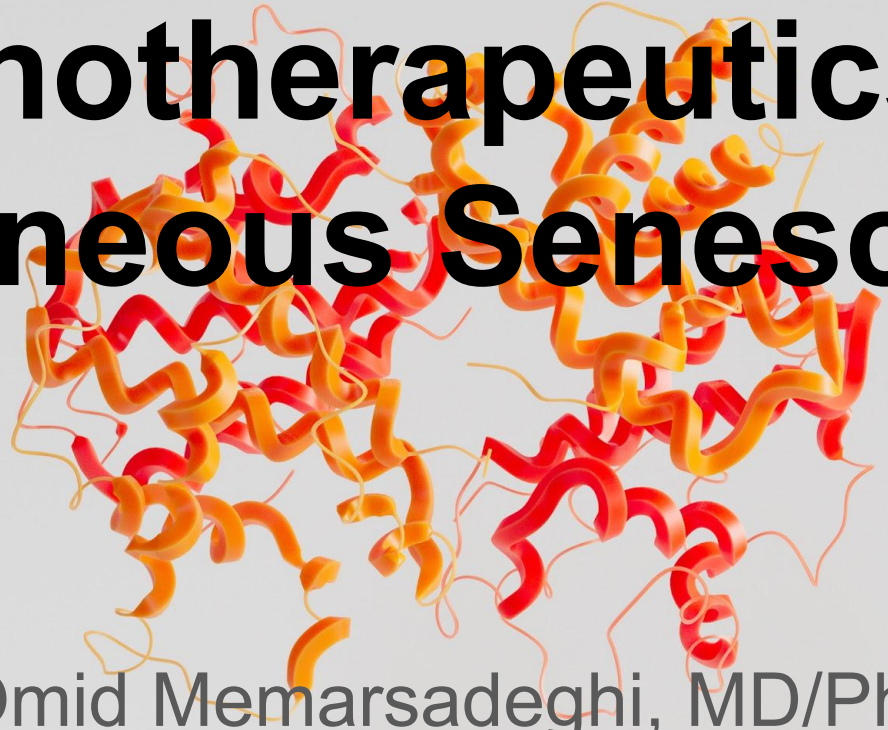
بسم الله الرحمن الرحیم

Senotherapeutics in Cutaneous Senescence



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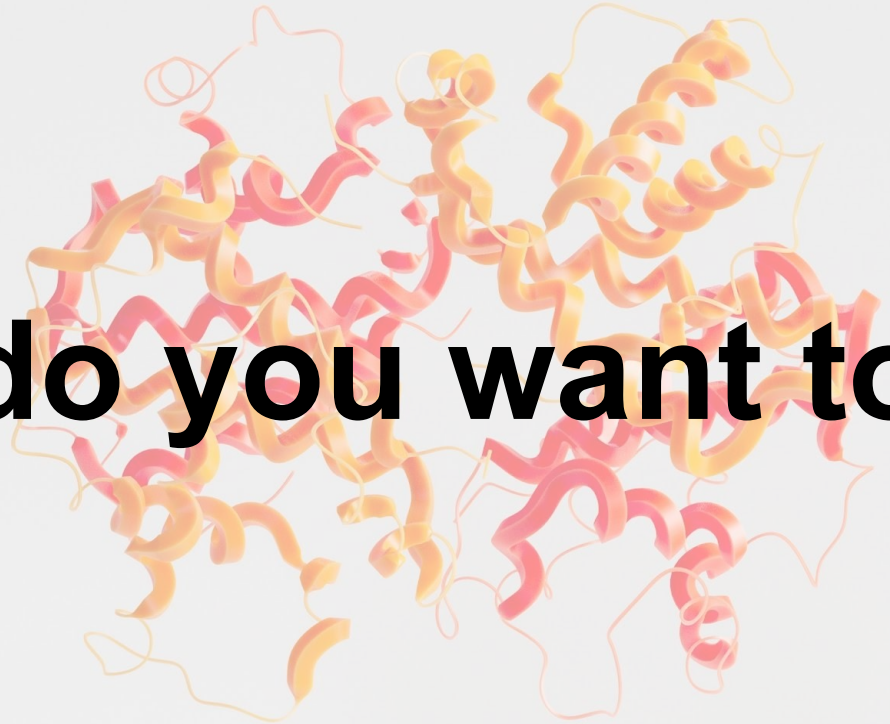


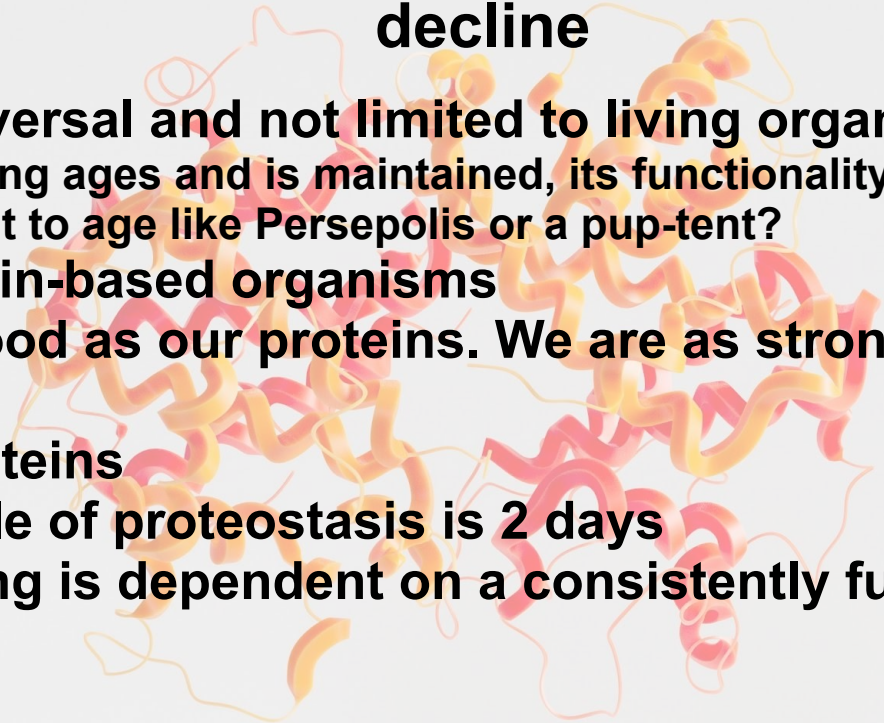
How do you want to age?

