Preventive Effects of Red Ginseng on an Aging Hallmark - Senescence

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ABSTRACT

By 2030, 1 in 6 people in the world will be aged 60 years or older, increasing the importance of interventional therapies for age-associated diseases. Research investigating cultural dietary differences recognized that the use of herbal medicine supplementation such as processed *Panax ginseng*, referred to as red ginseng (RG), increases lifespan in Asian countries by facilitating healthy aging. However, the mechanism for how RG prevents, delays, or reverses aging-related diseases is unknown. First, we used mouse embryonic fibroblasts (MEF) cells to determine whether RG reduces cellular senescence. The result was RG reduced cellular senescence on MEFs. Then, we assessed how RG facilitates healthy aging by identifying cellular and molecular mechanisms of hepatic senescence pathways in an aged mouse model. This study compared three animal groups: 9-week-old young mice, 18-month-old aged mice, and RG-treated 18-month-old mice. To evaluate how RG delays hepatic cellular senescence, we analyzed the expression levels of the main senescence effectors (p53, p15, and p16) as well as apoptosis markers (caspase-3 and cleaved caspase-3) in the liver and primary hepatocytes. The aged mice treated with RG showed significantly reduced expression levels of p53, p15, and p16 in primary hepatocytes and showed the same trends in liver samples compared to the control aged mice. RG supplementation attenuated hepatic cellular senescence by downregulating p53, p16, p15, and cleaved caspase-3. Therefore, our results suggest that RG could be a novel interventional agent for delaying cellular senescence. Our findings provide fundamental information that RG has the potential to be a widely used therapeutic agent to reduce the incidence of age-associated diseases.

BACKGROUND

Cellular senescence, one of the hallmarks of aging, refers to a permanent cell cycle arrest and is accelerated during the aging process. A senolytic, a small molecule that eliminates senescent cells, can be a potential strategy for extending healthy lifespan and ameliorate age-associated diseases. Red ginseng (RG) prepared via a steaming and drying process repeated 2-3 times from fresh ginseng (*Panax ginseng* C.A. Meyer) has been reported to have physiological benefits against reactive oxygen species, inflammation, and oncogenesis which are common cues to induce aging. Therefore, the present study was aimed to investigate the effect of RG on cellular senescence.

METHODS

➢ **Animals**

- Male C57BL/6(J) mice were randomized into three groups: distilled water orally administered 9-week-old mice (Young; n=6), distilled water orally administered 18-month-old mice (Old; n=6), and 300 mg/kg RG in distilled water orally administered 18-month-old mice (Old+RG; n=7) for 4 weeks.
- The body weight and food intake were monitored every two days.
- 6 h-fasting blood glucose levels were measured every two weeks.
- We measured mouse body composition at Week 4 via nuclear magnetic resonance (NMR) spectroscopy.
- At Week 4, glucose tolerance test (GTT; 2 g/kg glucose) and insulin tolerance test (ITT; 1.0 IU/kg) were performed by measuring blood glucose level from the mice tail veins.

➢ **Primary mouse embryonic fibroblasts (MEFs) isolation**

MEFs were isolated from E13.5 embryos of 9-week-old pregnant C57BL/6J female mice (n=3), and then incubated for 3 days for further experiments.

➢ **Measurement of senescence-associated β-galactosidase (SA-β-gal)**

- MEF cells with or without RG treatment (5 μg/mL) were irradiated with a 20 Gy of γ-ray (NIA).
- The irradiated cells were subjected to SA-β-gal staining using a Senescence β-Galactosidase Staining Kit.

➢ **Immunoblotting**

For protein analyses, we conducted immunoblotting. Membranes were blocked in blocking reagent for 1 h at room temperature and incubated with primary antibodies overnight at 4 °C as follows: caspase-3, cleaved caspase-3, p53, p16, p15, and β-actin.

➢ **Statistical analysis**

All experimental data are expressed as the mean ± standard error of the mean (SEM). Quantification analyses for WB band density among three groups was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. Student t-test was used for immunoblotting between two groups.

RESULTS

Red ginseng delays cellular senescence on MEFs

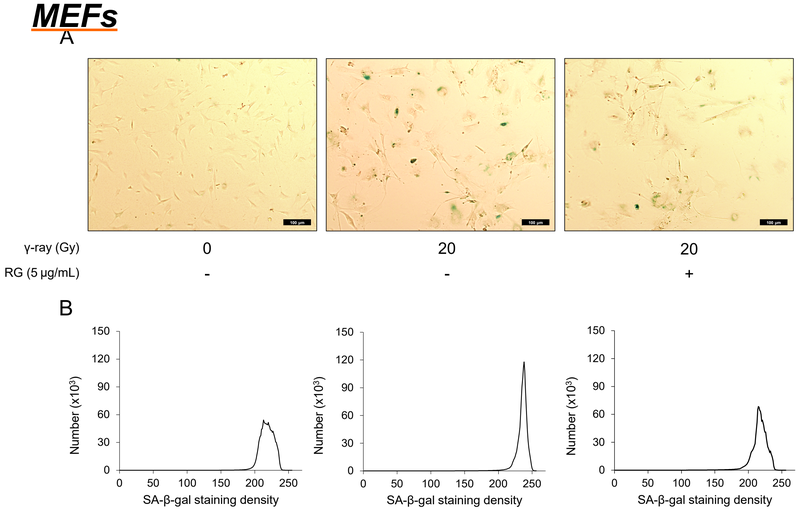


Figure 1. Representative morphologies of senescence-associated β-galactosidase (β-gal) stained (bluish-green color) primary mouse embryonic fibroblasts (MEFs) with 0 Gy, 20 Gy, 20 Gy+RG (5 μg/mL) (scale bar = 100 μm) (A) and SA-β-gal quantification plots based on staining density (B).

