

9th IPCE CONFERENCE
PISA – 30 June and 1 July

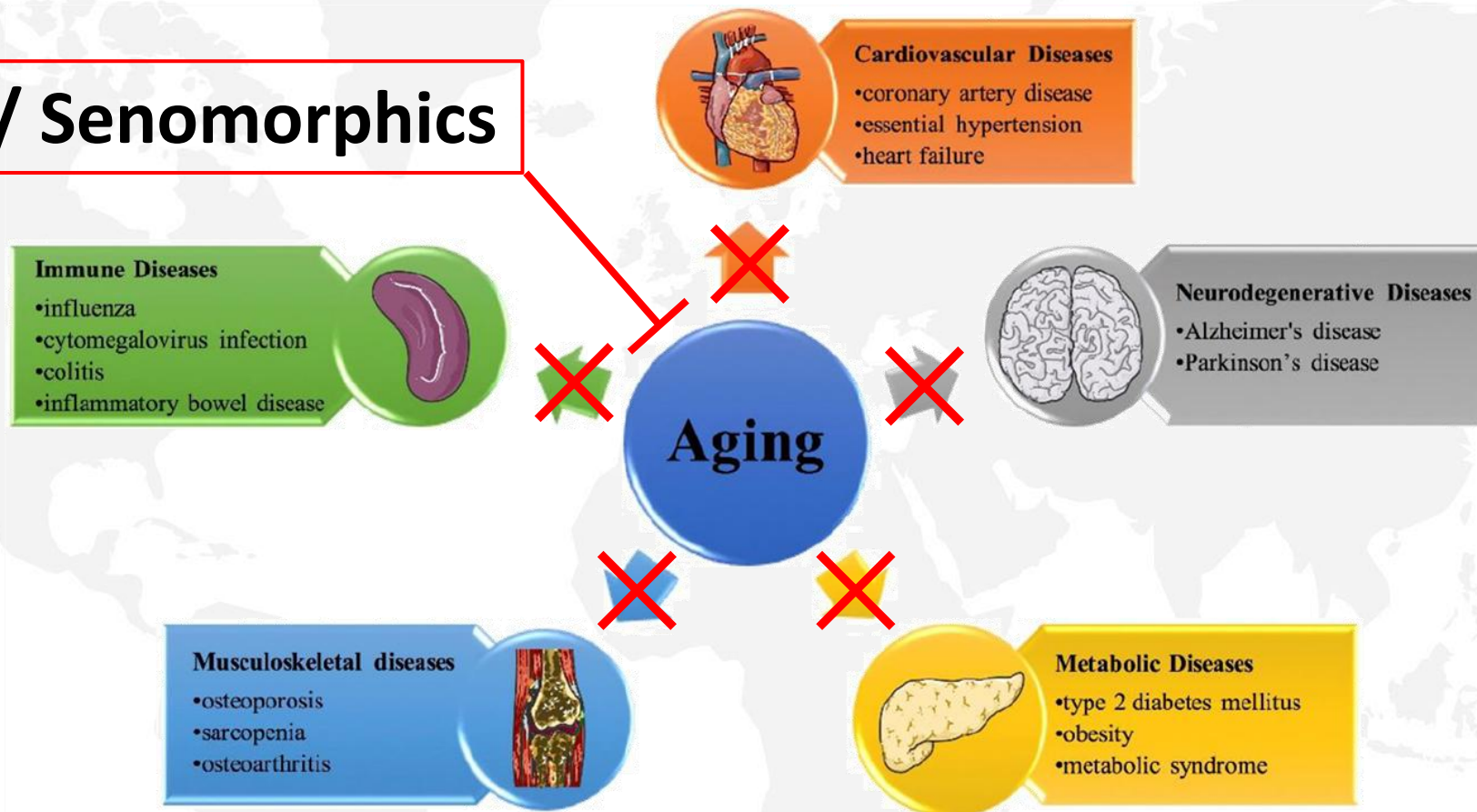
Breaking the senescence chain: Identification of cellular senescence inducing microRNA

Yuri SATO¹, Eriko ITAI², Jiaxu CHEN², Eri KAWASHIMA², Takashi TERAMURA²,
Toyofumi Nagatsuka², Kazuhiko KAJI², Toru ATSUGI²

¹ KOSE R&D France, KOSE Corporation, 5 Avenue Lionel Terray, 69330, Meyzieu, France

² Research Laboratories, KOSE Corporation, 48-18, Sakaecho, Kita-ku, Tokyo 114-0005, Japan

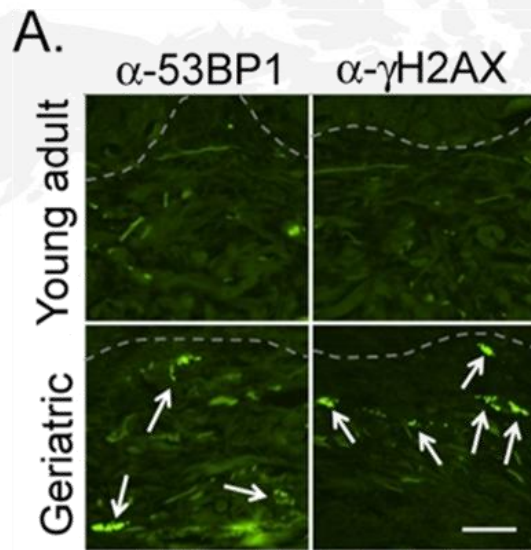
Senolytics/ Senomorphics



Yu-Qing Ni *et al.* Aging Dis. 2021

Anti-aging/rejuvenation research
targeting aging cells is expanding.

Accumulation of senescent cells induces senescence propagation

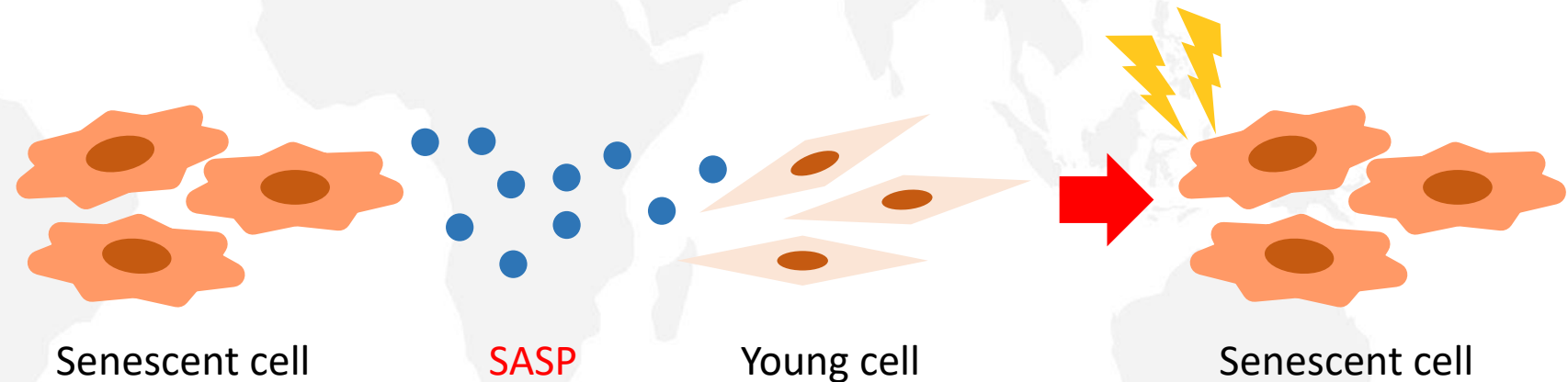
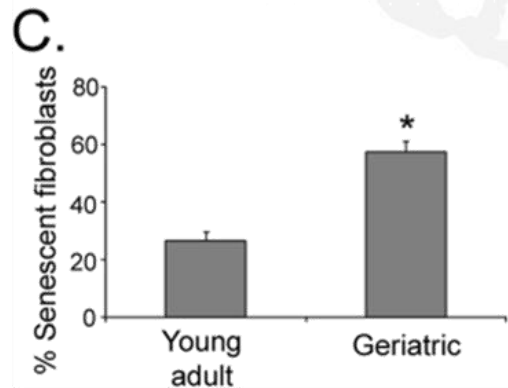


Long-term retention of senescent cells leads to...

- **Accelerated aging of surrounding cells/tissues**
- **Formation of the cancer microenvironment**

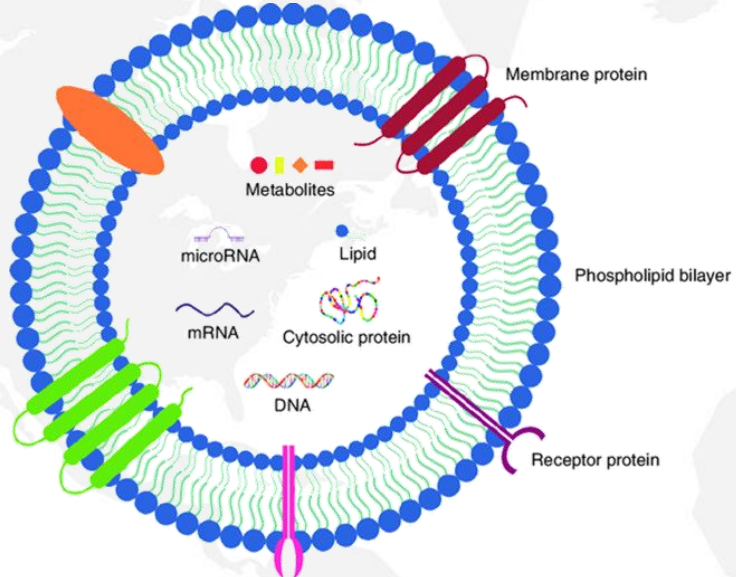
Alla Zorina *et al.* Int. J. Mol. Sci. 2022

What mechanisms mediate the “senescence propagation”?
→ **SASP** (senescence-associated secretory phenotype)