2500 YEARS OLD

25 YEARS OLD

Aging: Aging is characterized by a gradual functional decline

- Aging is universal and not limited to living organisms
  - As a building ages and is maintained, its functionality changes
  - Do we want to age like Persepolis or a pup-tent?
- We are protein-based organisms
- We are as good as our proteins. We are as strong as our weakest proteins.
- ~100,000 proteins
- Average cycle of proteostasis is 2 days
- Staying young is dependent on a consistently functional proteome



I'm a Dermatologist and Aging to me is…

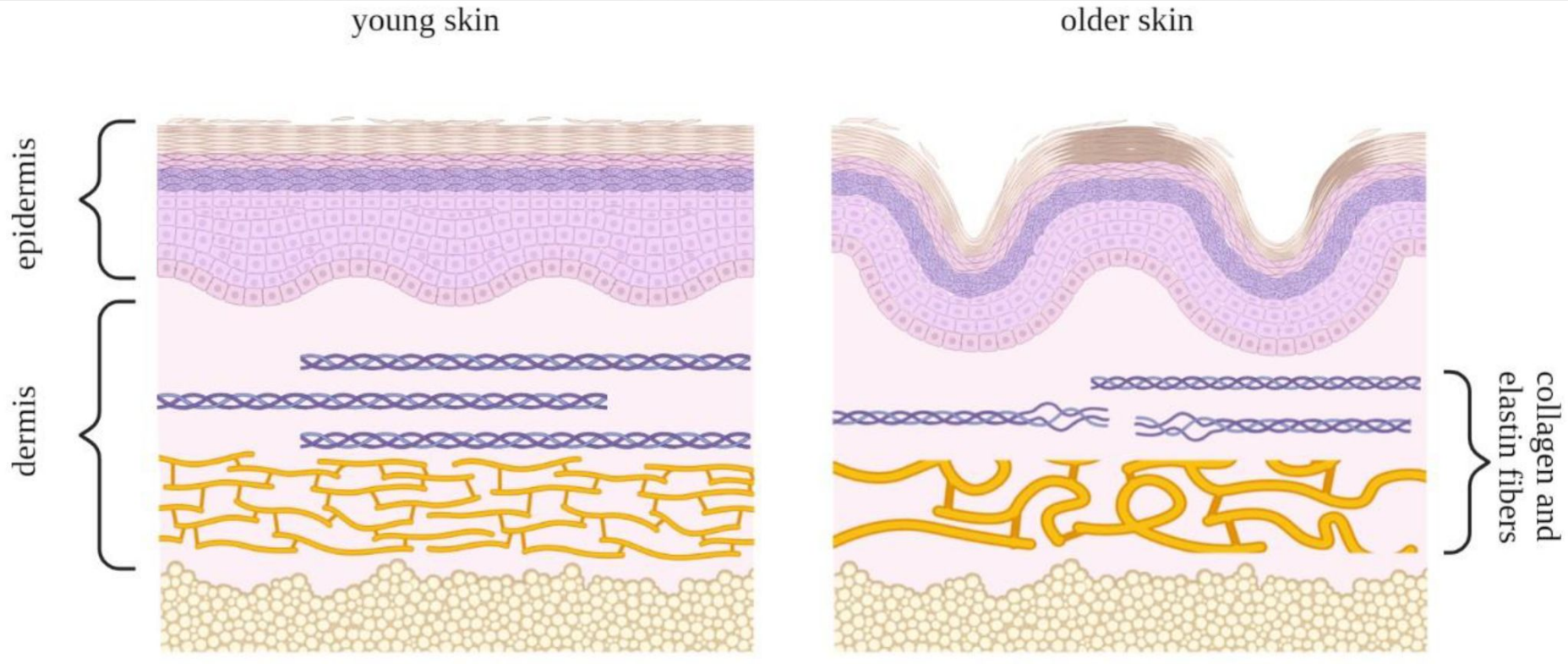
Wrinkling, melasma, erroneous wound healing processes, graying, and partial or total hair loss

The skin proteome also changes.

Vital functions such as thermoregulation, immunological and nervous skin responses, and cutaneous vascular responses are disrupted.

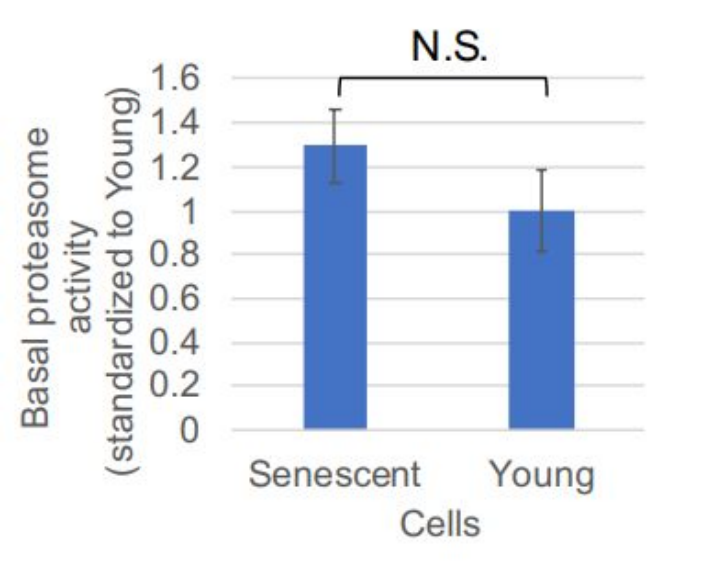
A kind of hypoproliferation vs hyperproliferation occurs in terms of pigment/pigmentary cells (solar lentigo, dyschromia, decreased melanocytes in sun-protected areas), appendages (sebaceous hyperplasia), epithelial cells (seborrheic keratoses, hairloss), even skin tags, fat pad redistribution, submental fat….

But There is More To It…



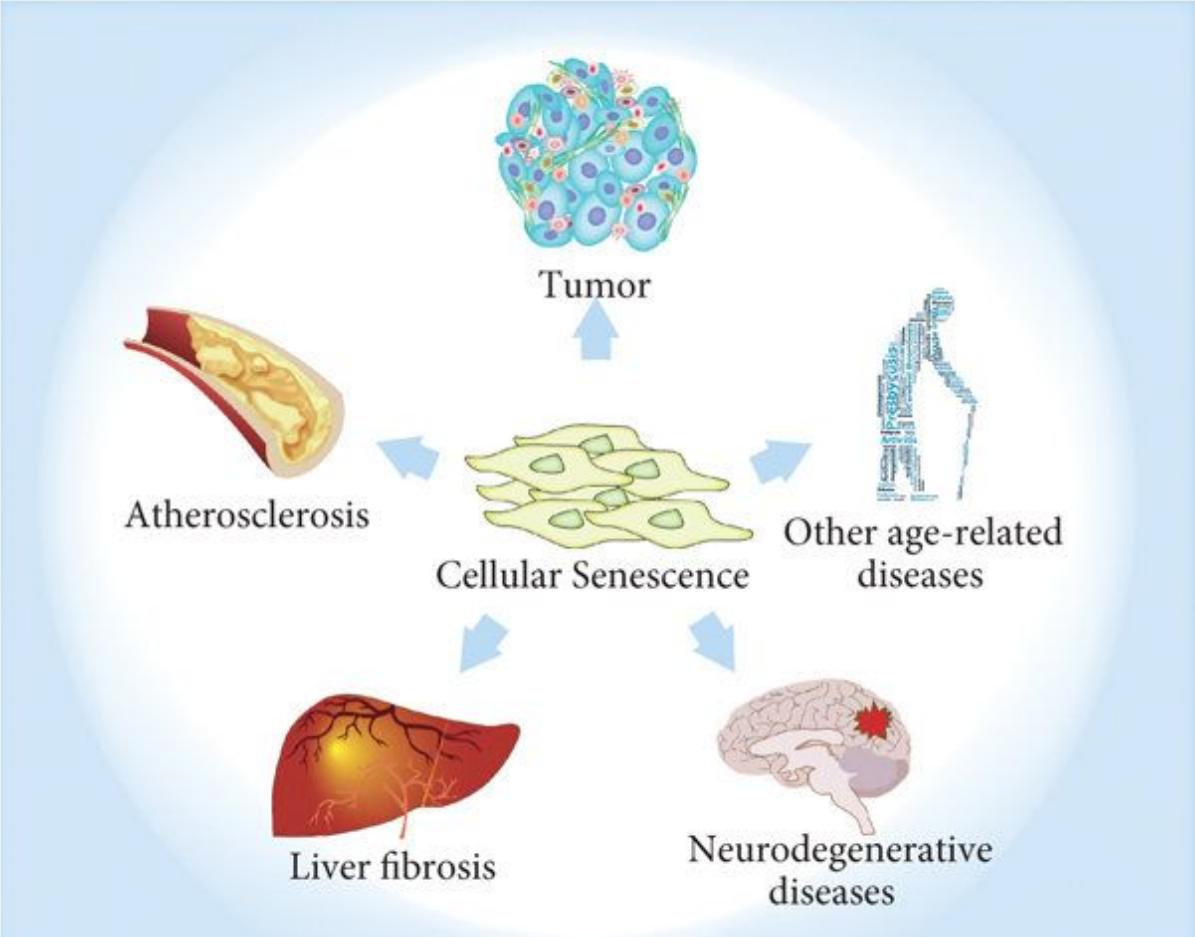
Both intrinsic and extrinsic elements determine the progression of a cellular and proteome change in skin aging more so than any other organ. Therefore, skin is an ideal model for aging.

