The dysfunction of dermal fibers in photoaging is caused by the impairment of mitochondrial function

BL-92

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Introduction:

What is photoaging in dermis?

Photoaging is that aging symptom is accelerated by chronic sun exposure, and photoaged skin is characterized by appearance of deep wrinkles, sagging and pigment spots. Among Sun lights, ultraviolet (UV) lights are responsible for progression of photoaging. It is observed as typical alterations that collagen fibers and oxytalan fibers composed by fibrillin-1 (FBN1) are disappeared in the upper dermis.

These alterations are able to be reproduced by UVA irradiation to fibroblasts which are mother cells for formation of both fibers (Fig. 1)

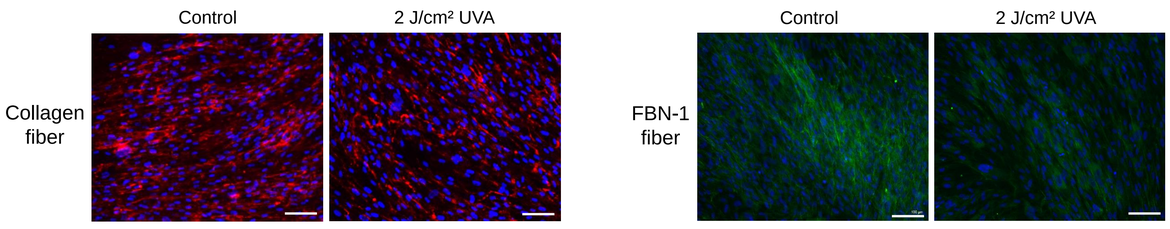


Fig. 1. Influence of UVA irradiation on dermal fibers in fibroblasts.
(red; collagen, green; FBN-1, blue; nuclei, scale bars; 100 μm)

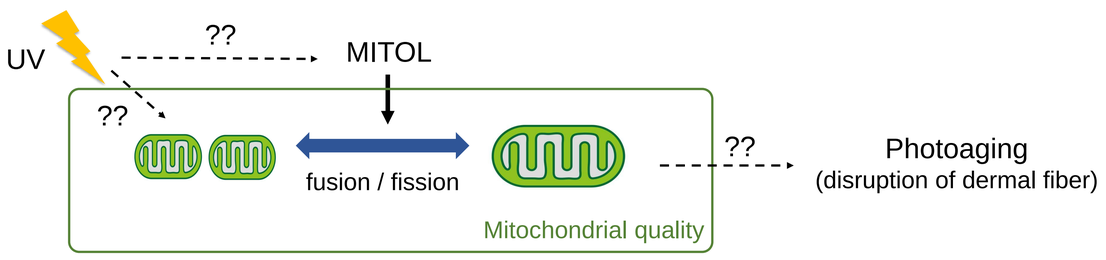
The influence of mitochondrial impairment on progression of photoaging

Mitochondria are key intracellular organelles that maintain the cellular homeostasis by supplying energy, adenosine triphosphate (ATP).
Although mitochondrial dysfunction has been observed in cells of UV-exposed skin, the relationship between the progression of photoaging and the impairment of mitochondria is not fully understood.

In general, quality of mitochondria is maintained by the balance between fission and fusion, and mitochondria which is fissioned excessively reduces their function to supply ATP. Recently, It is known that mitochondrial ubiquitin ligase (MITOL) plays a role to maintain the mitochondrial quality by removing impaired mitochondria.

