A link between mitochondrial mass increase by enhanced lipogenesis and respiratory defect in cell senescence

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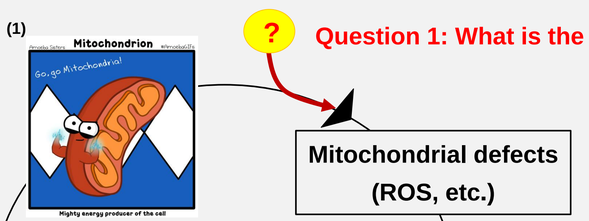
ABSTRACT

Mitochondrial theory of aging is one of the plausible hypotheses to explain underlying mechanism of aging process. In short, progressive accumulation of mitochondrial respiratory defects increases oxidative stress and resultant damaged macromolecules, thereby driving cell senescence and aging process. However, detailed mechanisms of how mitochondrial defects are triggered during the senescent process are not clearly established. In this study, we hypothesize that increased mitochondrial mass by enhanced lipogenesis may be one of the causes of mitochondrial defects during cellular senescence. At first, we investigated whether increment of mitochondrial mass is accompanied with mitochondrial defects during senescence. In stress-induced senescence (SIS), both cellular mitochondrial mass and elongated form of mitochondria were clearly increased with mitochondrial respiratory defect. Unexpectedly, mitochondrial biogenesis (mitochondrial DNA copy number, mitochondrial transcription, and mitochondrial protein expressions) was declined, despite increased mitochondrial mass. We also found that augmented lipogenesis in cell senescence and enhanced lipogenesis can induce cellular senescence. These results imply that increase of mitochondrial mass by enhanced lipogenesis without mitochondrial biogenesis may cause imbalanced or diffused respiratory complex formation, leading to mitochondrial defect in cellular senescence and aging process.

INTRODUCTION

What is the central mechanism of aging process?

Mitochondrial theory of aging

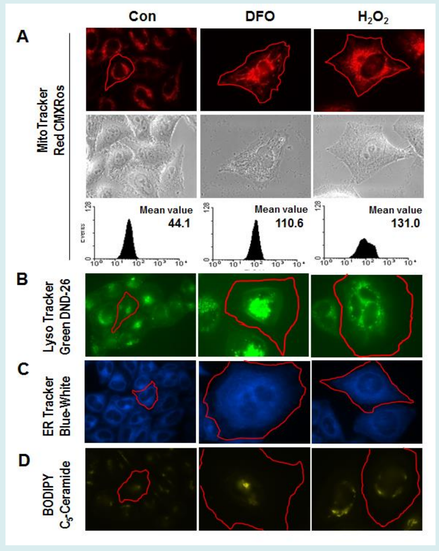


(2) J Am Geriatr Soc. 1972 Apr;20(4):145-7.
(3) Biol Signals Recept 2001;10:162–175

Question. 2: Can enhanced lipogenesis induce mitochondrial defect? ?

Other senescent phenotypes

Question. 3: If yes, what is the underlying mechanism? ?



Enhanced Lipogenesis

Increase in organellar mass

Increase in mitochondrial mass & elongated mitochondria

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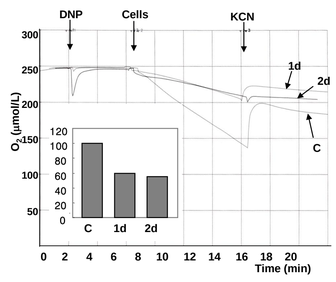
Hypothesis:
Increased mitochondrial mass by enhanced lipogenesis may be one of the causes of mitochondrial defects during cellular senescence.

RESULTS

Increase in mitochondrial mass is commonly accompanied with respiratory defect in SIS.

Mitochondrial Respiratory Defect



J Biol Chem. 2003 Dec 19;278(51):51577-86. Epub 2003 Sep 25

Fig.1 Mitochondrial O_2 consumption rate was measured after treatment without (*c*) or with 0.5mM DFO for one (*1d*) or 2 days (*2d*). Maximum respiration rates of DFO-treated cells were determined as a DNP-uncoupled versus KCN-inhibited O2 consumption rate and expressed as a percentage of control (*insert*).

Elongated mitochondria

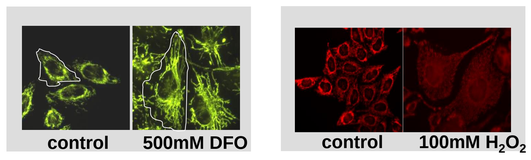


Fig.2 Higher magnified mitochondrial images were taken from stress-induced senescence model. DFO and H_2O_2 (used to induce senescence) treated mt-YFP and RFP Chang cells with a Plan-Neofluar, 1.3NA oil immersion. Objective with 1.6X optovar and Axiovert 200M fluorescence microscopy.