- The majority of dysfunctional proteins come from cells
- One cell, The Senescent Cell, is especially involved in inducing dysfunction

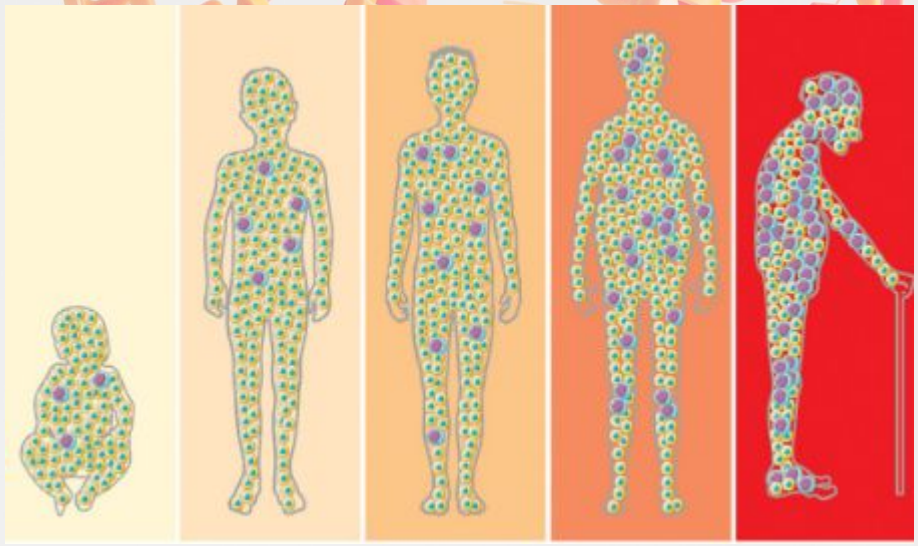


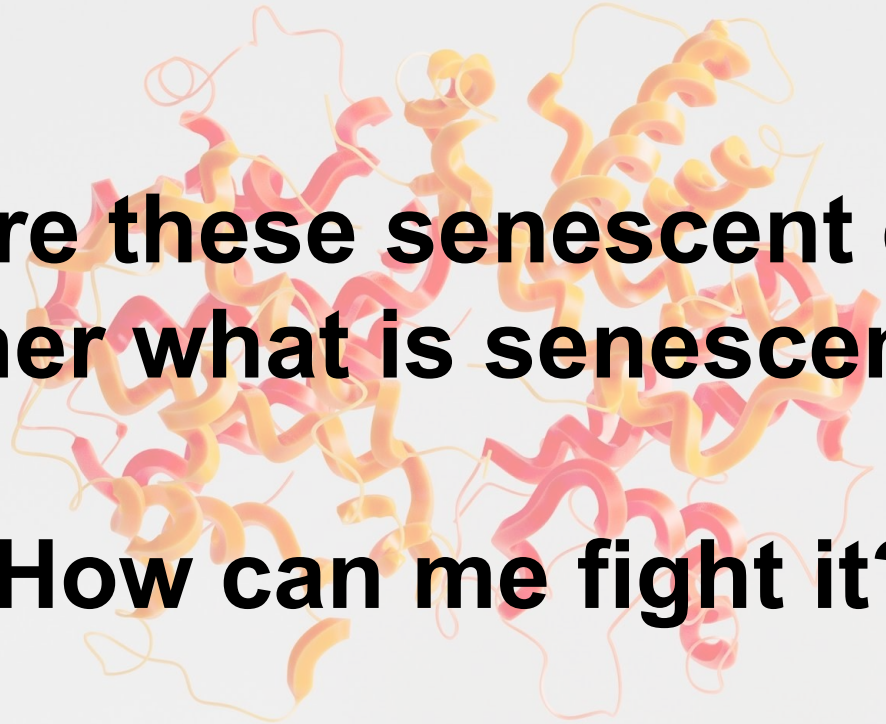
Sabath, N. et al. Cellular proteostasis decline in human senescence. *Proc. Natl Acad. Sci. USA* 30, 202018138 (2020).

Involvement of senescence in disease



- We are a mosaic of cells and some are senescent and some are not.
- With Aging or disease, the number of senescent cells increase





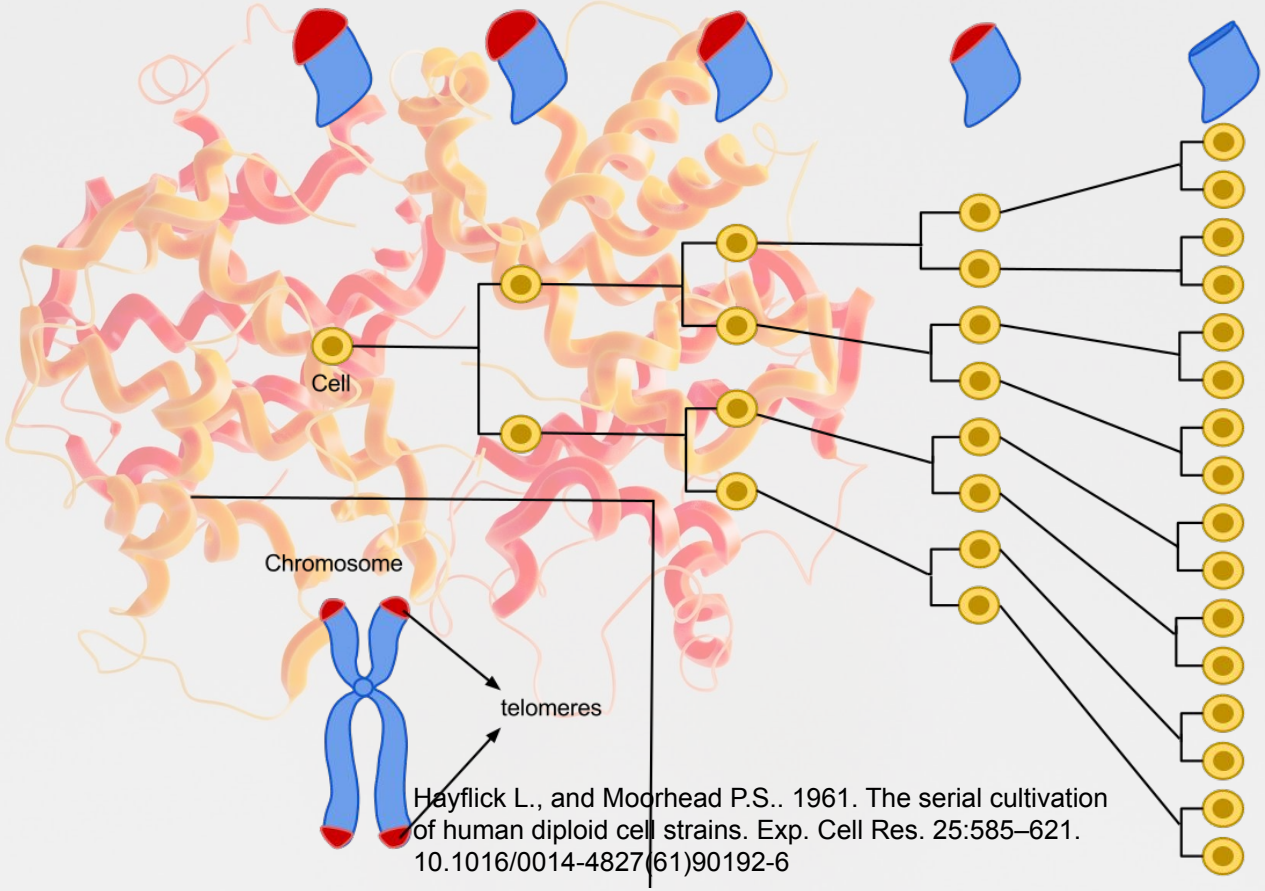
What are these senescent cells, or rather what is senescence?