Secretory factors that may trigger the senescence propagation

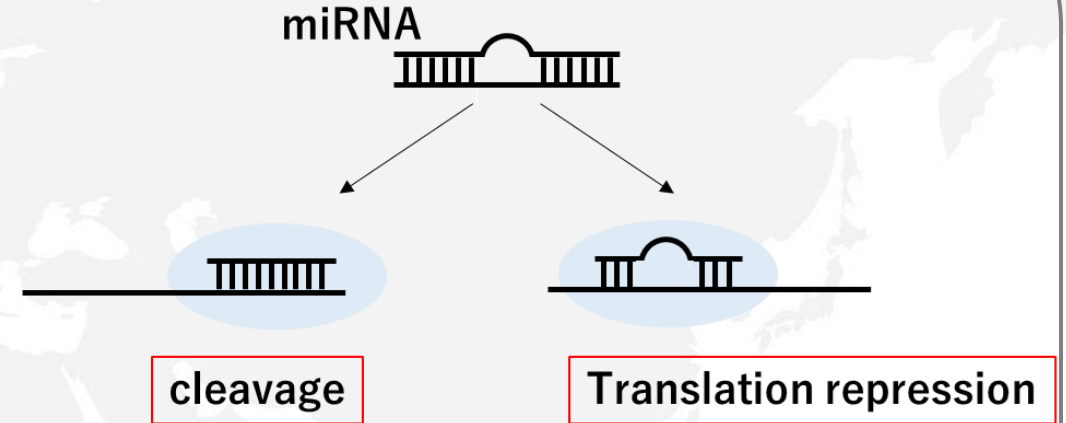
Extracellular vesicles (EVs)



- Membrane vesicles 30-150 nm in diameter
- Including mRNA, lipids, and proteins
- Biomarkers of various diseases

Nihat Dilsiz. Cell. Future Sci OA. 2020

microRNA (miRNA)

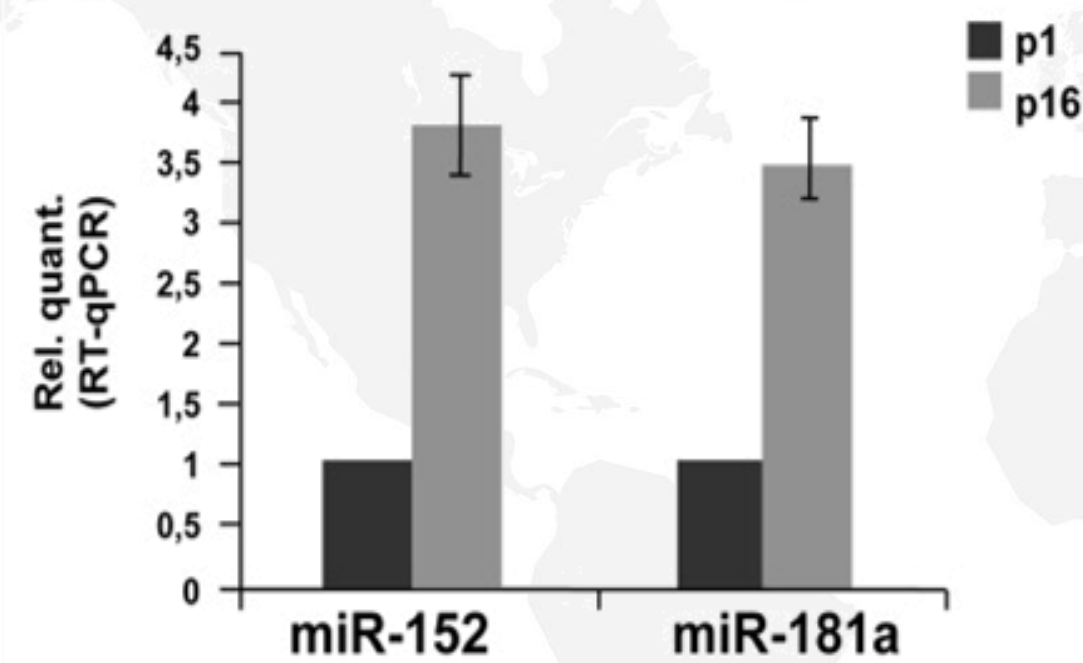


- Short RNA of approx. 20 bases
- Inhibition of targeting gene translation
- One of the EV-including factors