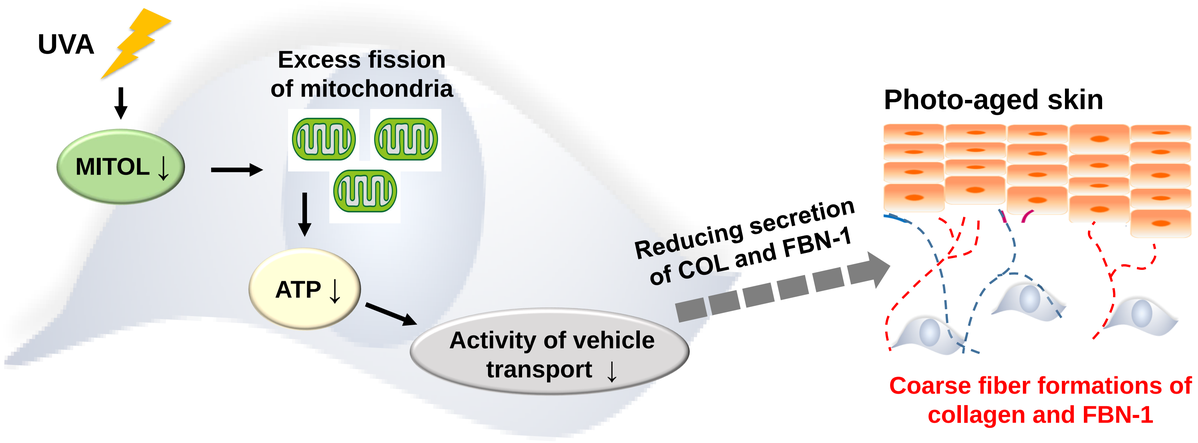
The purpose of this study

is to clarify whether lowered quality of mitochondria is an upstream-cause of the initiation and/or the progression of photoaging.



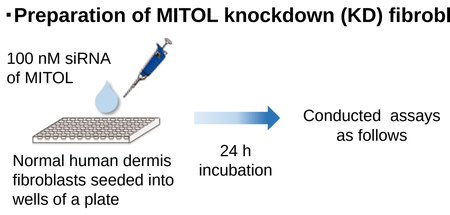
Conclusions:

Insufficient supplying of ATP by the UVA-induced depletion of MITOL is the most fundamental cause of the initiation and progression of photoaging.

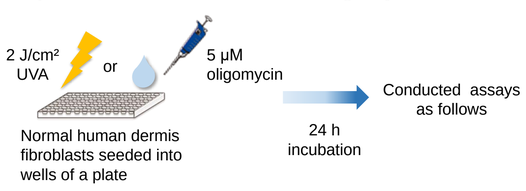


Materials & Methods:

・Preparation of MITOL knockdown (KD) fibroblasts



・Preparation of UVA-irradiated or oligomycin※-treated fibroblasts



※Oligomycin: ATP synthase inhibitor

・Assay items

- **Mitochondrial mass:** Mitochondria in cells were stained using a MITO-ID® Green detection kit.
- **Intracellular ATP:** Intracellular ATP levels in cell lysates were measured using a CellTiter-Glo®2.0 Assay kit.
- **Mitochondrial reactive oxygen species (ROS):** The mitochondrial ROS levels in cells were measured using a MitoSOX™.
- **mRNA expression:** Using total RNAs in cells extracted by Cells-to-CT kit, mRNA expressions were quantified with real-time PCR.
- **Immunostaining:** Type I collagen and FBN-1 were visualized as fluorescent images by immunostaining using corresponding antibodies.
- **Type I collagen and FBN-1:** Amounts of type I collagen and FBN-1 in cells and secreted into the culture medium were quantified by ELISA.

Results & Discussion:

① UVA irradiation impaired mitochondrial quality and was reduced MITOL expression in fibroblasts.