How can me fight it?

Cells that Stop Dividing

1961: The Hayflick limit deliberates that the average cell will divide around 50 times before reaching a stage known as senescence.

This is referred to as cellular senescence

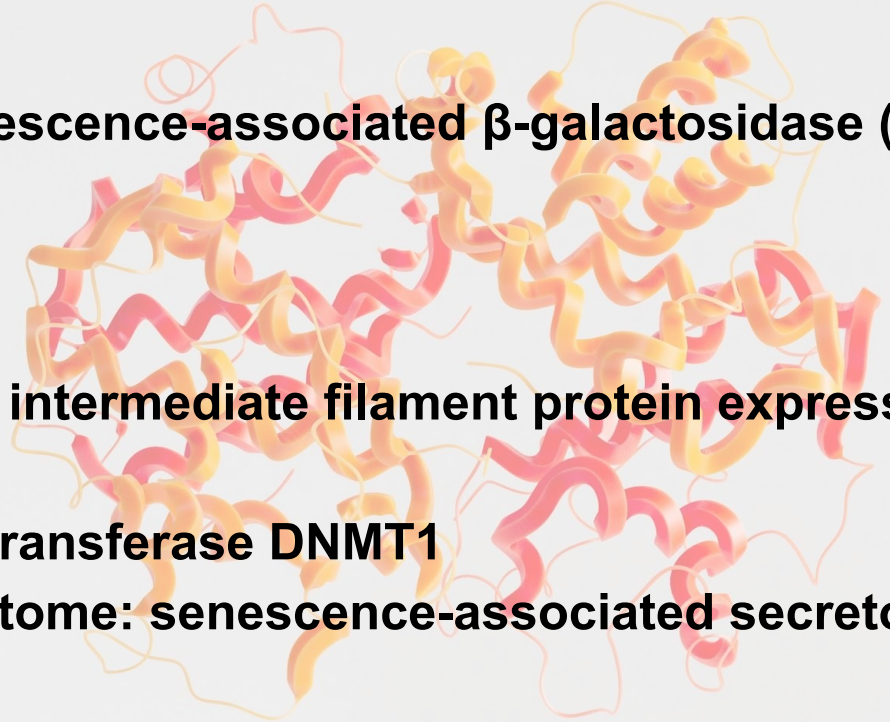


Hayflick L., and Moorhead P.S.. 1961. The serial cultivation of human diploid cell strains. Exp. Cell Res. 25:585–621. 10.1016/0014-4827(61)90192-6

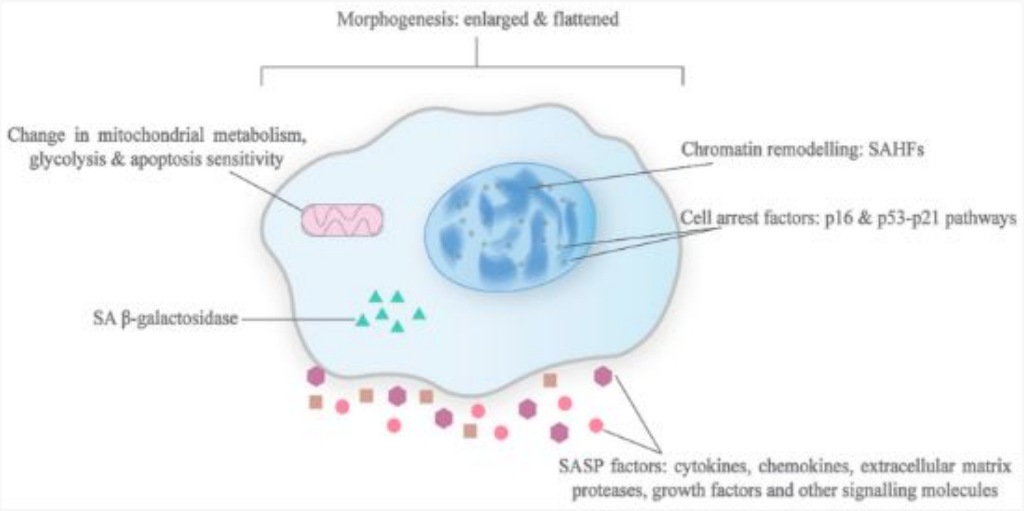
Senescence is….

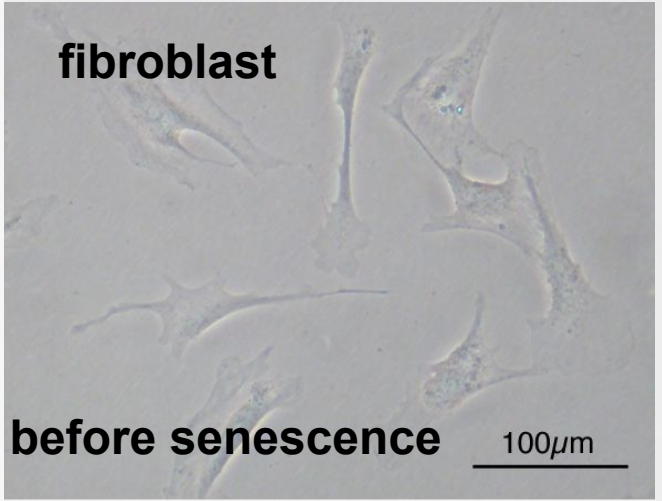
- Physiologic senescence vs pathologic senescence
- A response to stress signals.
  - genomic instability and telomere attrition
  - Inflammation
  - Environmental stress like UV or gamma irradiation
  - Carcinogenesis
- It is needed for tissue homeostasis and wound healing
- Distinct phenotype: larger cytology, chromatin remodeling, metabolic reprogramming, dysregulated autophagy, and the implementation of a complex secretome
- Resistant to apoptosis: stay alive through pro-survival/anti-apoptotic pathways
- The growth arrest is implemented by the activation of p16INK4a/Rb and p53/p21CIP1 tumor suppressor networks: Hence biomarkers of senescence.
- Secrete a specific secretome: Senescence Associated Secretory Phenotype (SASP)
- **“Zombie cells”**