Intracellular miRNAs fluctuating with aging

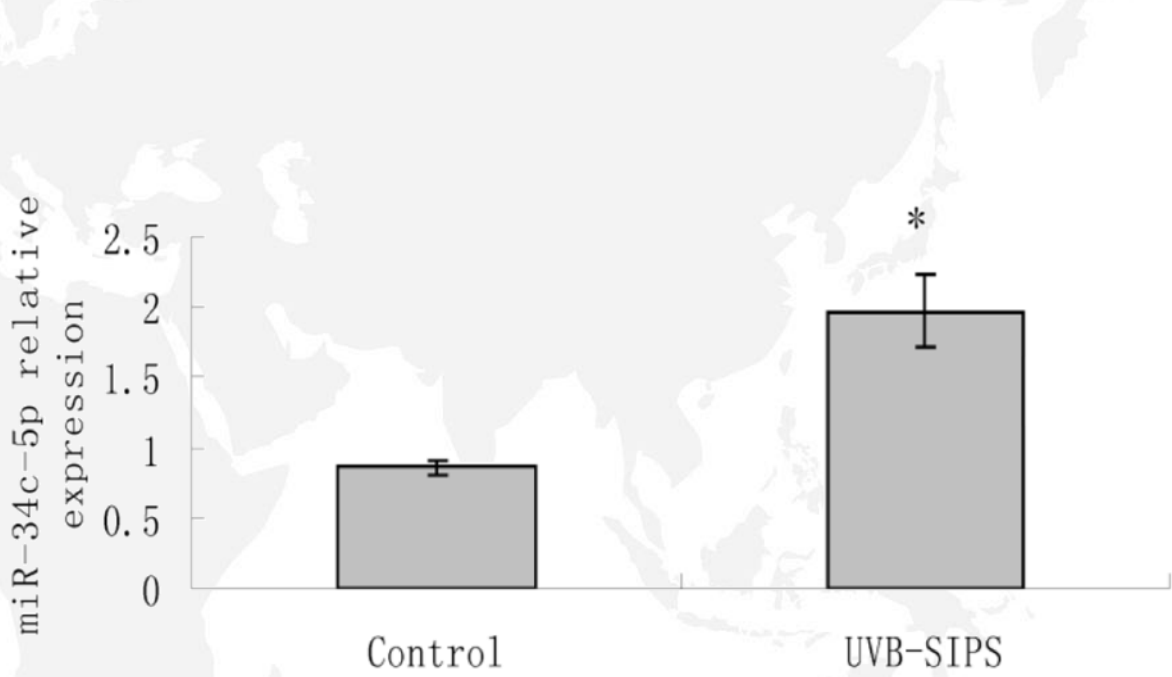
Previous Reports

In vitro aging



Mara Mancini *et al.* Aging (Albany NY). **2012**

Photoaging



Bing-rong ZHOU *et al.* Int J Biol Sci. **2013**

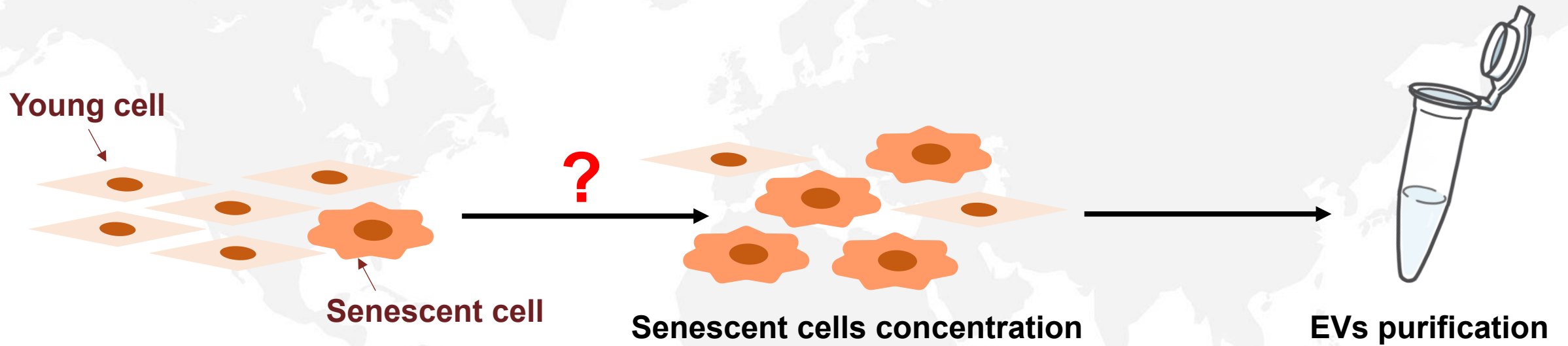
The purpose of this study

Exploring the aging-related microRNA in dermal fibroblasts derived EVs



Identification of novel senescence chain-related factors

Strategy of this study



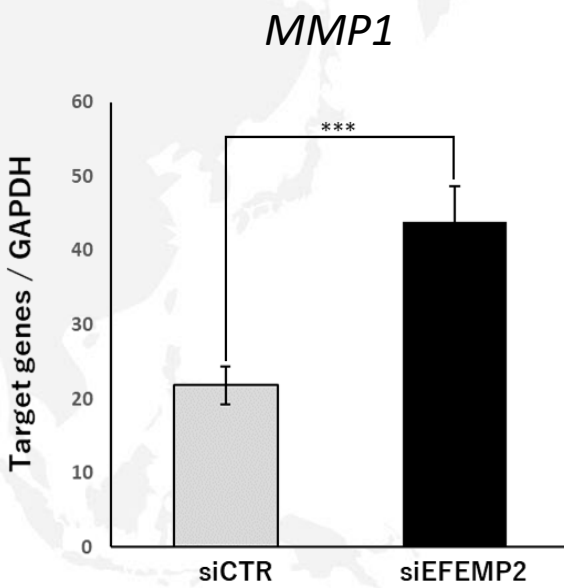
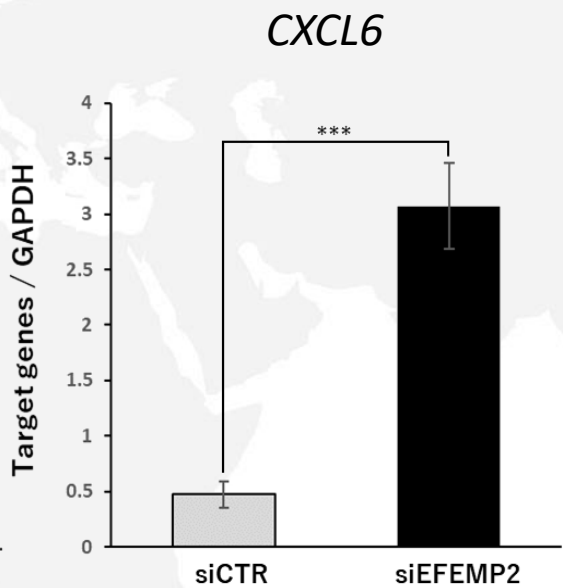
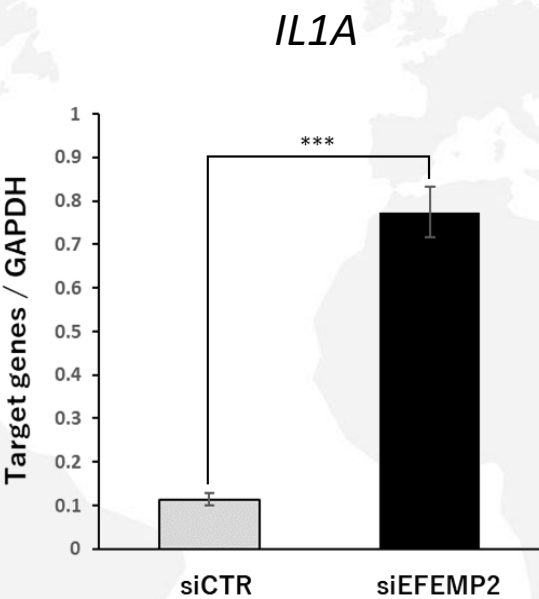
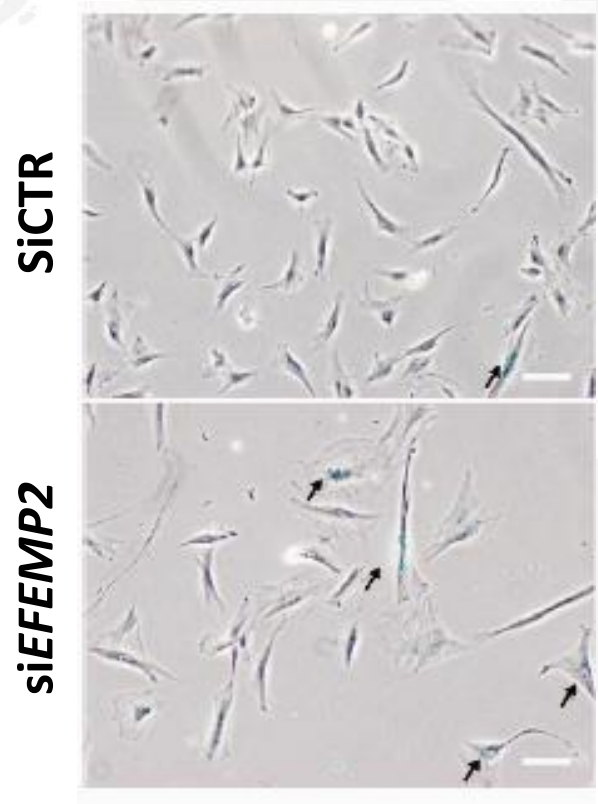
→ Exploring novel senescence spreading factor (miRNA)

Identification of *EFEMP2* as aging inhibitory gene

Our Previous Research

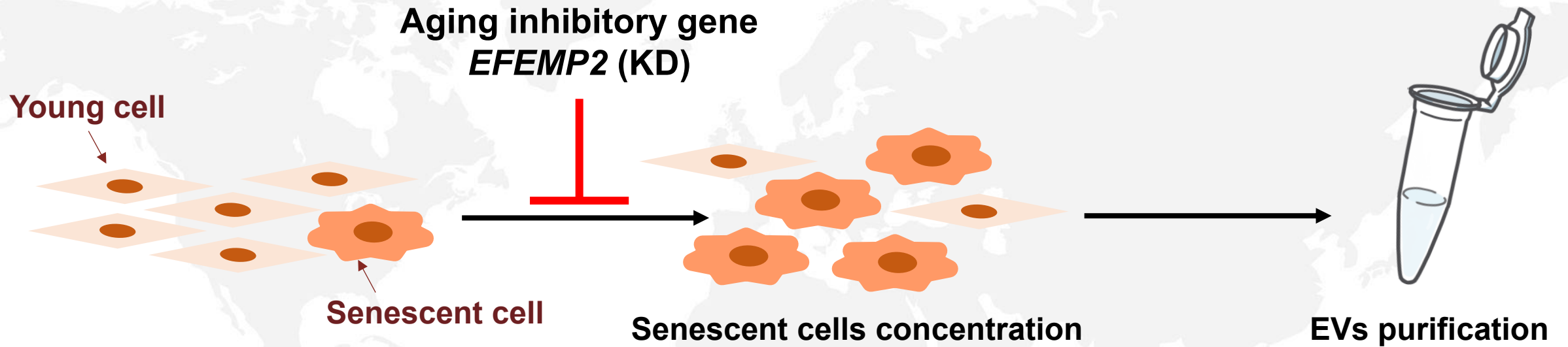
EFEMP2 KD

→ induced cellular senescence



Eriko Itai et al. Exp Dermatol. 2023 Partially modified

Strategy of this study



Induction of senescence by KD of *EFEMP2*
→ Exploring novel senescence spreading factor (miRNA)

Research design

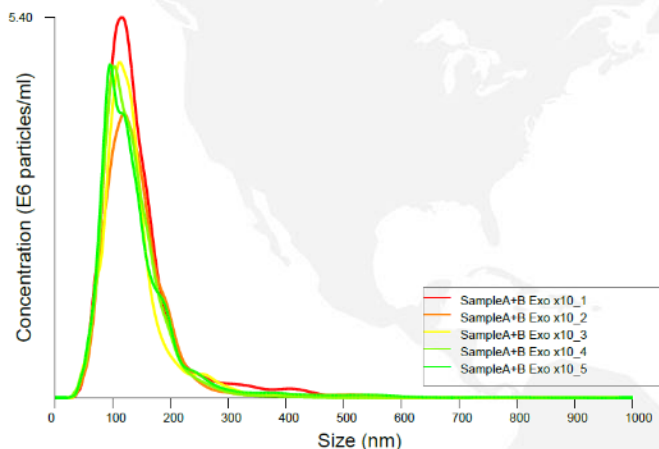
1. EVs purification and microarray of EVs-derived miRNA
2. Functional analysis of miR-570-3p in cells
3. *Ex vivo* culture of excised human skin with miR-570-3p
4. Analysis of cosmetic agents modulating miR-570-3p

Research design

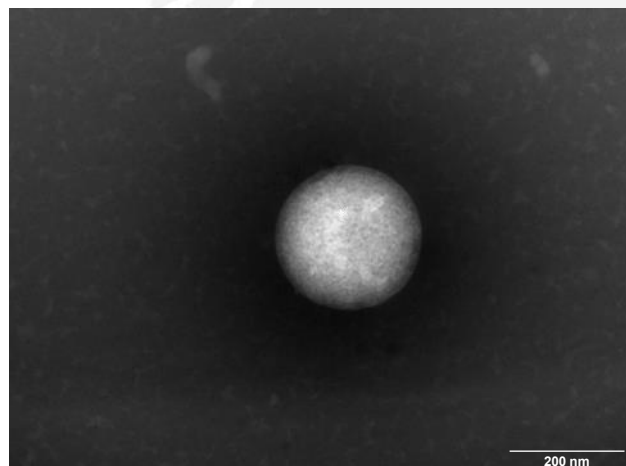
1. EVs purification and microarray of EVs-derived miRNA
2. Functional analysis of miR-570-3p in cells
3. *Ex vivo* culture of excised human skin with miR-570-3p
4. Analysis of cosmetic agents modulating miR-570-3p

EVs purification from dermal fibroblasts

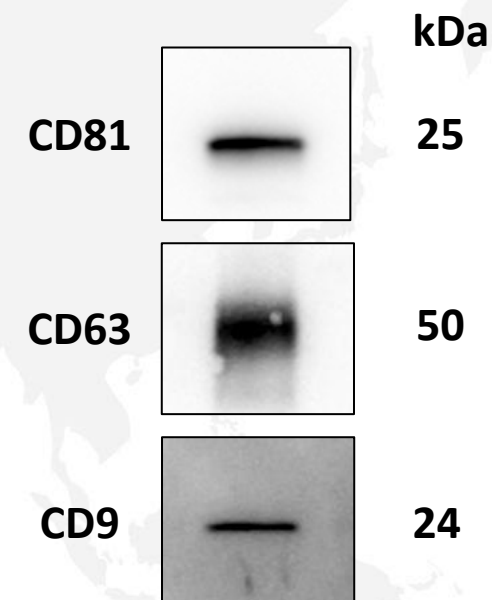
Particle size (Nanosight)



Particle imaging (TEM)



