and RT-PCR.

2.1 Inhibition of ionizing radiation and hydrogen peroxide-induced ROS by NAC

We examined the effects of IR and H_2O_2 on the intracellular ROS level. Young IMR-90 cells at PD 39 were exposed to 4 Gy of IR or 100 μM H_2O_2, and the intracellular ROS levels were then measured using DCF fluorescence. As shown in Fig. 1A, ROS levels were significantly increased by IR (1.6 fold) and H_2O_2 (1.9 fold). Similar ROS increases were observed in old IMR-90 cells (2.6 fold) at PD 55. These increases of ROS level by IR and H_2O_2 were reduced by NAC, a well-known antioxidant compound.

2.2 Inhibition of ROS-induced senescence-associated β-galactosidase activity by NAC

To confirm the induction of cellular senescence by IR and H_2O_2, SA-β-gal activity was determined as a cellular senescence marker [1]. Young IMR-90 cells at PD 39 were exposed to IR and H_2O_2, and the SA-β-gal activity level was measured using FDG. As shown in Fig. 1B, SA-β-gal activity was increased marginally yet significantly by the use of IR (1.17±0.01 fold) and H_2O_2 (1.25±0.15 fold). The increase of SA-β-gal activity was observed in old IMR-90 cells at PD 55 (1.65±0.12 fold). In accordance with the results of ROS levels, NAC sufficiently reduced the IR and H_2O_2-induced SA-β-gal activities to normal control levels. This result suggests that increased intracellular ROS levels mediate cellular senescence induced by IR and H_2O_2.

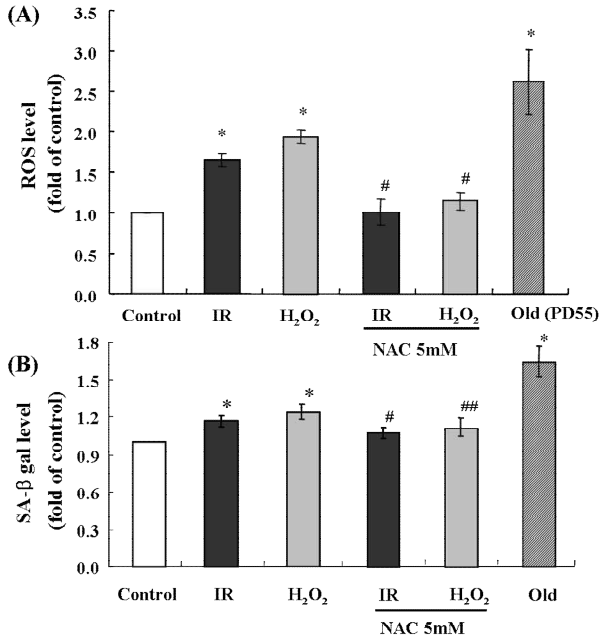

Fig. 1. Effects of ionizing radiation on ROS level and senescence-associated β-galactosidase activity in IMR-90 cells. (A) The intracellular ROS level was detected using a DCFH-DA. (B) The SA-β-gal activity was measured using the FDG. The results represent the mean ±SD from three separate experiments. *$p < 0.05$ vs control, $^{\#}p < 0.05$ vs IR alone, and $^{\#\#}p < 0.01$ vs H_2O_2 alone.

2.3 Inhibition of ROS-induced mitochondrial DNA common deletion by NAC

Next, we investigated whether ROS plays a role of an inducer of mtDNA common deletion, which is one of the aging markers [2,3]. As expected, IR and H_2O_2-treated cells, and old non-treated cells, showed higher levels of mtDNA deletion compared to young non-treated cells (Fig. 2). The treatment of NAC again abated the increased mtDNA deletion in IR and H_2O_2-treated cells, indicating that ROS is an inducer of mtDNA common deletion in IMR-90 cells.