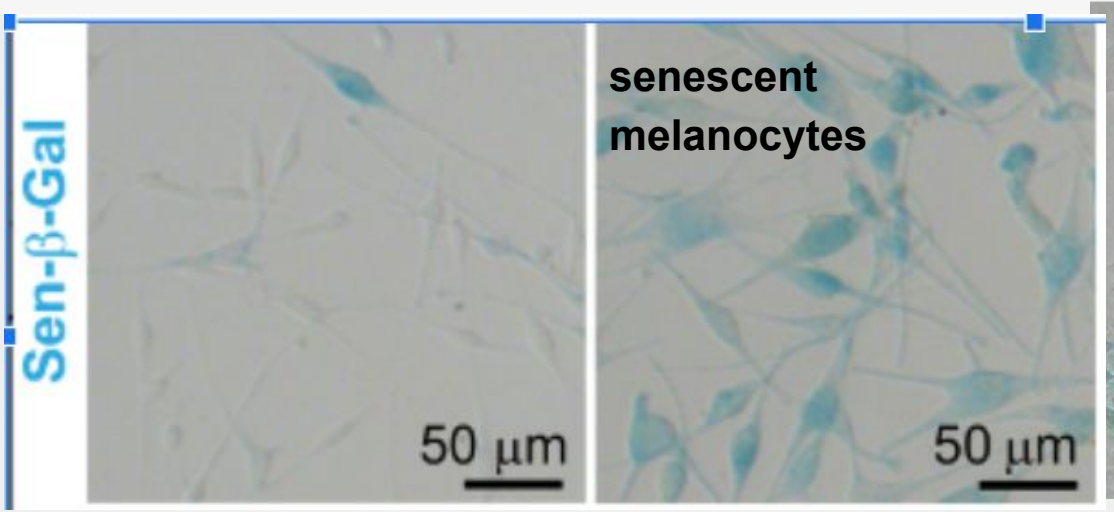
Biomarkers of Senescence

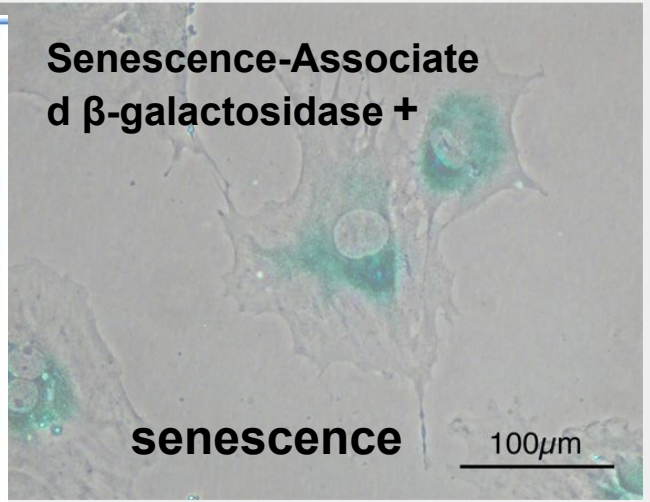


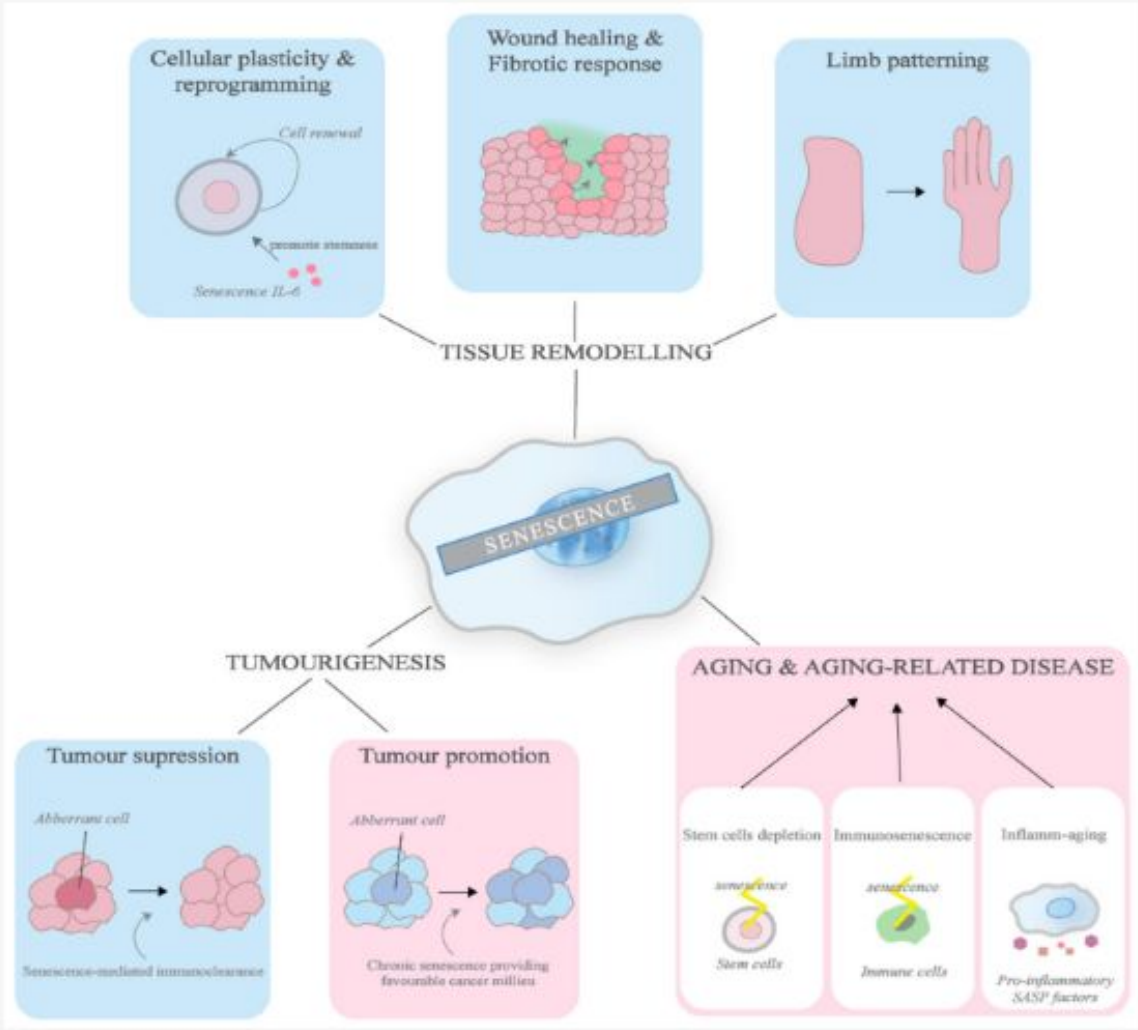
- Elevated senescence-associated β-galactosidase (SA-β-gal) activity
- ⇧ p16INK4a
- ⇧ p21ClP1
- ⇧ P53
- ⇩ Lamin B1, an intermediate filament protein expressed in all somatic cells
- ⇩ DNA methyl-transferase DNMT1
- Unique secretome: senescence-associated secretory phenotype (SASP)