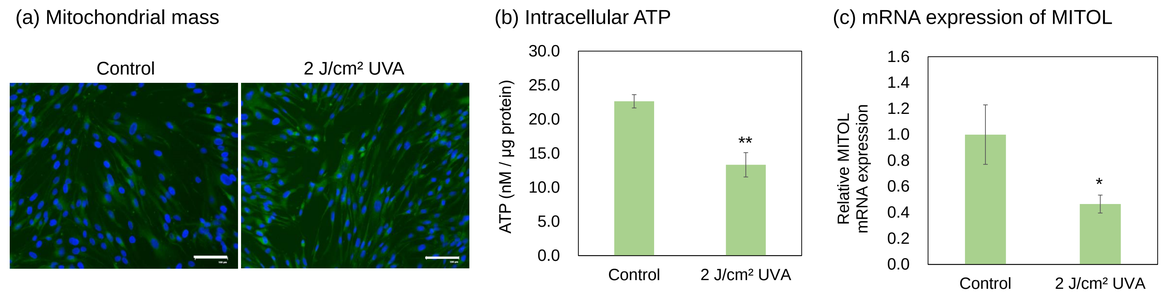


Fig. 2. Influence of UVA irradiation on mitochondrial functions in fibroblasts.
(a) Images of mitochondrial mass in fibroblasts (green; mitochondria, blue; nuclei, scale bars; 100 μm). (b) Intracellular ATP content at 24 h after UVA irradiation. (c) mRNA expression level of MITOL at 1 h after UVA irradiation. Means ± S.D., *p<0.05, **p<0.01 vs. control, n=3.

② MITOL-KD impaired mitochondrial quality in fibroblasts.

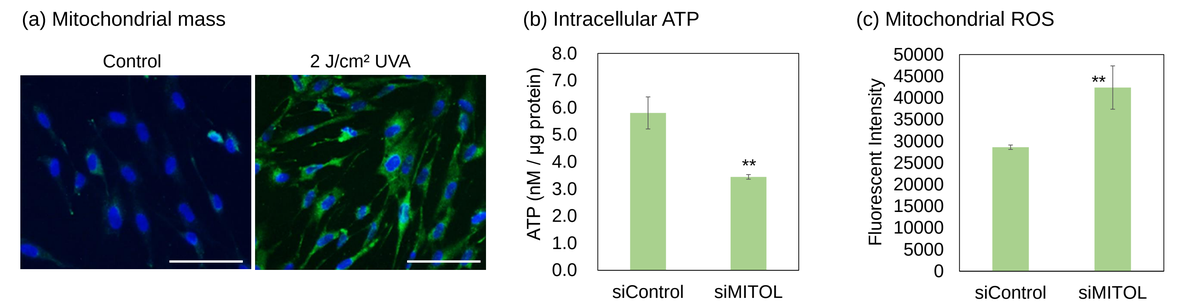


Fig. 3. Influence of MITOL-KD on mitochondrial functions in fibroblasts.
(a) Images of mitochondrial mass in fibroblasts (green; mitochondria, blue; nuclei, scale bars; 100 μm). (b) Intracellular ATP content. (c) Mitochondrial ROS levels. Means ± S.D., *p<0.05, **p<0.01 vs. siControl, n=3.

③ MITOL-KD in fibroblast caused coarse formations of dermal fibers.

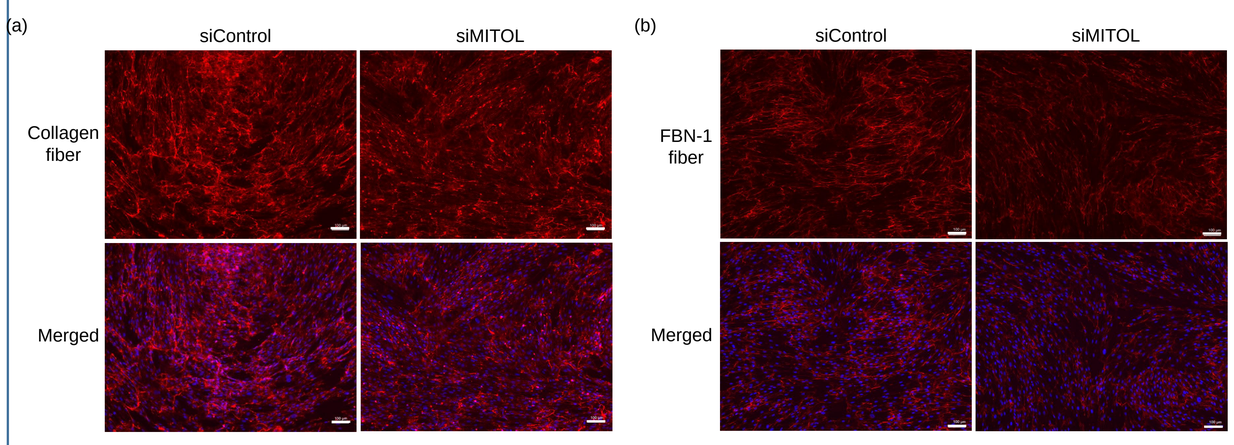


Fig. 4. Immunohistochemical staining showing the influence of MITOL-KD in fibroblasts for 7 days on (a) collagen fibers and (b) FBN-1 fibers (red; fibers, blue; nuclei, scale bars; 100 μm)

