Chronic induction of senescence marker in gamma-irradiation mice

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1. Introduction

Aging or senescence occurs at organ level as well as cell level. Most senescent cells are characterized by morphological changes into large, flat, and multinucleated phenotypes[1]. Also senescence cells show a stable and long-term loss of proliferative capacity although viability and metabolic activity are maintained. The *in vivo* senescence or aging has been studied using various animal models. However, until now, the mechanisms of senescence or aging *in vivo*. Ionizing radiation (IR) is a well-known inducer of oxidative stress and DNA damage, which is one of the important causes of aging process. Therefore, in this study, we wanted to find out whether IR can induce or accelerate aging process in mice by investigating the long-term effects of IR on the various senescence markers in the tissues.

2. Methods and Results

Groups of 8-week-old C57BL/6 mice were exposed to whole-body irradiation of ^{137}Cs γ-rays at a single dose (5Gy) or fractionated doses (1Gy × 5 times, 0.5Gy × 10 times, or 0.2Gy × 25 times). At two, four, six months after irradiation, lung, liver and kidney tissues were isolated from mice. Two-months old and 24-months old mice were used as young and old controls, respectively.

2.1 Effect of IR on mtDNA common deletion in mouse tissues

The mtDNA common deletion (3867 bp) was detected by nested PCR. Mitochondrial DNA common (mtDNA) deletion is accumulated with aging in various tissues of human and mice [2], and has been proposed as a marker of natural aging. We also observed similar results that mtDNA common deletion was accumulated at higher levels in lung, liver, and kidney tissues of old (24-month-old) mice compared to young (2-month-old) mice (Fig. 1A). As shown in Fig. 1B, mtDNA common deletion was increased in the lung, liver, and kidney of irradiation groups compared to the non-irradiation age-matched groups. Interestingly, the level of mtDNA common deletion was lowest in single irradiation group compared to fractionated irradiation groups at all time points and in all tissues examined, which suggested that fractionated irradiation is more efficient in inducing mtDNA deletion than single irradiation.

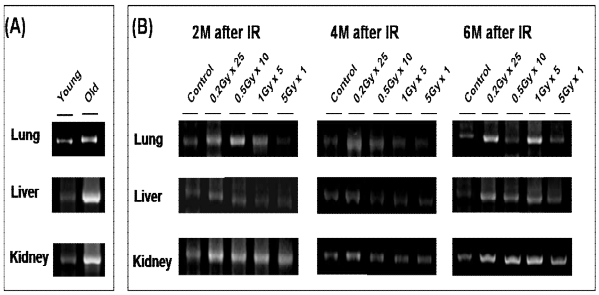

Fig 1. Induction of the mitochondrial DNA common deletion (3867bp) in the tissues of irradiated C57BL/6 mice. (A) Tissues isolated from young (2-month-old) and old (24-month-old) C57BL/6 mice were analyzed for mtDNA common deletion. (B) 8-week-old C57BL/6 mice were exposed to ionizing radiation at a single dose (5Gy×1), or fractionated doses (0.2Gy×25, 0.5Gy×10, or 1Gy×5) at the age of 2 months, and they were sacrificed at 2, 4, 6 months later after IR exposure.

2.2 Effect of IR on SA β -gal staining in mouse kidney

SA β-gal has been recommended as a biomarker of cellular senescence both *in vivo* and *in vitro*, and it can be easily detected by X-gal staining [3-4]. The kidney tissue samples were snap frozen in OCT compound (Sakura Finetek, Japan). Seven-micrometer-thick fresh frozen sections were mounted on saline coating slide glass (Pau Marienfeld GMBH & Co., Germany) and fixed in 10% formalin for 10min at room temperature. The sections were washed in PBS and were incubated in the staining solution (1mg/ml X-gal, 5mM potassium ferricyanide, 5mM potassium ferrocyanide, 2mM $MgCl_2$, 150mM NaCl, citric acid-sodium phosphate, pH6.0) for 16 hours without CO_2 and then counterstained with eosin (Junsei Chemical Co., Janpan). We examined SA β-gal positive cells in the kidney of irradiated mice to confirm the induction of senescence by IR. Interestingly, SA β-gal positive cells were increased in the kidney of old(24-month-old) mice than young(2-month-old) mice(Fig. 2A). Furthermore, the fractionated irradiation (1Gy×5, 0.5Gy×10 and 0.2Gy×25) resulted in more prominent increase of SA β-gal positive cells than the

single irradiation (5Gy×1) (Fig. 2B). These results showed that IR elicited a gradual and but persistent increase of SA β-gal positive cells in the kidney, and that fractionated irradiation increased the SA β-gal positive cells more effectively than single irradiation.