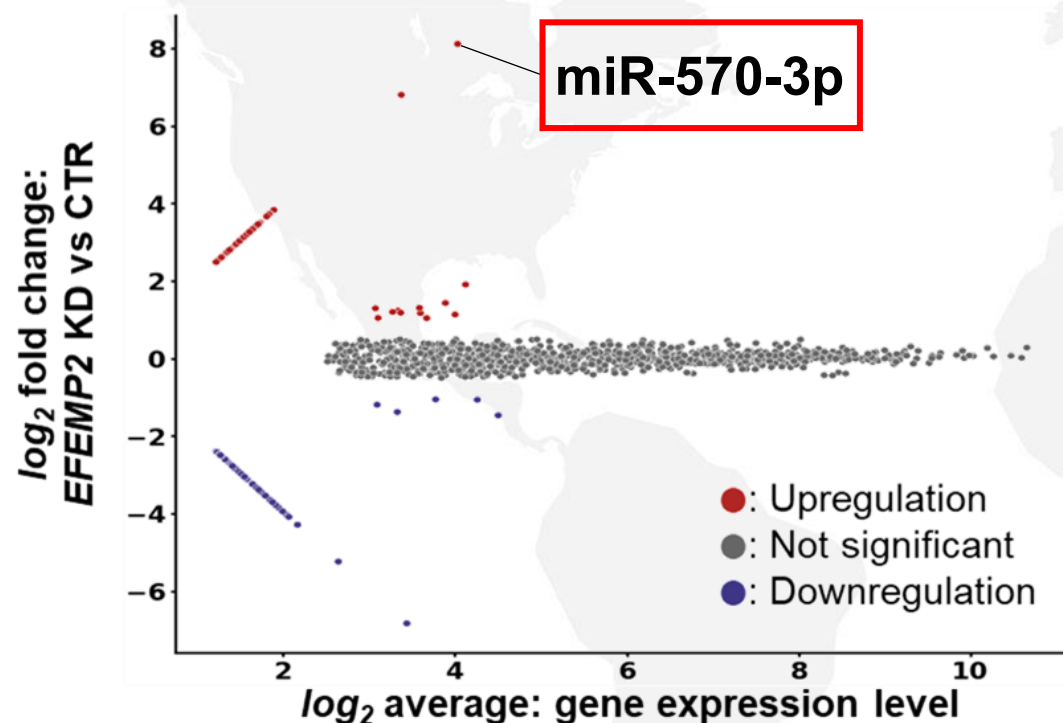
Marker protein (WB)



Cells used in this study were approved by the Ethics Committee of the Japan Pharmaceutical University (Approval number: 27-04)

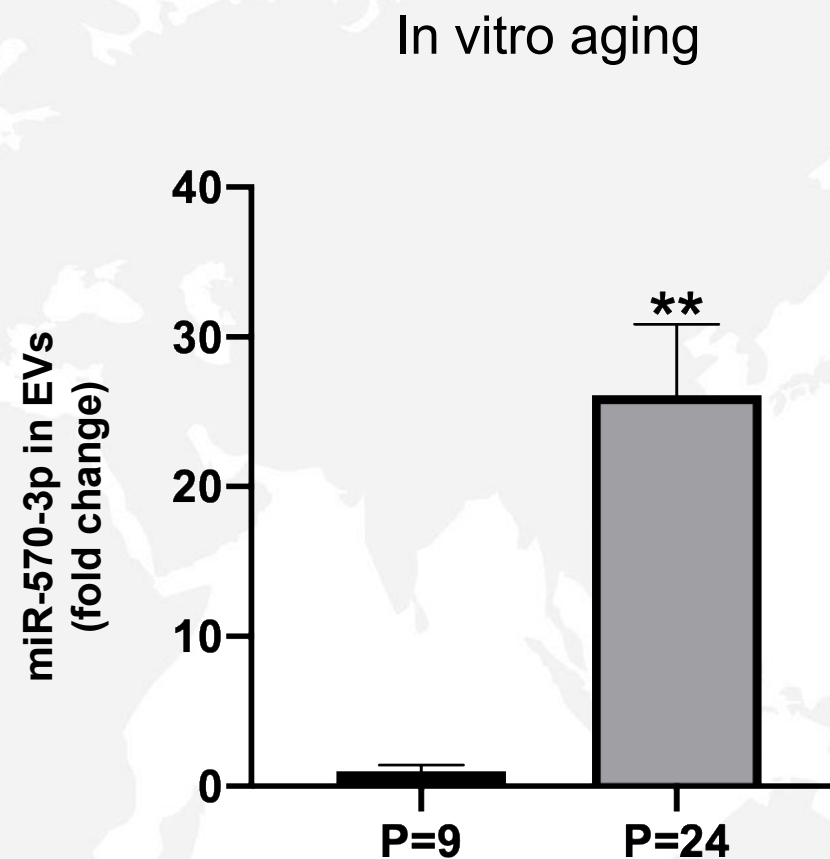
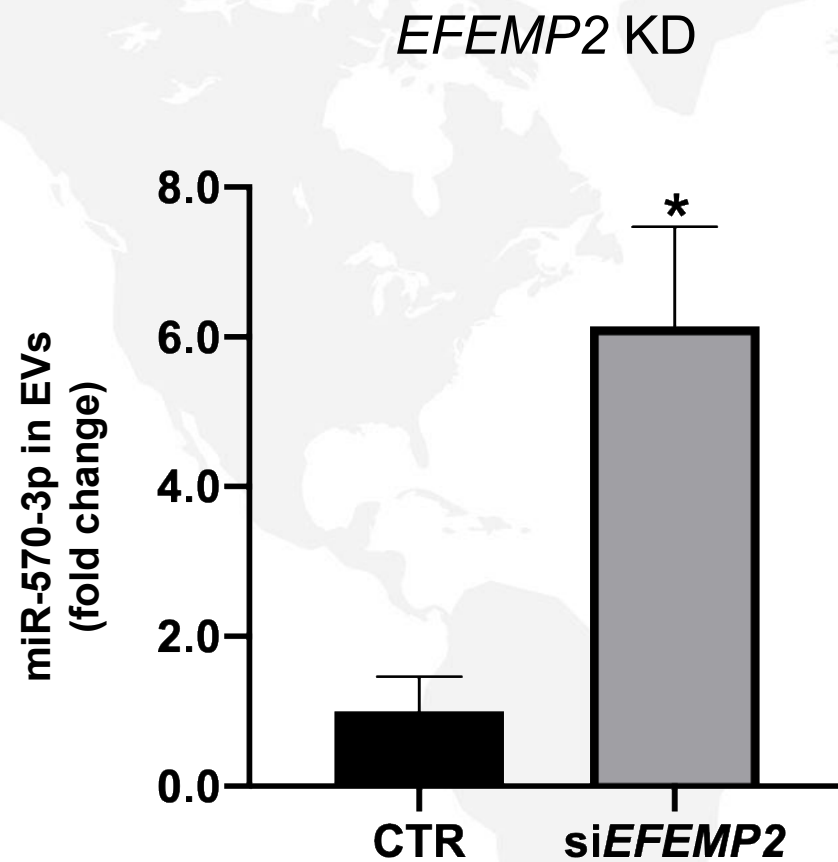
miR-570-3p increased by cellular senescence in EVs

Alteration of miRNAs in the EVs by *EFEMP2* KD



miRNA	ratio (vs CTR)
hsa-miR-570-3p	276.5
hsa-miR-3613-5p	111.5
hsa-miR-4773	14.2
hsa-miR-4746-5p	13.6
hsa-miR-3184-3p	13.3
hsa-miR-1908-3p	13.2
hsa-miR-7152-3p	12.7
hsa-miR-1972	11.4
hsa-miR-6734-3p	11.2
hsa-miR-7153-5p	11.0

miR-570-3p increased by cellular senescence in EVs

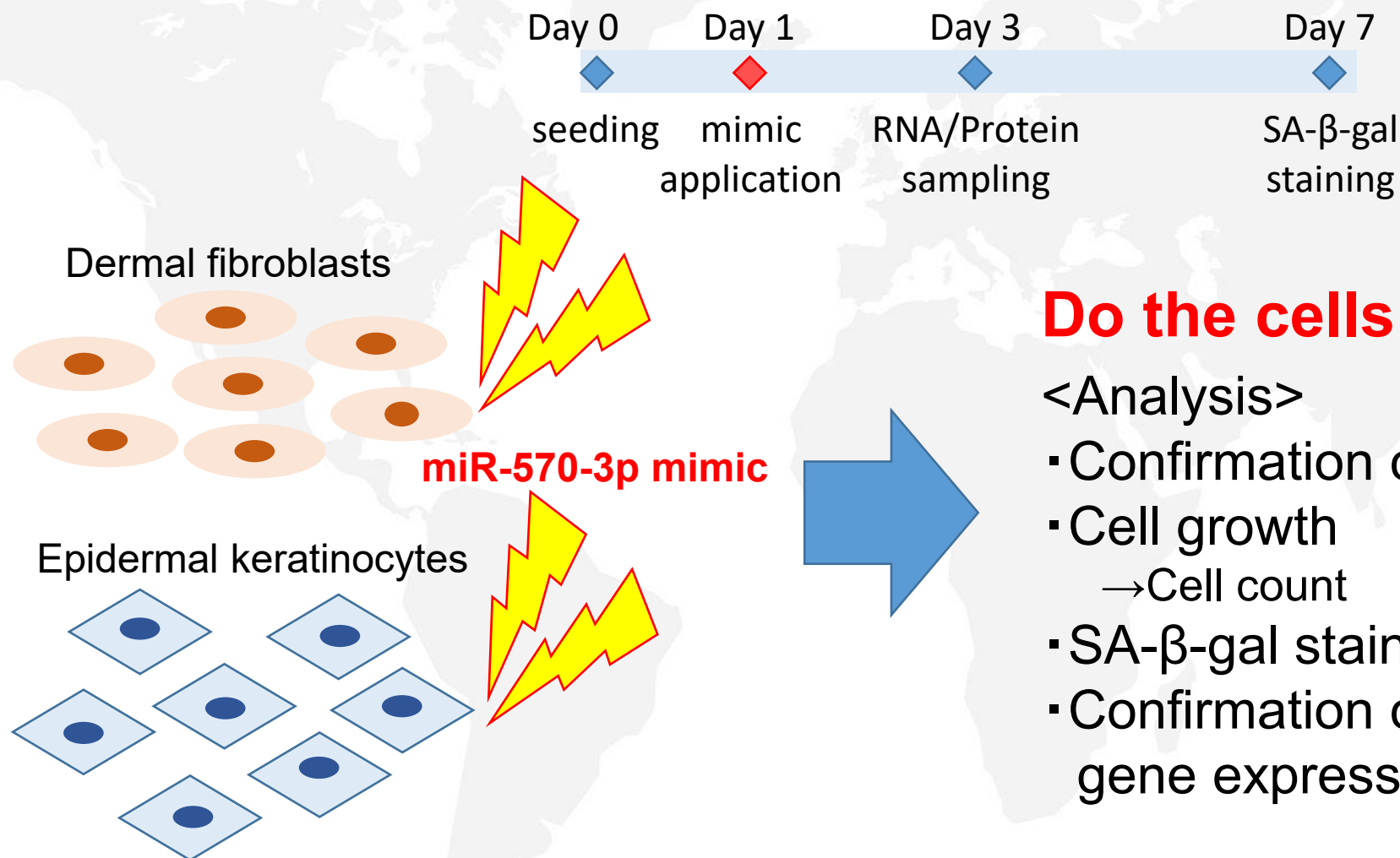


Research design

1. EVs purification and microarray of EVs-derived miRNA
- 2. Functional analysis of miR-570-3p in cells**
3. *Ex vivo* culture of excised human skin with miR-570-3p
4. Analysis of cosmetic agents modulating miR-570-3p

Experimental design

Objective: To investigate the function of miR-570-3p in skin cells.