④ MITOL-KD in fibroblasts suppressed secretions of Collagen and FBN-1.

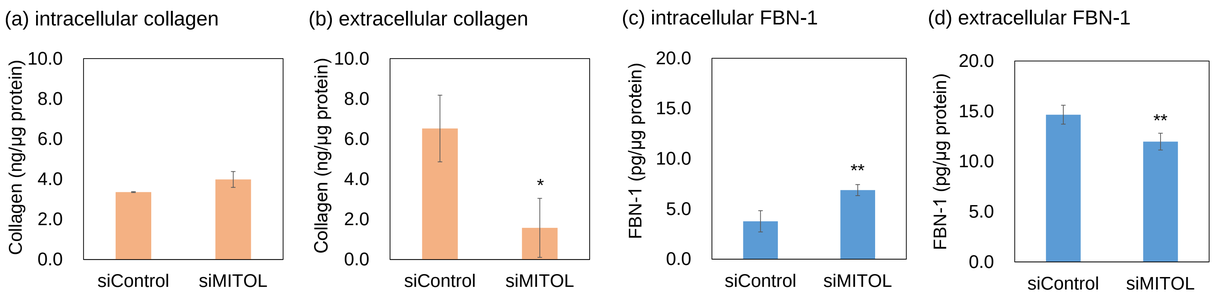


Fig. 5. Intracellular or secreted fiber proteins, Collagen and FBN-1, of MITOL-KD fibroblasts. (a) Intracellular type I collagen content. (b) Extracellular type I collagen content. (c) Intracellular FBN-1 content. (d) Extracellular FBN-1 content.
Means ± S.D., *p<0.05, **p<0.01 vs. siControl, n=4.

⑤ Depletion of ATP supplying in fibroblasts reduced secretions of Collagen and FBN-1.

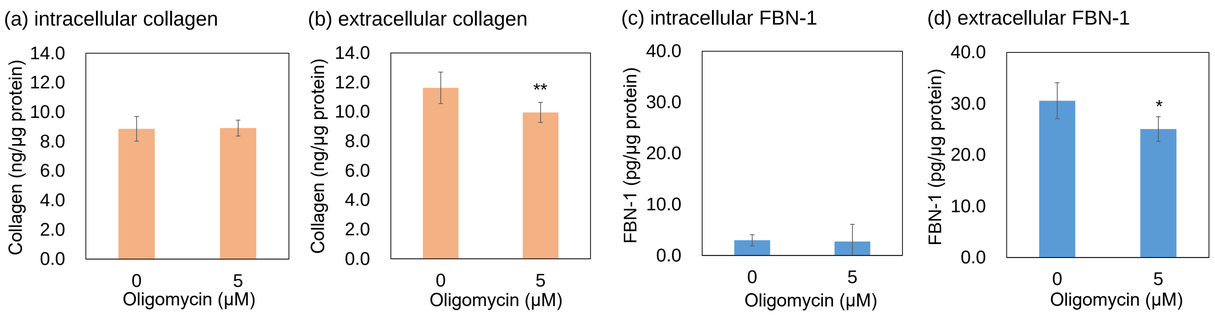


Fig. 6. Intracellular or secreted fiber proteins, Collagen and FBN-1, of oligomycin-treated fibroblasts. (a) Intracellular type I collagen content. (b) Extracellular type I collagen content. (c) Intracellular FBN-1 content. (d) Extracellular FBN-1 content.
Means ± S.D., *p<0.05, **p<0.01 vs. 0 μM, n=4.