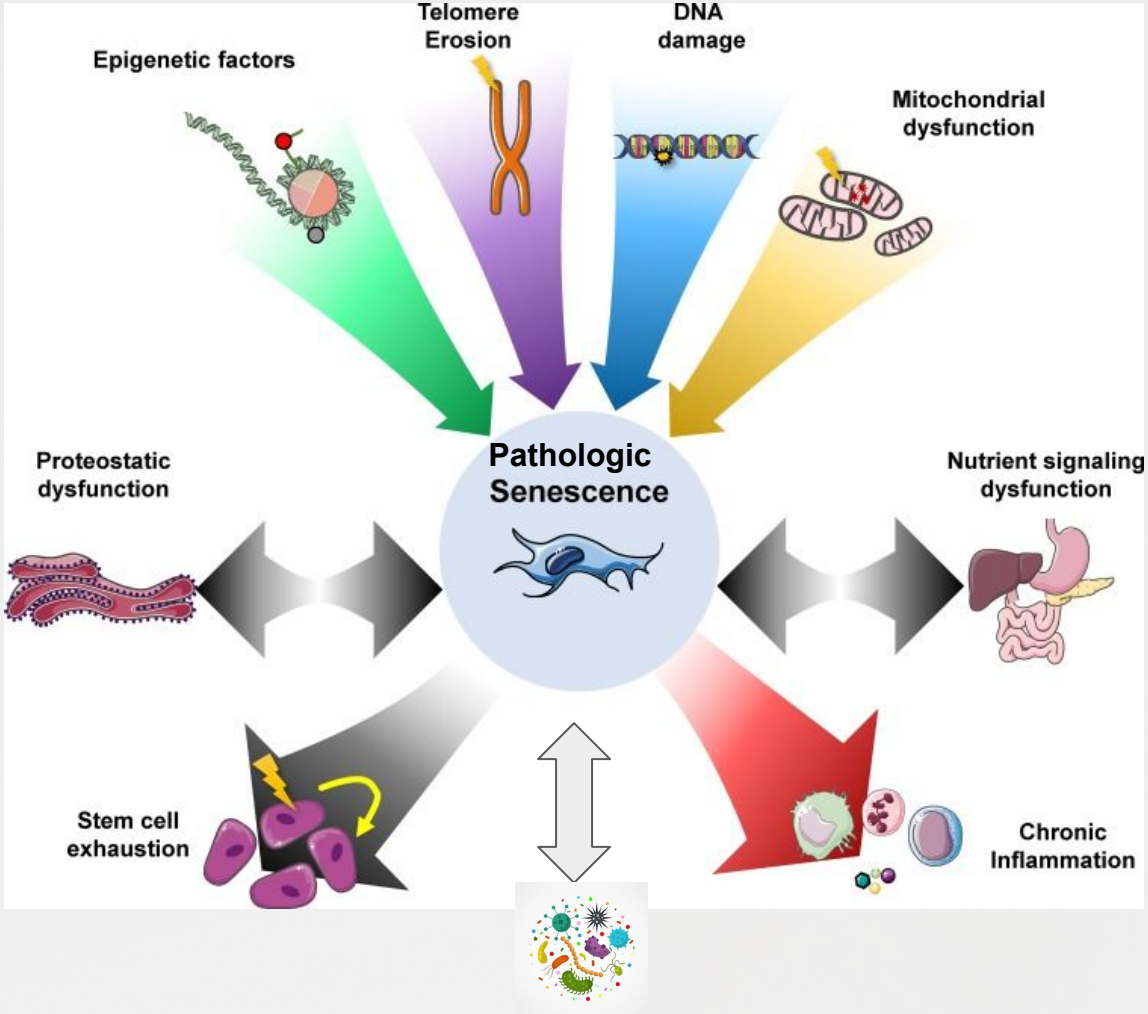

Morphogenesis: enlarged & flattened
Change in mitochondrial metabolism, glycolysis & apoptosis sensitivity
Chromatin remodelling: SAHFs
Cell arrest factors: p16 & p53-p21 pathways
SA β-galactosidase
SASP factors: cytokines, chemokines, extracellular matrix proteases, growth factors and other signalling molecules