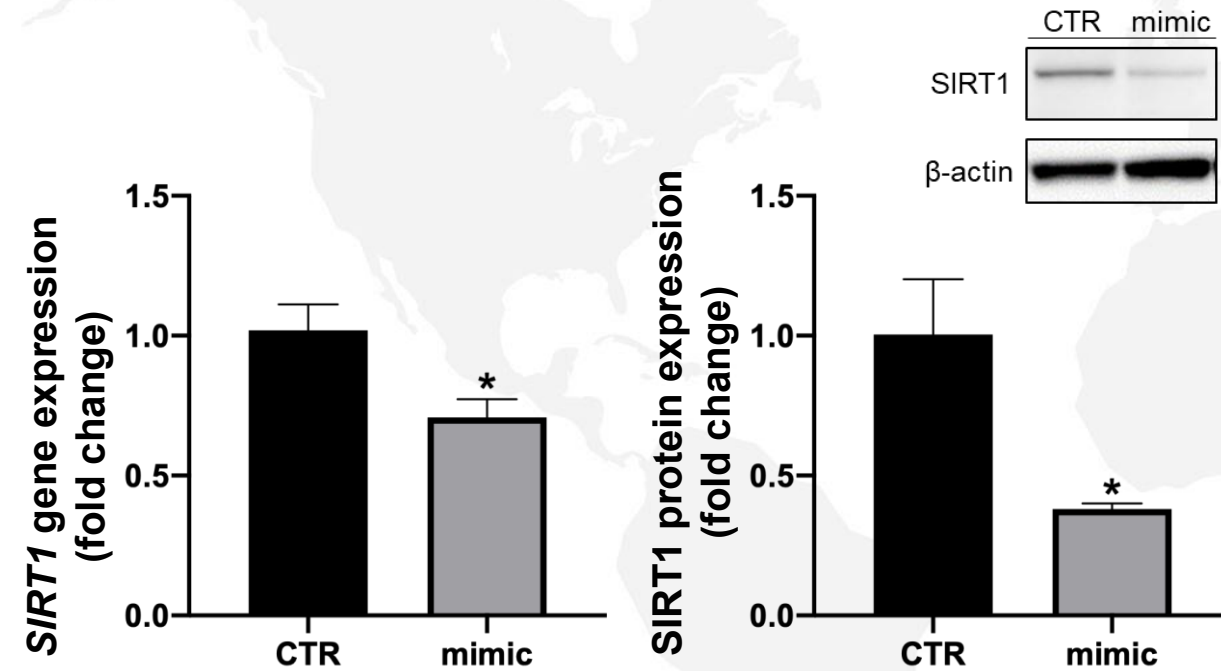
Do the cells get aged?

<Analysis>

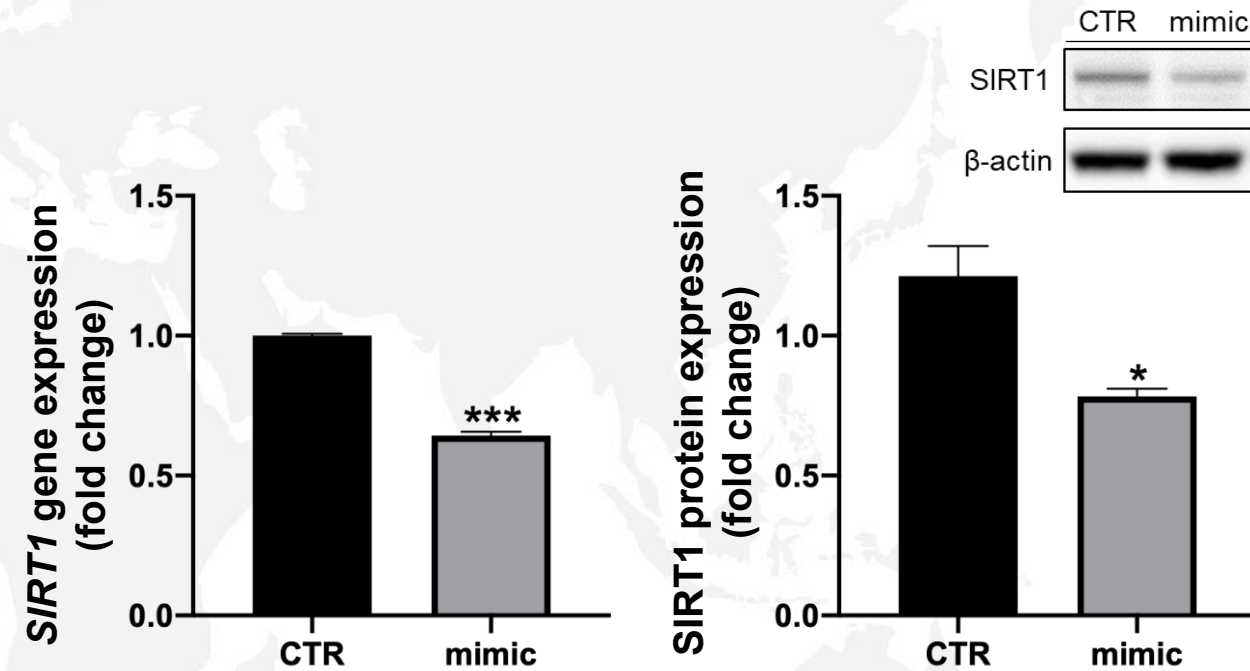
- Confirmation of SIRT1 expression
- Cell growth
→ Cell count
- SA- β -gal staining
- Confirmation of senescence marker gene expression

miR-570-3p inhibits SIRT1 expression in skin cells

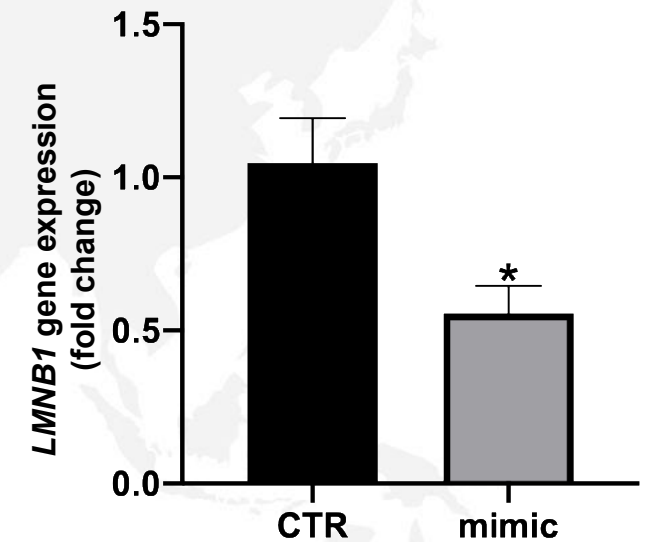
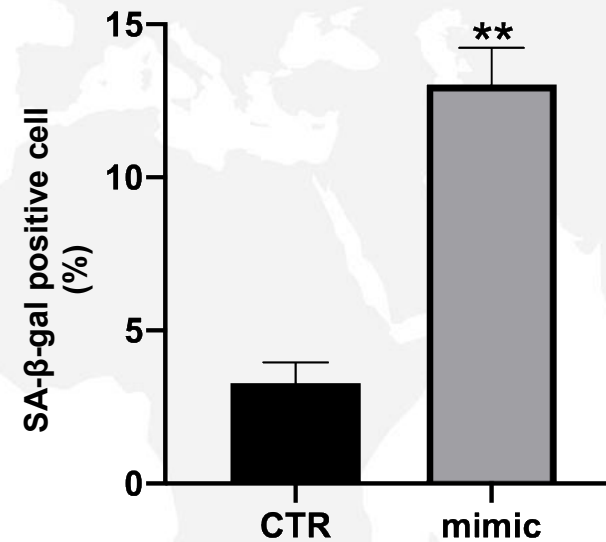
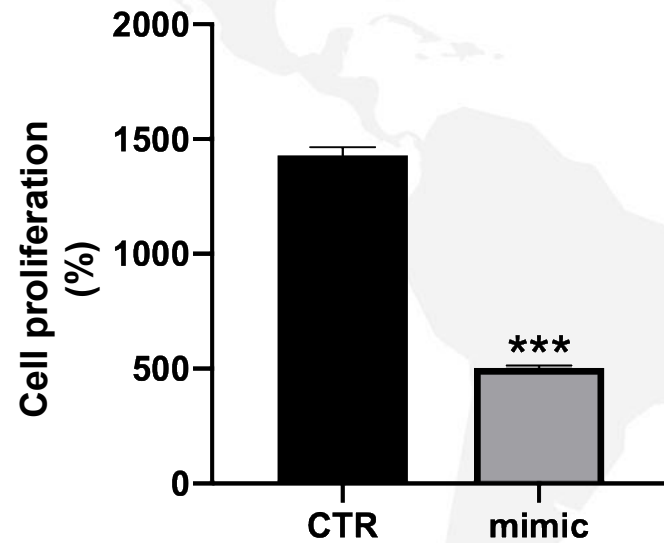
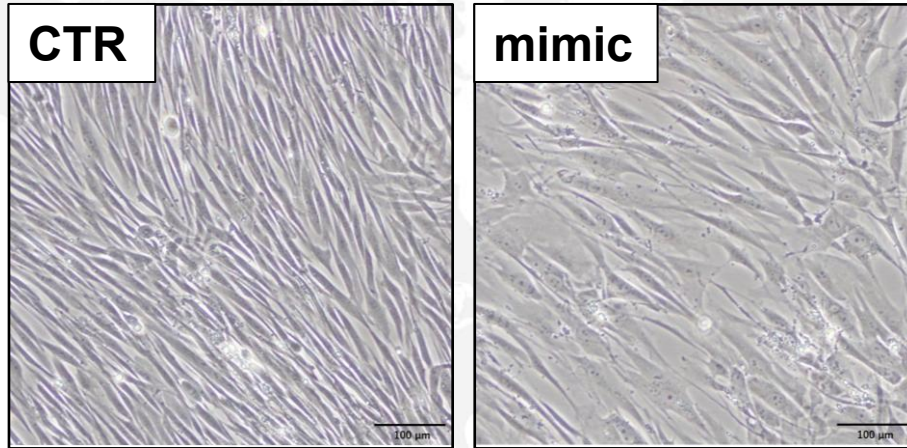
Dermal fibroblasts



