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BASIC AND TRANSLATIONAL RESEARCH

Metformin alleviates auditory cell senescence by mitophagy induction

Sung-Il Cho, Eu-Ri Jo, Hee Sun Jang
Department of Otolaryngology-Head and Neck Surgery, Chosun University College of Medicine, Gwangju, Republic of Korea

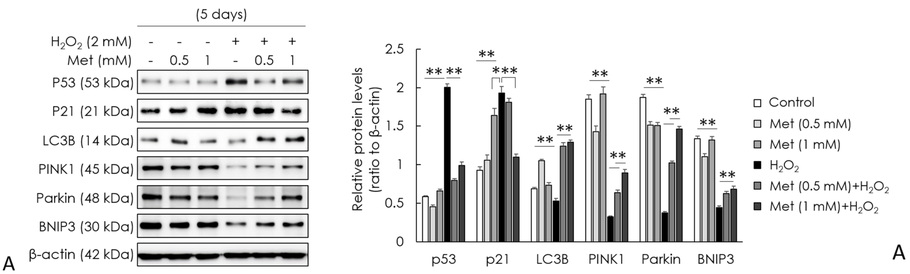
Introduction

- Mitochondrial dysfunction is considered a hallmark of aging and leads auditory cell death by adenosine triphosphate (ATP) depletion, impaired iron homeostasis, and excessive ROS formation.
- Mitophagy is mitochondria specific autophagy which turnovers damaged mitochondrial components through autophagic delivery to lysosomes.
- Mitophagy enhancement by pharmacological agents is a considerable strategy for treating various age-associated disorders.
- Metformin (N, N-dimethylbiguanide) is a hypoglycemic agent originally derived from an herb French lilac Galega officinalis and has become the first-line drug to treat type 2 diabetes mellitus.
- Reactivation of mitophagy using metformin might attenuate senescence in the auditory cells. In the present study, we aimed to investigate the effect of metformin on the prevention of senescence in auditory cells.

Materials and Methods

- For senescence induction, HEI-OC1 cells were treated with 2 mM H_2O_2 for 1 h.
- The cells were pre-treated for 2 h with 1 mM metformin and incubated with 2 mM H_2O_2 for 1 h. After washing with PBS, the cells were incubated in fresh culture medium for 5 days and used for further experiments.
- The isolated cochlear explants from C57BL/6J mice were incubated with 1 mM metformin for 2 h and treated with 0.5 mM H_2O_2 for 5 h. The tissues were incubated in fresh culture medium for 3 days and used for experiments.

Results



Results

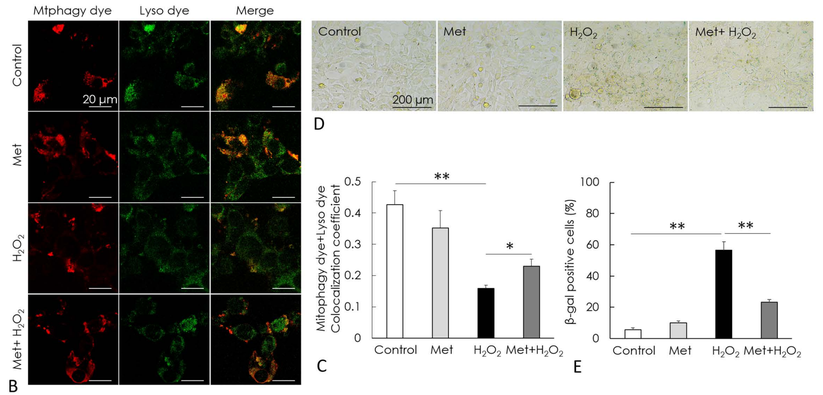
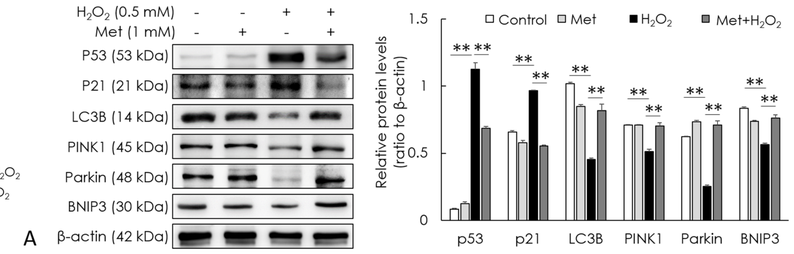


