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KOBAYASHI

Senolytics restore the contraction of the collagen gel based on senescent dermal fibroblasts culture : A novel insight into the therapeutic approach to skin aging

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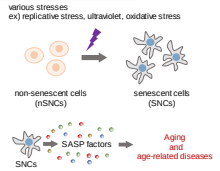
Introduction

Cellular Senescence



Induction of cellular senescence

- Cellular senescence is induced by various stresses and arrest growth irreversibly.

Characterization of senescent cells (SNCs)

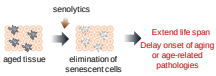
Beneficial effects

- SNCs prevent genetically unstable propagation and promote wound healing, tissue repair.

Deleterious effects

- SNCs damage surrounding tissues via the secretion senescence-associated secretory phenotype (SASP) factors such as inflammatory cytokine and protease.
- Accumulation of senescent cells contributes to aging and age-related pathologies in various organs.

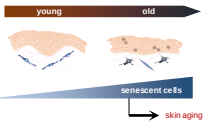
Strategies to target SNCs therapeutically



Senolysis and senolytics

- Accumulating evidence demonstrates that clearing of SNCs from tissue (senolysis) has a protective effects against aging and age-related pathologies.
- Senolytics are drugs that can selectively eliminate SNCs.
- To data, several senolytics including ABT263 has been reported.
- There are few reports on the effects of senolytics on the skin.

Relationship between cellular senescence and skin aging



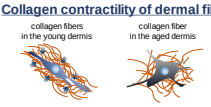
SNCs in skin

- SNCs are accumulated in the skin including dermis with age.

Senescent dermal fibroblasts and skin aging

- senescent dermal fibroblasts are considered to be associated with skin aging.
- How these senescent fibroblasts contribute to skin aging has not been fully elucidated.

Collagen contractility of dermal fibroblasts



	Age-related change
quantity and thickness of collagen bundles	decreased
orientation of collagen fiber	altered
collagen contractility of dermal fibroblast	unknown

Roles of collagen in dermis

- Collagen fibers are major component of extracellular matrix in dermis.
- The contraction of collagen fibers by dermal fibroblasts provide tensile strength and elasticity to prevent skin aging such as wrinkling and sagging.

Collagen contractility and skin aging

- The disturbance of collagen structure cause signs of skin aging such as wrinkling and sagging.
- Collagen fibers density decreases with age.
- Age-related changes in collagen contractility of dermal fibroblast are not well understood.

We investigated the change in collagen contractility of SNCs and examined the effects of senolytics on collagen contraction.

Materials & Methods

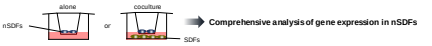
Experiment 1. Induction of cellular senescence

Normal human dermal fibroblasts were treated with **doxorubicin (DXR)** for 3 days to induce cellular senescence.
After treatment, these cells were subjected to **SA-β gal staining** and immunofluorescence staining for **senescence marker (γH2AX)**.

Experiment 2. The influences of SDFs on collagen gel contraction

The contraction of **non-senescent dermal fibroblasts (nSDFs)** and **senescent dermal fibroblasts (SDFs)** induced by DXR containing collagen gel, referred to as **young dermal model** and **aged dermal model** respectively, were observed with microscopy at indicated times after release from dish. The acquired images were analyzed with ImageJ and the area of collagen gel was calculated.

Experiment 3. The influences of SDFs on the gene expression in nSDFs



nSDFs were cultured with or without SDFs for 3 days using a culture insert system.
After culture, comprehensive gene expression in the nSDFs was analyzed by **DNA microarray**.

Experiment 4. The effects of senolytics on the aged dermal model

The contraction of aged dermal model in the presence or absence of **ABT263*** with or without **Z-VAD-FMK**** was observed. The acquired images were analyzed with ImageJ and the area of collagen was calculated. Senescence marker (γH2AX) and collagen orientation in the aged dermal model were observed by **immunofluorescence** and **second harmony generation (SHG) with multiphoton microscopy**, respectively.