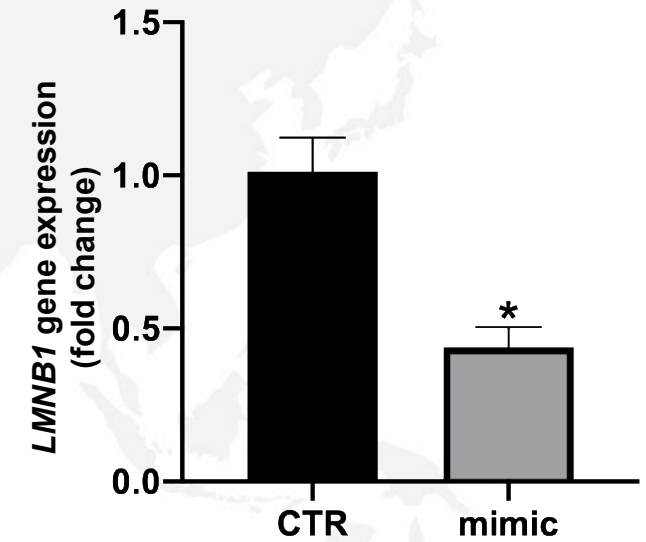
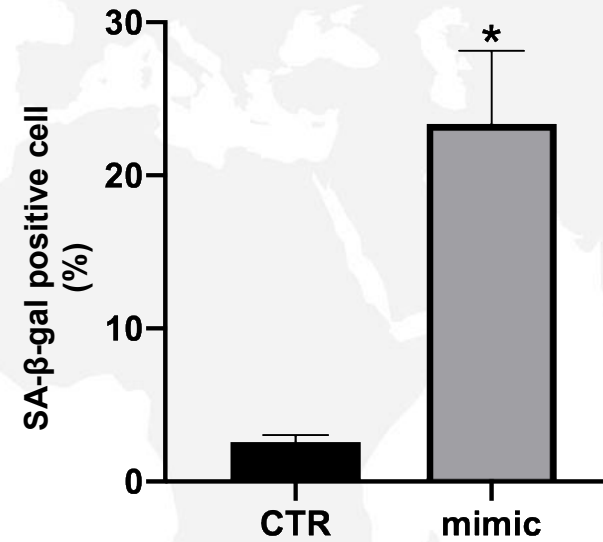
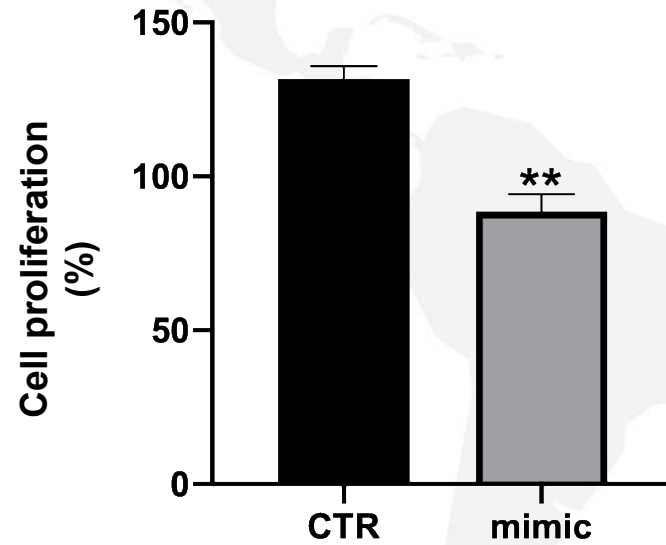
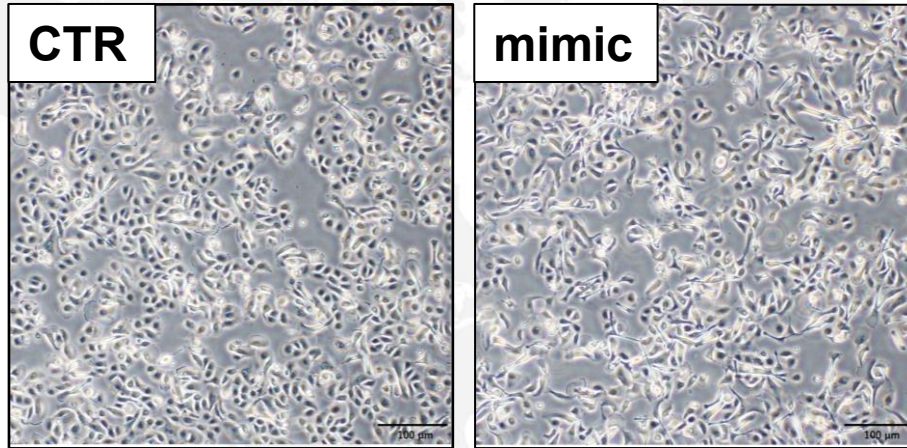
Epidermal keratinocytes



miR-570-3p induces cellular senescence in dermal fibroblasts

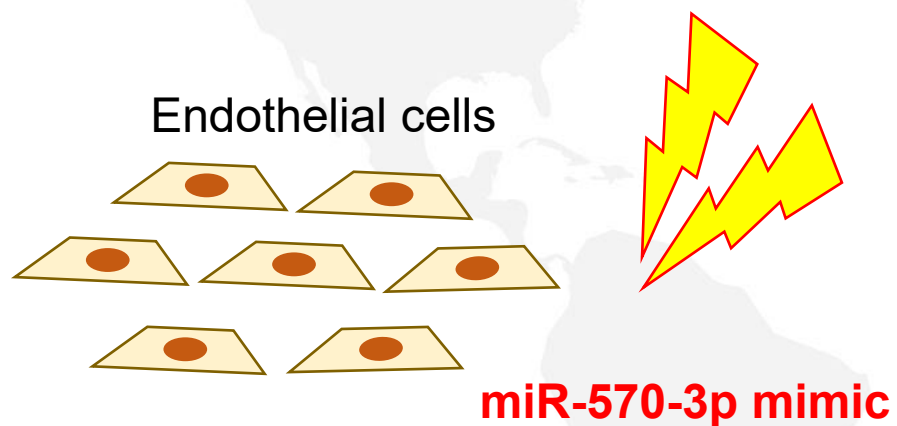
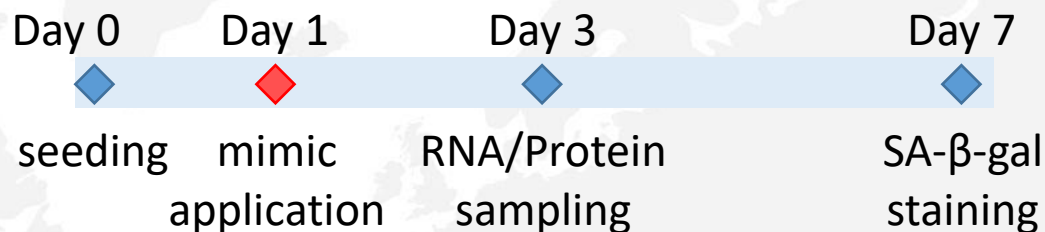


miR-570-3p induces cellular senescence in epidermal keratinocytes



Experimental design

Objective: To investigate the function of miR-570-3p in endothelial cells.