Short-term red ginseng supplementation does not lead to metabolic changes

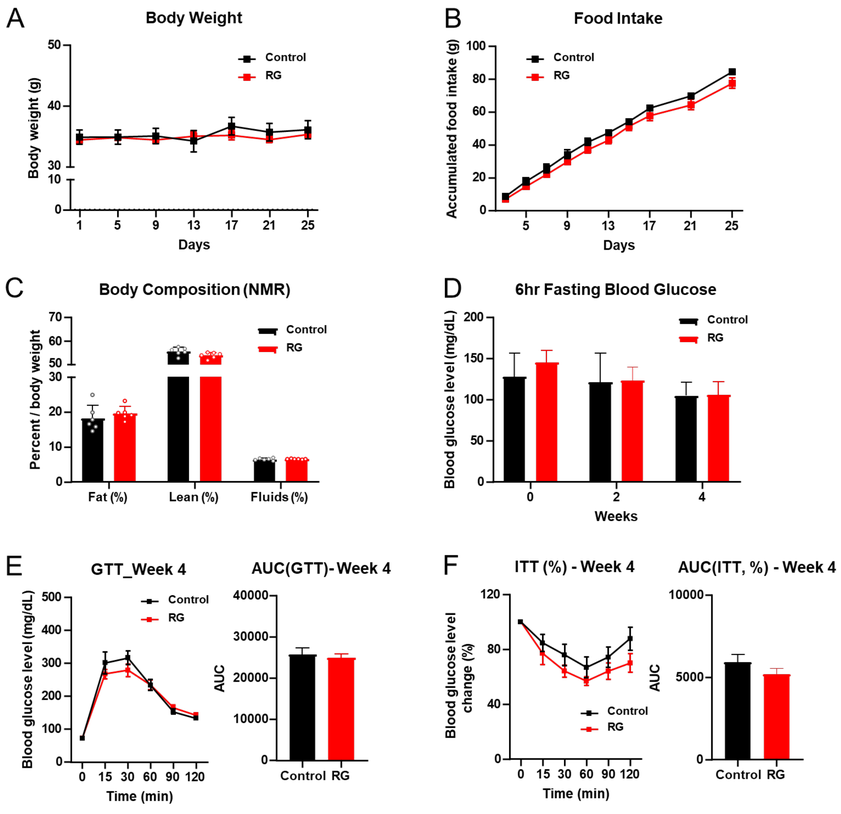
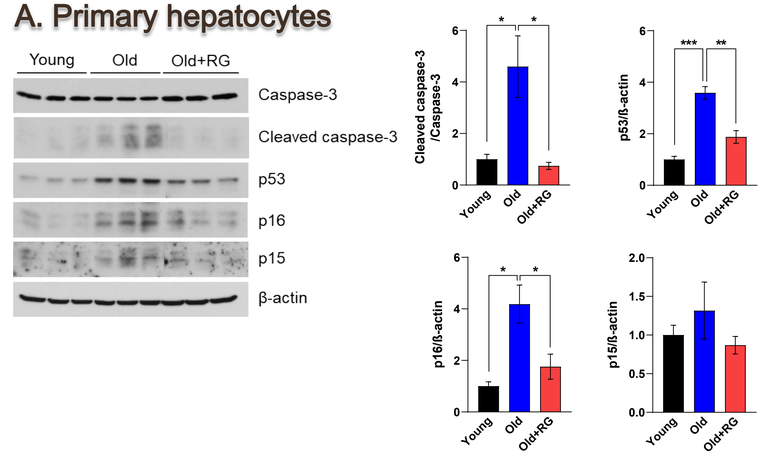


Figure 2. Body composition comparisons between Old and Old+RG groups: body weight (A), food intake (B), body composition (NMR) (C), 6 hr-fasting blood glucose (D), GTT (E), and ITT (F).

Red ginseng supplementation ameliorates canonical hepatic cellular senescence pathways in metabolically active organs in aged mice



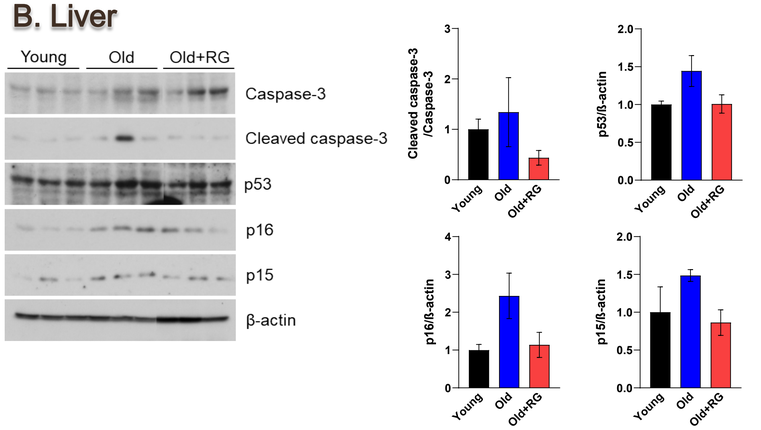


Figure 3. Protein expression levels of caspase-3, cleaved caspase-3, p53, p16, and p15 in primary hepatocytes (A) and liver (B) (n = 3 mice per group). *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001.

CONCLUSIONS

The current study indicates that RG may be a potential senolytic candidate to delay hepatic cellular senescence, resulting from a downregulated p53, p15/p16 pathway, and apoptosis pathway.

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