fibroblast
before senescence
100μm

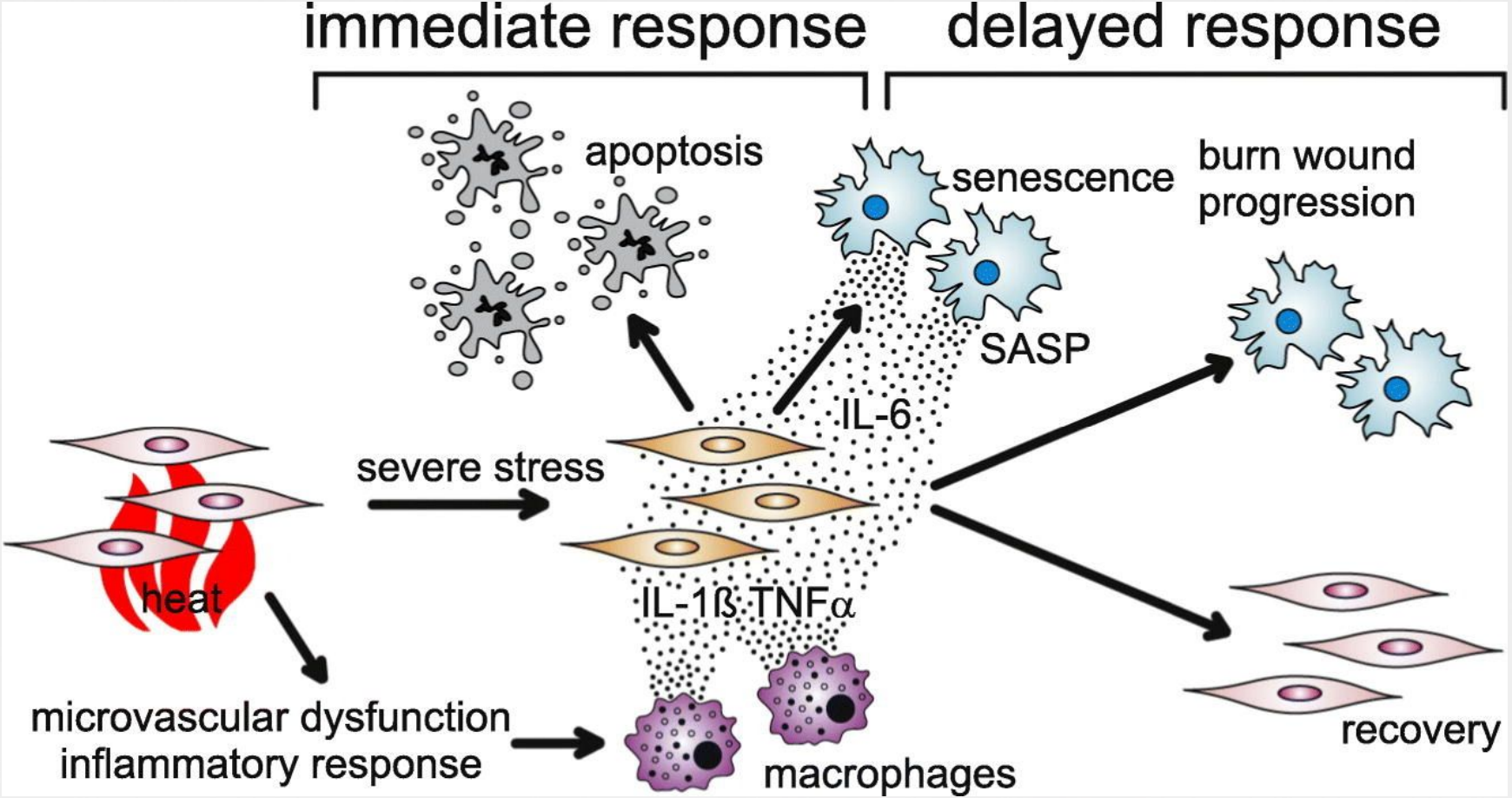

Sen-β-Gal
50 μm
senescent melanocytes
50 μm


Senescence-Associated β-galactosidase +
senescence
100μm

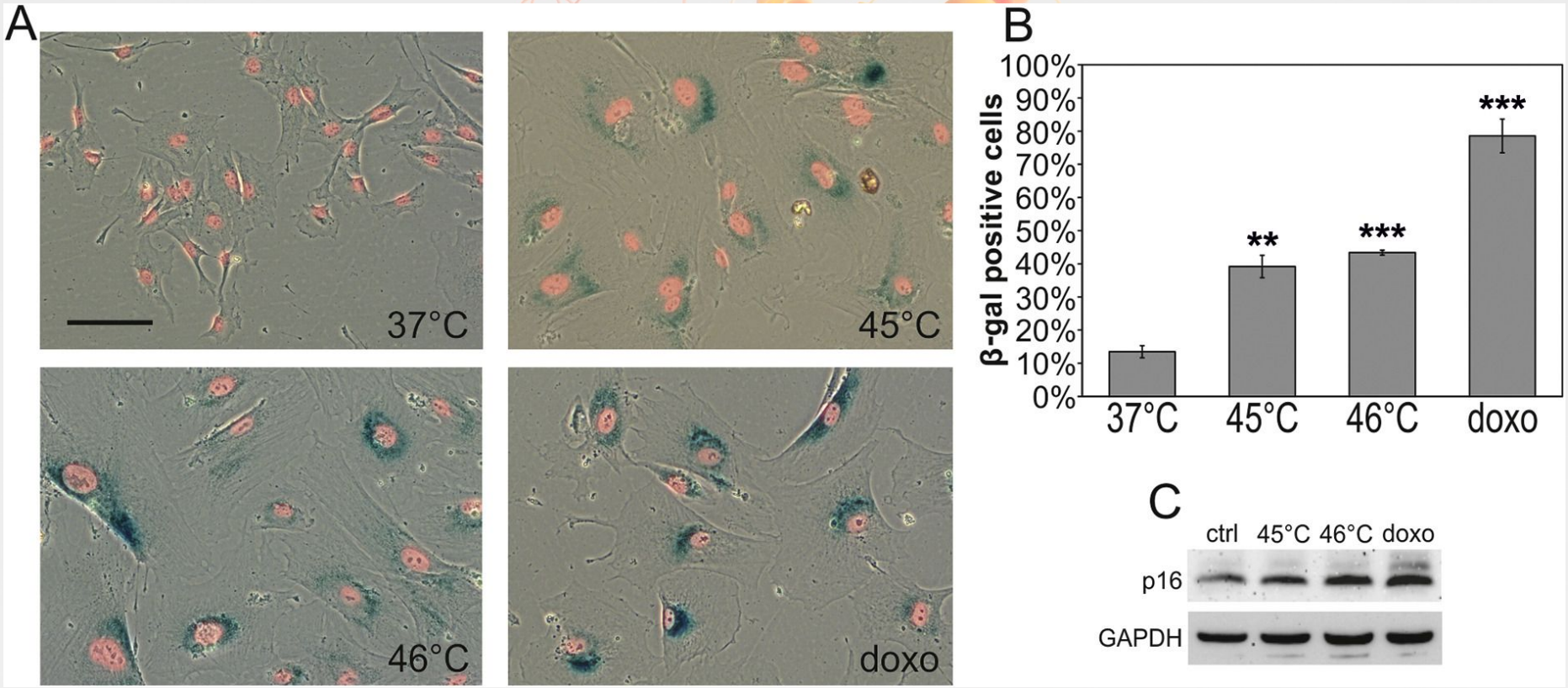

Cellular plasticity & reprogramming
Cell renewal
promote stemness
Senescence IL-6
Wound healing & Fibrotic response
Limb patterning
TISSUE REMODELLING
SENESCENCE
TUMOURIGENESIS
Tumour supression
Abberrant cell
Senescence-mediated immunoclearance
Tumour promotion
Abberrant cell
Chronic senescence providing favourable cancer milieu
AGING & AGING-RELATED DISEASE
Stem cells depletion
senescence
Stem cells
Immunosenescence
senescence
Immune cells
Inflamm-aging
Pro-inflammatory SASP factors


Telomere Erosion
DNA damage
Epigenetic factors
Mitochondrial dysfunction
Proteostatic dysfunction
Pathologic Senescence
Nutrient signaling dysfunction
Stem cell exhaustion
Chronic Inflammation
Microbiome

Heat-Induced Senescence



More Heat….More Senescence



Lasers can Cause Senescence ….Potential Public Health Catastrophe

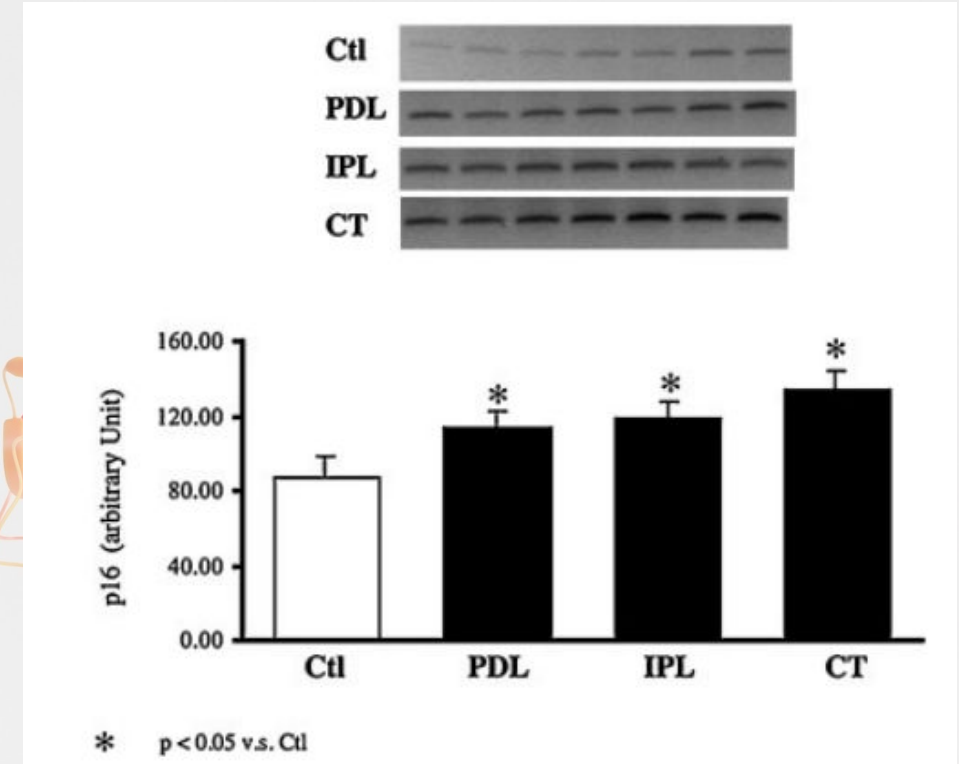
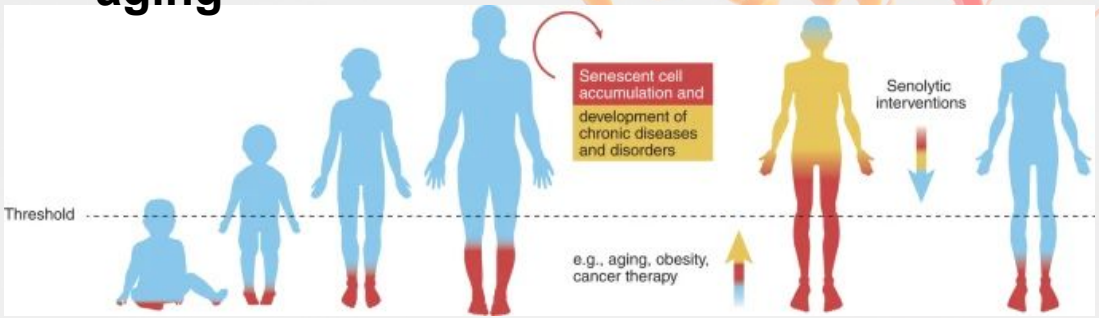