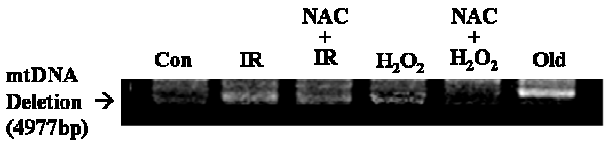

Fig. 2. Effects of ionizing radiation on mtDNA deletion in IMR-90 cells. The mtDNA deletion was detected using a nested PCR assay. The population doubling (PD) number of cells for radiation groups was PD 39, while that of the old group was PD 55.

2.4 Effects of ROS on mitochondrial biogenesis gene expression

To identify the effects of IR and H_2O_2-induced ROS on mitochondrial biogenesis, we examined the changes in mRNA transcription of the genes involved in mitochondrial biogenesis. The relative abundances of mRNA transcript encoding for nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (TFAM) were examined using RT-PCR. The expressions of NRF-1 and TFAM were markedly lower in IR and H_2O_2-treated cells and old cells (Fig. 3A and B). The NRF-1 mRNA expression was decreased to 0.60±0.05 by IR, and 0.63±0.08 fold by H_2O_2, and to 0.6±0.04 in old cells compared to non-treated young cells. TFAM mRNA expressions were reduced to 0.8±0.06 by IR and 0.7±0.07 by H_2O_2 and to 0.8±0.06 in old cells compared to non-treated young cells. Decreased mRNA expressions of NRF-1 and TFAM using IR and H_2O_2 were recovered to the level of young control cells through the use of NAC treatment. The above results demonstrate that the expression of mitochondrial biogenesis genes was affected by IR and H_2O_2-induced intracellular ROS.

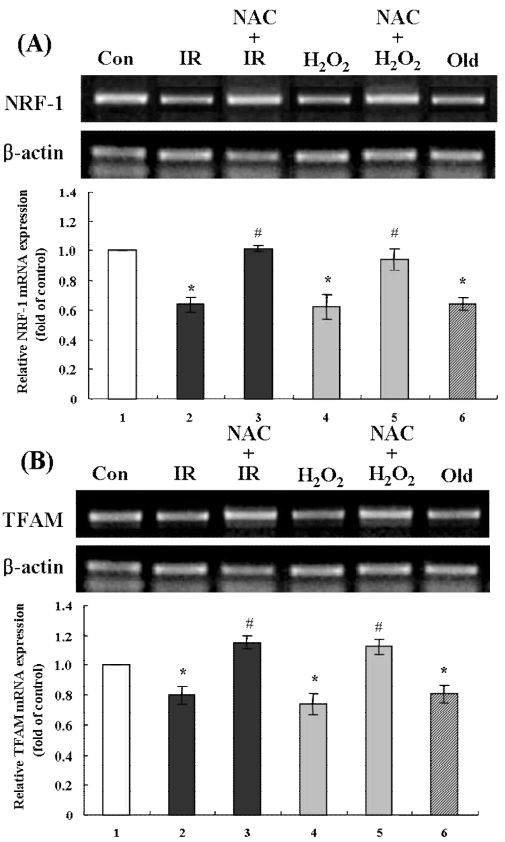

Fig. 3. Effects of ionizing radiation on mitochondrial biogenesis genes in IMR-90 cells. After harvesting the cells, the total RNA was isolated and the mRNA levels of NRF-1 and TFAM were detected using RT-PCR. Densitometric quantification for each gene is shown as a relative change of NRF-1 and TFAM mRNA expression compared to the control after normalizing with β-actin. The results represent the mean ±SD from three separate experiments. *$p < 0.05$ vs control and $^{\#}p < 0.05$ vs IR and H_2O_2 alone.

3. Conclusions

Our results suggest that ROS is a key inducer of mtDNA deletion in cellular senescence induced by IR, which may be mediated by the regulation of mitochondria biogenesis genes, and that a change of mitochondrial biogenesis genes may mediate this process. This study will contribute toward research on the mechanisms of mtDNA deletion and its related degenerative disorders, such as Alzheimer's and Parkinson's diseases, and further more aging process.

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