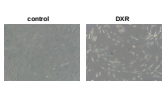
*ABT263 has been reported to eliminate SNCs through activation of apoptosis pathway in SNCs

**Z-VAD-FMK is a inhibitor of apoptosis

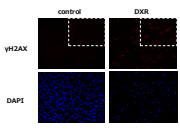
Results & Discussion

Experiment 1. Induction of cellular senescence

SA-β gal staining



immunofluorescence

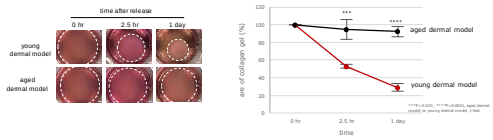


Doxorubicin induced dermal fibroblast senescence.

Results & Discussion (continued)

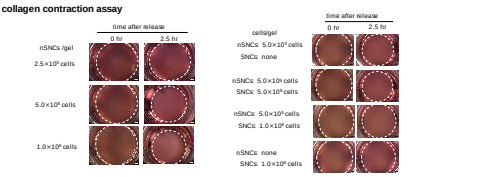
Experiment 2. The effects of SDFs on collagen gel contraction

collagen contraction assay



The contraction of the aged dermal model was drastically lower than that of the young dermal model.
➡ The collagen contractility of SDFs is decreased.

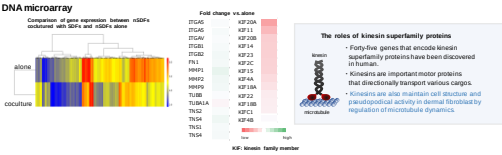
collagen contraction assay



Decreased collagen gel contraction due to SDFs was also observed even in the presence of an adequate number of nSDFs sufficient to contract the gel.
➡ SDFs decrease the collagen contractility of nSDFs .

Experiment 3. The influences of SDFs on the gene expression in nSDFs

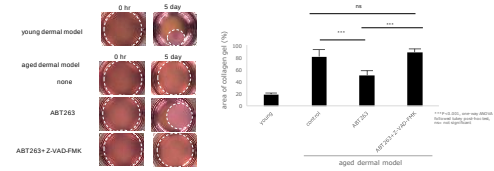
DNA microarray



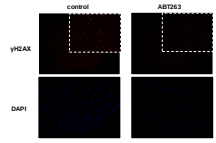
The expression of many kinesin super family genes was decreased in nSDFs cocultured with SDFs
➡ Kinesins in dermal fibroblasts might be involved in ability of these cells to contract collagen.

Experiment 4. The effects of senolytics on the aged dermal model

collagen contraction assay



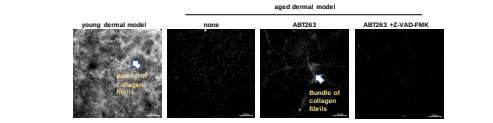
immunofluorescence



- **The contraction of aged dermal model treated with ABT263 was observed.**
- **Apoptosis inhibitor canceled the effects of ABT263 on collagen contraction of aged dermal model.**
- **ABT263 decreased the number of SDFs in aged dermal model.**

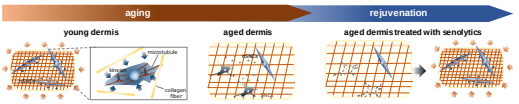
Removal of SDFs by senolytics recovers the collagen contraction.

SHG imaging



The collagen fiber orientation was observed in aged dermal model treated with ABT263.
➡ Removal of SDFs by senolytics recovers the collagen fiber orientation.

Conclusions



young dermis

Collagen contractility by dermal fibroblasts is strong, therefore skin tensile strength and elasticity are high.

aged dermis

SNCs are accumulated and collagen contractility is lowdue to decreased expression of kinesins which are important for pseudopodical activity in dermal fibroblast. These cause skin aging appearance such as wrinkling and sagging.

aged dermis treated with senolytics

Senolytics remove SNCs from aged dermis, and recovers the decrease of collagen contractility induced by SNCs, and may improve skin wrinkling and sagging caused by decrease of skin tension in aged person. Senolytics rejuvenate aged skin.

- **Low collagen contraction ability by SNCs cause changes in dermal ECM morphology with skin aging.**
- **Elimination of SNCs from aged dermis restores collagen contractility.**

Senolytics are effective therapeutic approach to skin aging.