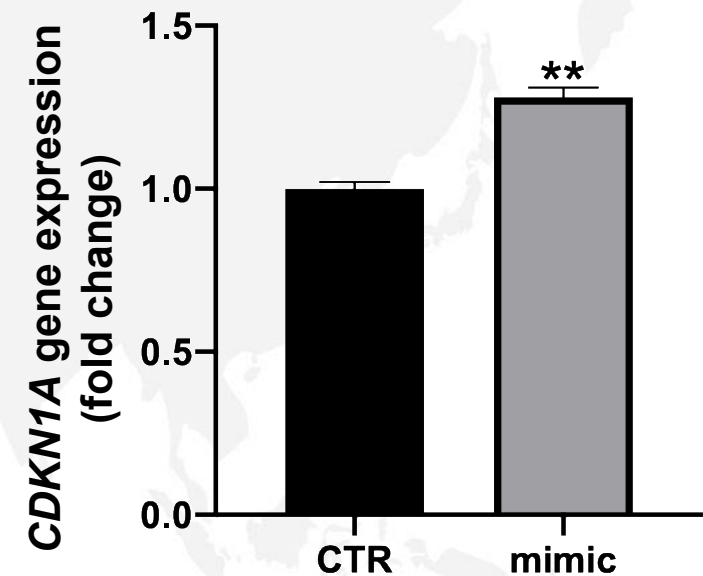
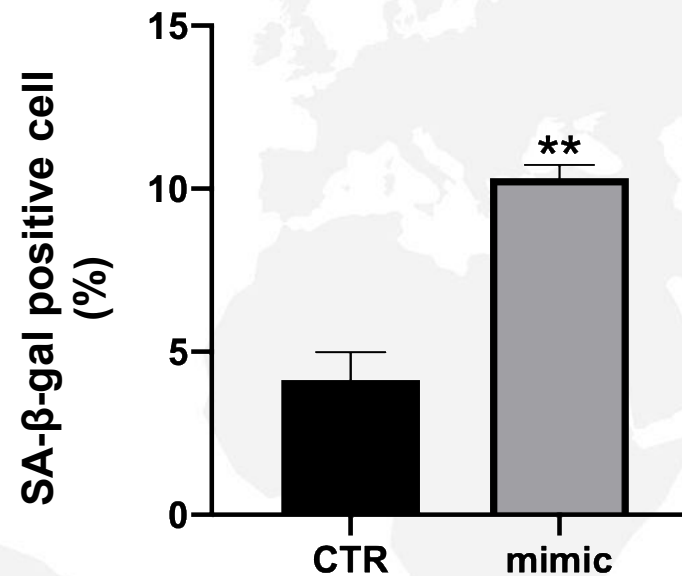
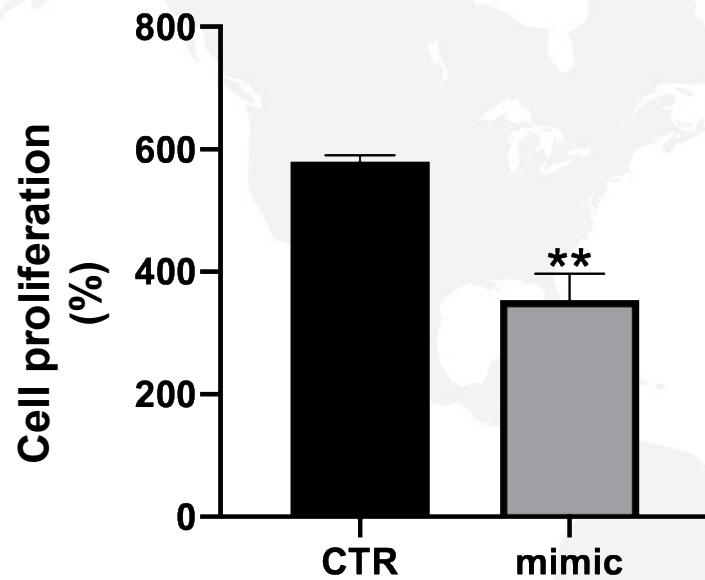


Do the cells get aged?

<Analysis>

- Cell growth
→ Cell count
- SA-β-gal staining
- Confirmation of senescence marker gene expression

miR-570-3p induces cellular senescence in endothelial cells

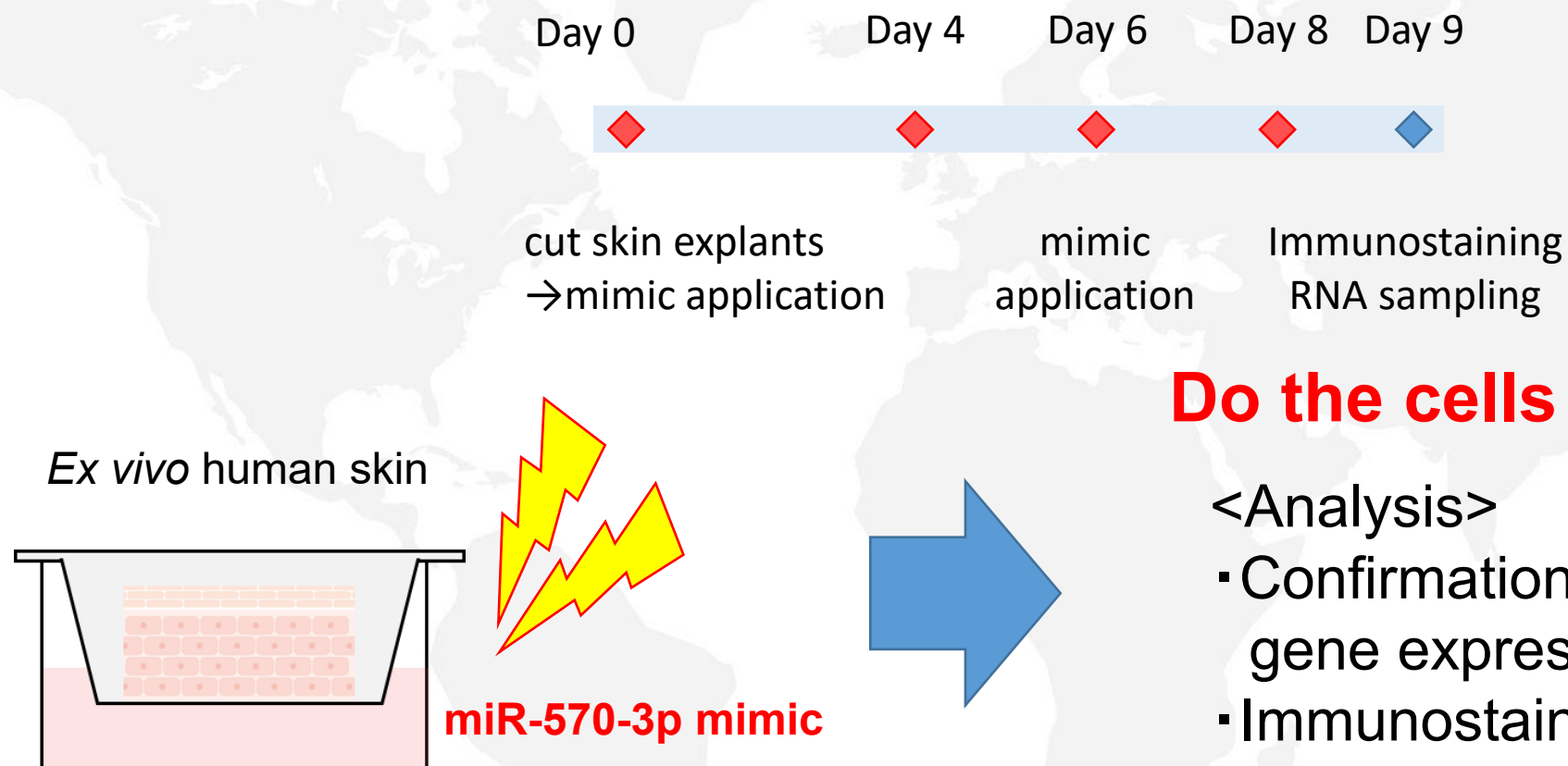


Research design

1. EVs purification and microarray of EVs-derived miRNA
2. Functional analysis of miR-570-3p in cells
3. *Ex vivo* culture of excised human skin with miR-570-3p
4. Analysis of cosmetic agents modulating miR-570-3p

Experimental design

Objective: To investigate the function of miR-570-3p in *ex vivo* human skin.

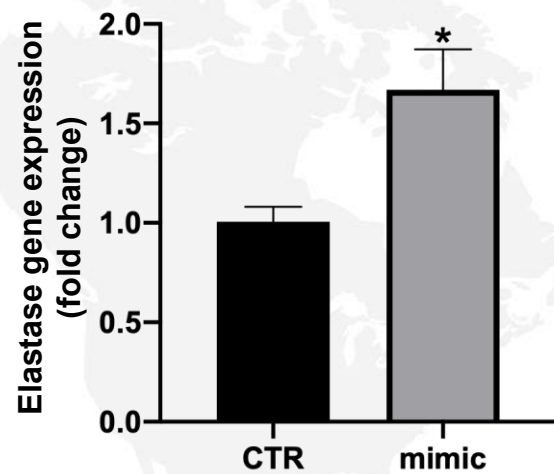


Do the cells get aged?

<Analysis>

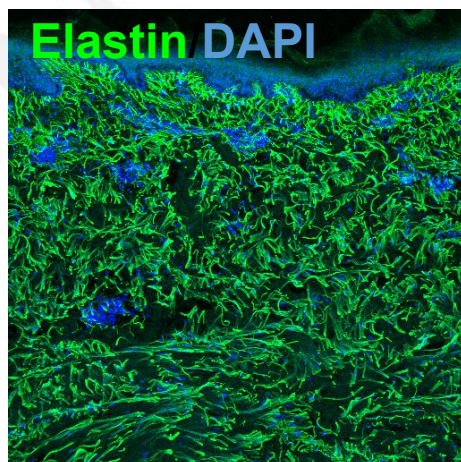
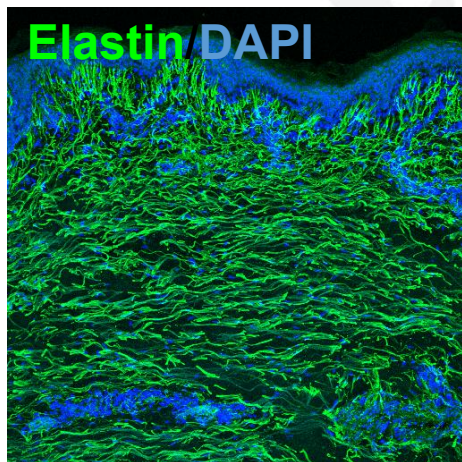
- Confirmation of *Elastase* gene expression
- Immunostaining for elastin