Fig 1. Effect of metformin on senescence and mitophagy in HEI-OC1 cells. (A) HEI-OC1 cells pre-treated with 0.5 and 1 mM metformin for 2 h, exposed with or without 2 mM H_2O_2 for 1 h, and incubated in culture medium for 5 days. Metformin pre-treatment significantly decreased the levels of senescence-associated p53 and p21, which are increased by H_2O_2 treatment of HEI-OC1 cells, and significantly increased mitophagy-related proteins, including PINK1, Parkin, and BNIP3, which are decreased by H_2O_2 treatment of HEI-OC1 cells. (B) Mitophagy examined using immunofluorescence analysis of mitophagy and lyso dyes. (C) Metformin pre-treatment has significantly increased the colocalization of mitophagy dye and lyso dye, which are decreased in the H_2O_2 -induced senescent HEI-OC1 cells. (D) Senescent cells examined using senescence associated beta galactosidase (β-gal) staining. (E) The β-gal positive cells of H_2O_2 -induced HEI-OC1 cells are significantly decreased by pre-treatment of metformin. (Met, metformin; *p<0.05, **p<0.01).



Results

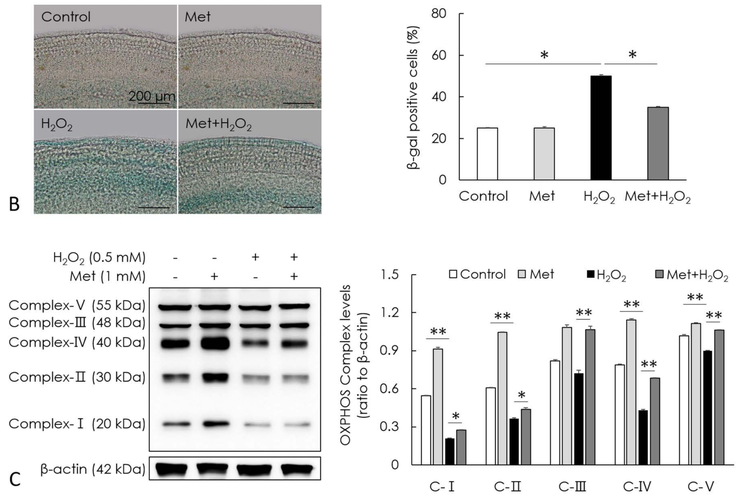


Fig 2. Effect of metformin on senescence and mitophagy in cochlear explants. (A) Cochlear explants pre-treated with 1 mM metformin for 2 h, exposed with or without 0.5 mM H_2O_2 for 5 h, and incubated in culture medium for 3 days. Metformin pre-treatment significantly decreased the levels of senescence-associated p53 and p21, which are increased by H_2O_2 treatment of cochlear explants, and significantly increased mitophagy-related proteins, including PINK1, Parkin, and BNIP3, which are decreased by H_2O_2 treatment of cochlear explants. (B) Senescent cells are examined using senescence associated beta galactosidase (β-gal) staining. The β-gal positive cells of H_2O_2 -induced cochlear explants are significantly decreased by pre-treatment of metformin. (C) The expression of oxidative phosphorylation (OXPHOS) proteins is examined using western blot analysis in cochlear explants. Metformin pre-treatment significantly increased the levels of all OXPHOS complexes I, II, III, IV, and V compared with those of H_2O_2 -induced cochlear explants. (Met, metformin; *p<0.05, **p<0.01).

Conclusion

- This study revealed that metformin enhanced mitophagy and effectively prevented premature senescence in auditory cells.
- Upregulation of mitophagy using metformin can be a preventive strategy for patients with age-related hearing loss.

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