Increase in mitochondria mass

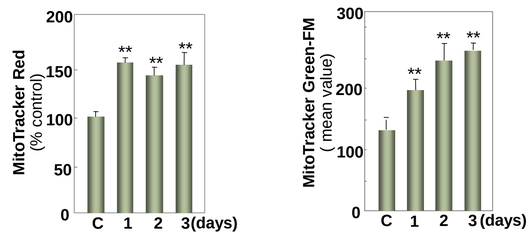
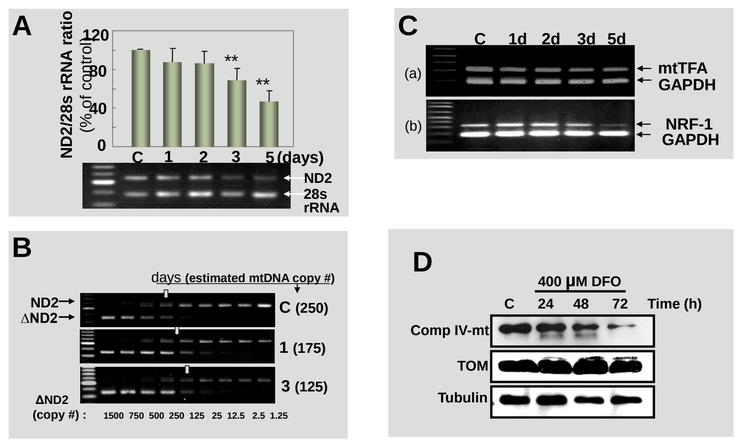


Fig.3 Intracellular mass of mitochondrial membrane was estimated by Flow cytometric analysis after staining Chang cells with CMXRos or MitoTraker Green-FM. **, $p < 0.01$.

Mitochondrial biogenesis is declined in SIS, despite increased mitochondrial mass.



Decrease in mitochondrial DNA copy number
Decrease in mitochondrial transcription, mtTFA
Decrease in mitochondrial protein expression

Fig.4 A, Intracellular mtDNA levels were analyzed by genomic PCR with a primer set for mitochondrial DNA ND2 and a primer set for 28S rRNA as control of nuclear DNA and the ratio of ND2 versus 28S rRNA was presented as percent of control. B, Intracellular mtDNA copy number of Chang cells exposed to DFO (0.5mM) was estimated by competitive PCR. C, RNA content of mtTFA and NRF-1 was estimated by RT-PCR. D, mitochondrial protein expressions were examined by western blot analysis *, $p < 0.05$; **, $p < 0.01$

Lipogenesis is augmented SIS

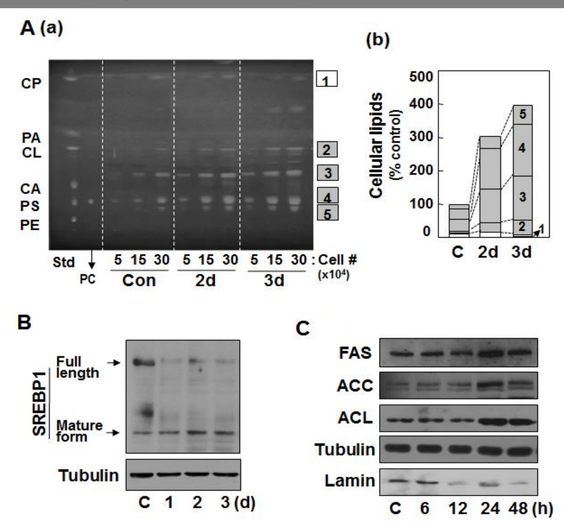


Fig.5. Chang cells were treated with 200 μM DFO for the indicated periods. *A*. Cellular lipid profile of senescent cells was obtained by Thin Layer Chromatography (TLC). Representative TLC image (a) and quantitative estimation (b) of cellular lipids extracted from different numbers of cells are shown. Standard lipid mixture (Std) containing 10 μg each was used. Cholesteryl palmitate (CP, 1) belongs to non-polar storage lipid, and cholesterol (CL, 2), cardiolipin (CA, 3), phosphatidyl choline (PC, 4) and phosphatidyl serine (PS, 5) belong to membrane lipids. 'd' in the x-axis stands for day. *B*. Western blot for SREBP1. Full length and mature form of SREBP1 were indicated by arrows. *C*. Western blot analyses for lipogenic enzymes.