miR-570-3p induces fragmentation of elastin in *ex vivo* human skin



CTR 100 nM

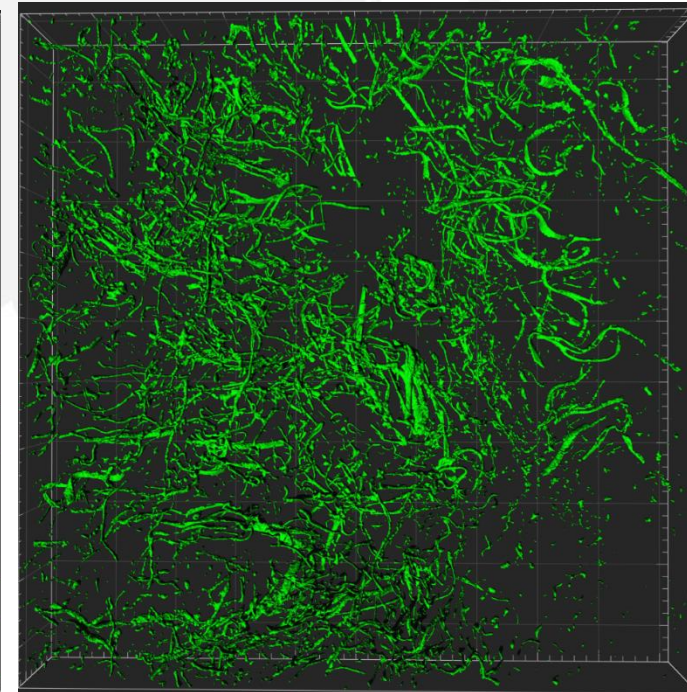
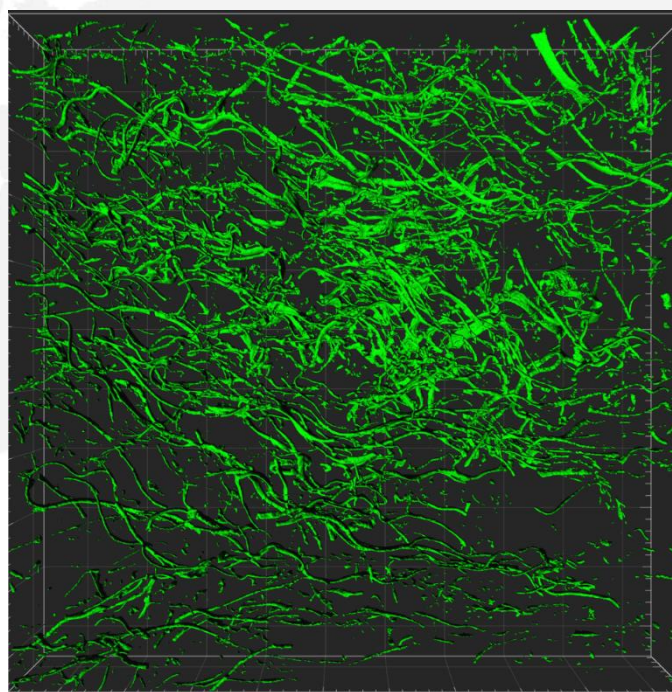
mimic 100 nM



3D rendering of elastin staining

CTR 100 nM

mimic 100 nM



Research design

1. EVs purification and microarray of EVs-derived miRNA
2. Functional analysis of miR-570-3p in cells
3. *Ex vivo* culture of excised human skin with miR-570-3p
4. Analysis of cosmetic agents modulating miR-570-3p

Experimental design

Objective: Identification of miR-570-3p regulator from Cosmetic Agents.

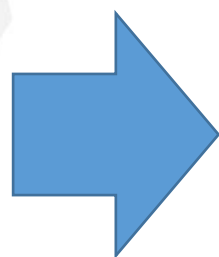
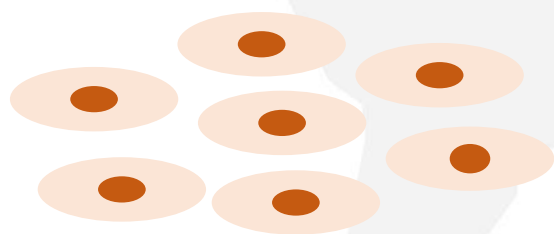


Rosa multiflora fruit extract

- Contains flavonoids
- Antioxidant activity
- Anti-inflammatory activity

It has been reported that flavonoids alter miR-570-3p expression.
→ We focused on this extract in this study.

Dermal fibroblasts



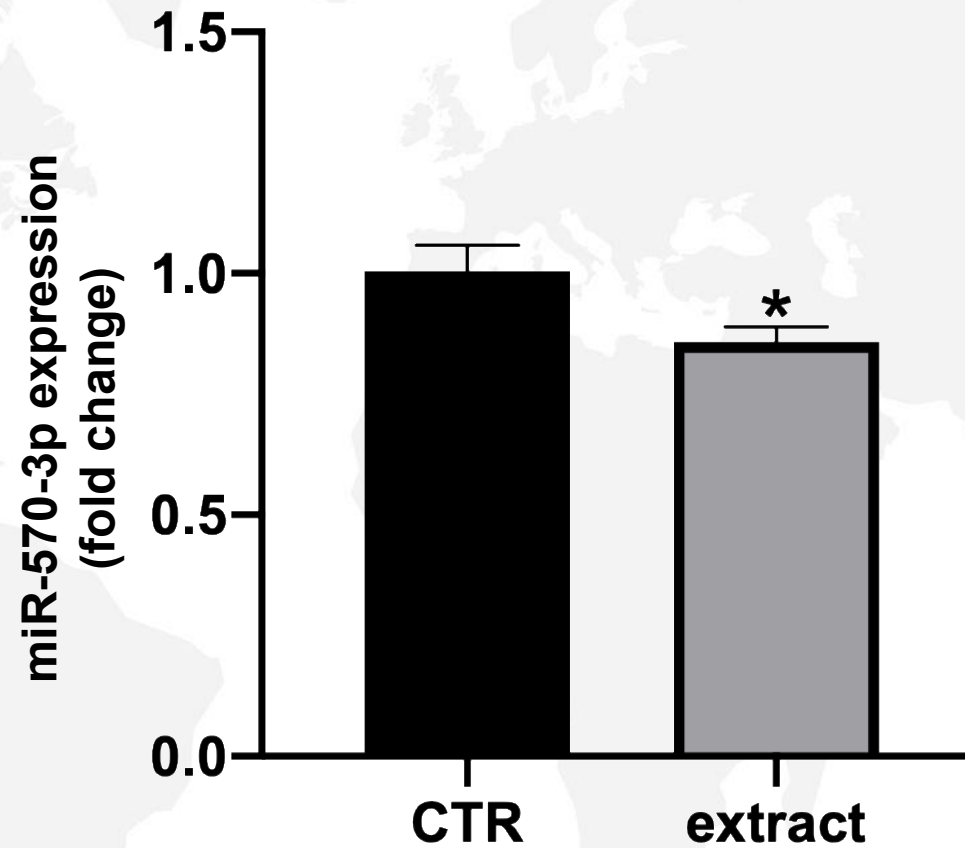
Rosa multiflora fruit extract

Does miR-570-3p decrease?

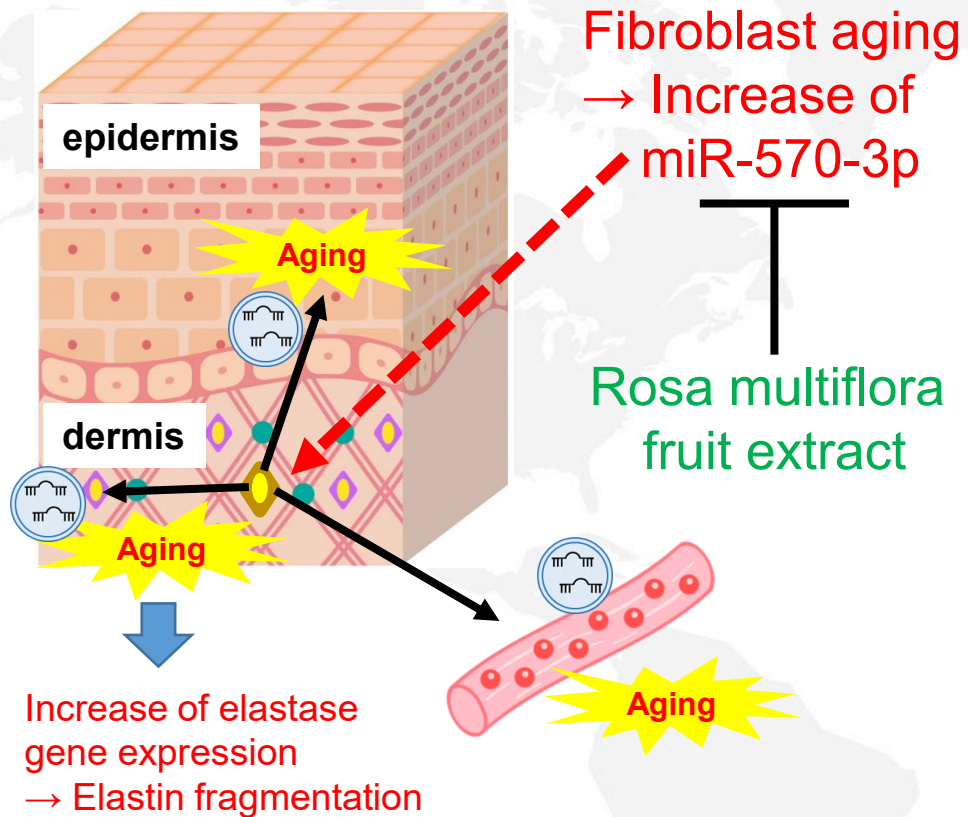
<Analysis>

- Confirmation of miR-570-3p expression

Rosa multiflora fruit extract reduces miR-570-3p expression



Conclusion



- miR-570-3p in EV increases with cell aging.
- miR-570-3p induces cellular senescence in fibroblasts, keratinocytes, vascular endothelial cells.
- In ex-vivo skin culture, miR-570-3p induces elastin fragmentation with an increase in elastase expression.
- Rosa multiflora fruit extract reduces the expression of miR-570-3p in fibroblasts.

Cosmetics that suppress miR-570-3p offer an innovative approach to interrupt the senescence chain and promote skin longevity.

Acknowledgment

I would like to express my sincere gratitude to all co-authors for their support and contributions.



Thank you !