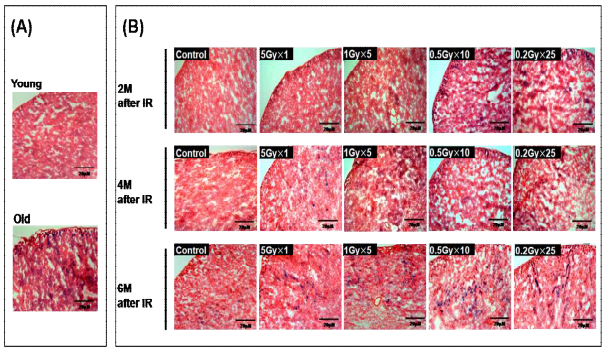

Fig 2. Increased SA β-gal positive cells in the kidney of irradiated C57BL/6 mice. (A) The kidney isolated from young (2-month-old) and old (24-month-old) C57BL/6 mice analyzed for SA β-gal staining. (B) The C57BL/6 mice exposed to ionizing radiation at a single dose (5Gy×1), or fractionated doses (0.2Gy×25, 0.5Gy×10, or 1Gy×5) at the age of 2 months were sacrificed at 2, 4, 6 months after radiation exposure and the kidney were examined for SA β-gal staining.

2.3 Effect of IR on p21 in mouse tissue

The p21 protein is important in the stress response and is the major transcriptional target of p53, and increased expression of p21 is a well-known biomarker for oxidative stress and cellular senescence[5]. The tissue lysates containing 25ug protein were resolved by SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked in 5% skim milk and hybridized for overnight at 4℃ with an anti-p21 mouse IgG antibody. We examined p21 protein level in the tissues of irradiated mice to confirm the induction of senescence by IR. P21 protein level was accumulated at higher levels in lung, liver, and kidney tissues of old (24-month-old) mice compared to young (2-month-old) mice (Fig. 3A). Interestingly, the fractionated irradiation groups showed higher levels of p21 compared to single irradiation groups(Fig. 3B). Which suggested that fractionated irradiation is more efficient in inducing p21 expression than single irradiation.

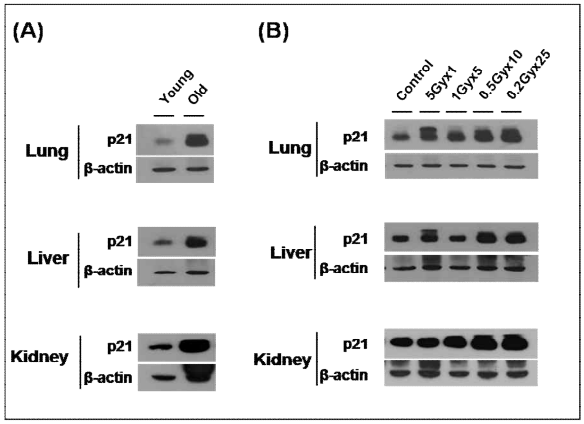

Fig. 3. Increased expression of p21 in the tissues of irradiated C57BL/6 mice. (A) Tissues isolated from young(2-month-old) and old(24-month-old) C57BL/6 mice were analyzed for p21 protein expression. (B) 8-week-old C57BL/6 mice were exposed to ionizing radiation at a single dose (5Gy×1), or fractionated doses (0.2Gy×25, 0.5Gy×10, or 1Gy×5) at the age of 2 months.

3. Conclusions

The mtDNA deletions of mouse tissues were increased in all IR group, and fractioned IR was more efficient in inducing mtDNA deletions than the single IR. Also, the level of SA β-galactosidase and p21 was increased by IR, which was more prominent by fractionated IR. In conclusion, our results showed that IR induced the various senescence biomarkers *in vivo* that persisted at least 6 months after irradiation, and fractionated irradiation showed more marked effects than single irradiation.

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