Enhanced lipogenesis by ADD1 overexpression induces senescence.

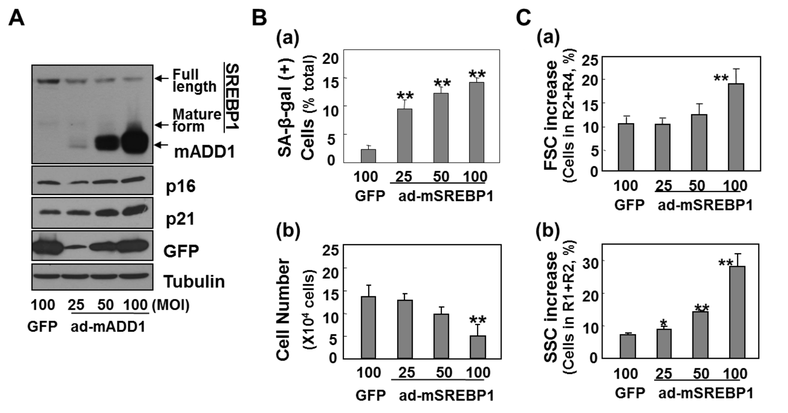
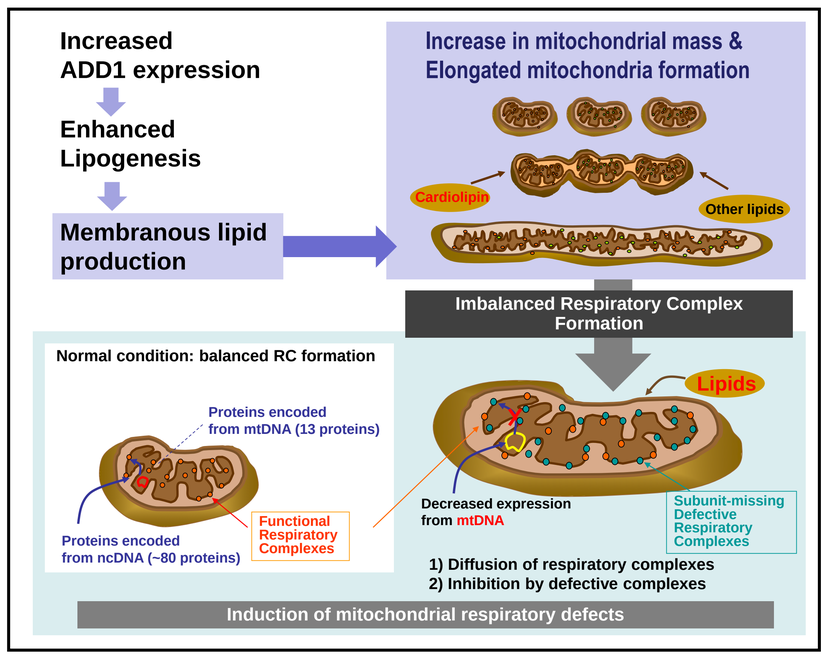


Fig.6 Chang cells were infected with recombinant adenovirus encoding mature form of ADD1 (ad-mADD1, mouse ortholgue of human SREBP-1; master regulator of lipogenesis) for 4 days. A, Western blot analysis of lipogenesis and senescence-related proteins. *Ba*, analysis of SA-β-galactosidase-positive cell population. *Bb*, cell growth rates compared by counting trypan blue-negative live cells. C, cell size (*a*) and cell granularity (*b*) analyzed by comparing FSC and SSC, respectively. *, $p < 0.05$; **, $p < 0.01$ *versus* ad-GFP.

Proposed Underlying Mechanism

Enhanced lipogenesis increases mitochondrial mass production without mitochondrial biogenesis (replication, transcription, and translation), thus resulting in imbalanced or diffused respiratory complex formation.



CONCLUSION

1. Mitochondrial respiratory defect is accompanied by increased mitochondrial mass generation without increased mitochondrial biogenesis (mitochondrial replication, transcription and translation).

2. Enhanced lipogenesis increases mitochondrial mass production.

3. Enhanced lipogenesis may be a cause of respiratory defect via imbalanced mitochondrial biogenesis.

REFERENCE

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(2) The biologic clock: the mitochondria? J Am Geriatr Soc. 1972 Apr;20(4):145-7.
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(5) Sterol regulatory element-binding protein (SREBP)-1-mediated lipogenesis is involved in cell senescence. J Biol Chem. 2010 Sep 17;285(38):29069-77.