Fig. 6. Immunoblotting analysis of p16 expression in mouse skin.

Chan, H. H. L., Yang, C. H., Leung, J. C. K., Wei, W. I., & Lai, K. N. (2007). *An animal study of the effects on p16 and PCNA expression of repeated treatment with high-energy laser and intense pulsed light exposure. Lasers in Surgery and Medicine, 39(1), 8-13.* doi:10.1002/lsm.20447

Persistent Senescence: SASP and Disease

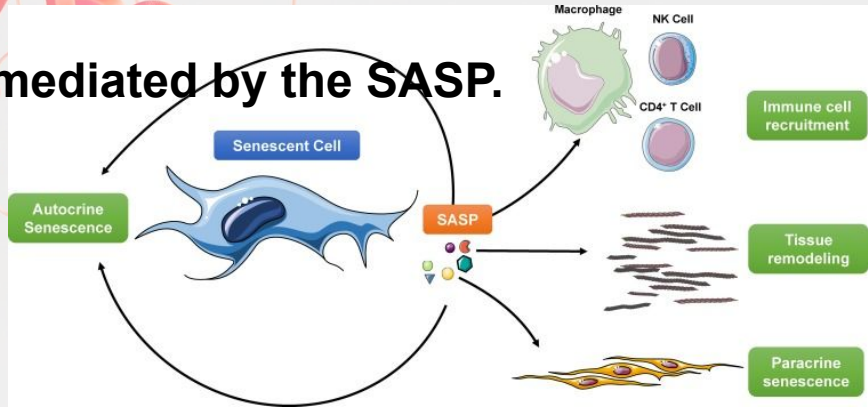
- **Serum from old mice induce senescence and disease in young syngeneic mice**
- **Destruction of senescent cells in old mice abrogates this ability to induce senescence and disease in young mice**
- **Old mice given young mice blood or saline + albumin (plasma dilution) had fewer hallmarks of aging and diseases of aging**
- **It is strongly assumed that SASP is a great contributor to the disease of aging**



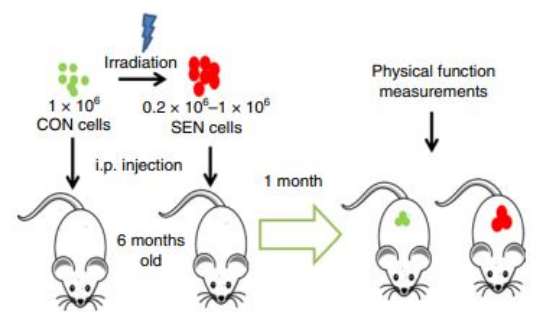
Jeon, O.H., Mehdipour, M., Gil, TH. et al. Systemic induction of senescence in young mice after single heterochronic blood exchange. Nat Metab 4, 995–1006 (2022). https://doi.org/10.1038/s42255-022-00609-6

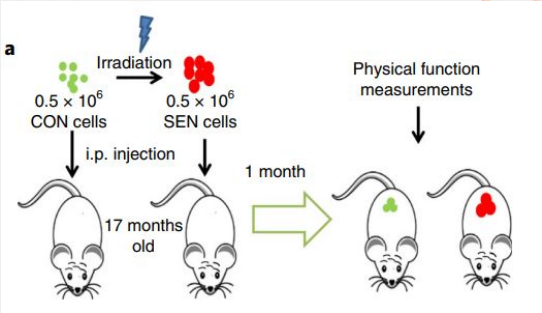
Senescence Associated Secretory Phenotype (SASP)

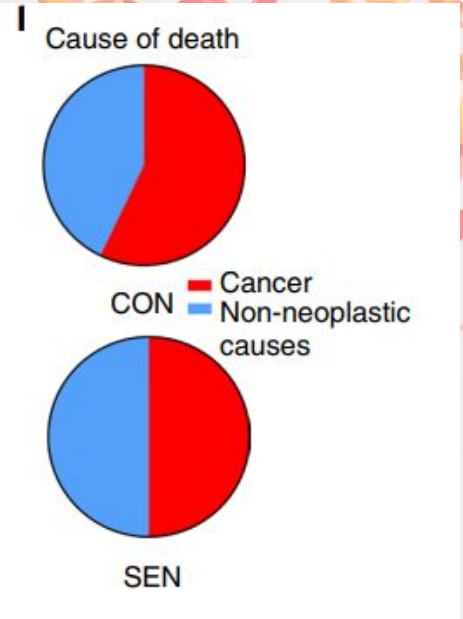
- **The specific combination of secreted factors is thought to depend on the cell type and the senescent inducer.**
- **However, many of the key effectors of the SASP and its regulatory mechanism seemed to be shared.**
- **SASP proteome is different between young and old, with young being more anti-inflammatory and old being more pro-inflammatory, proapoptotic and pro-fibrotic factors**
- **The disease of aging is thought to be mediated by the SASP.**



Transplanting senescent cells induces physical dysfunction in younger mice



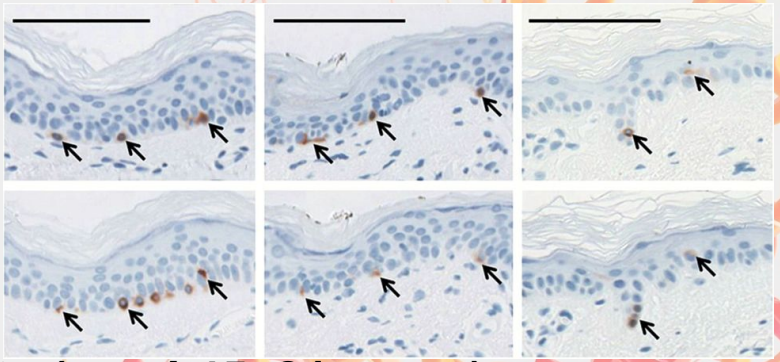




Transplanting senescent cells into middle-aged mice cause profound physical dysfunction and early death.

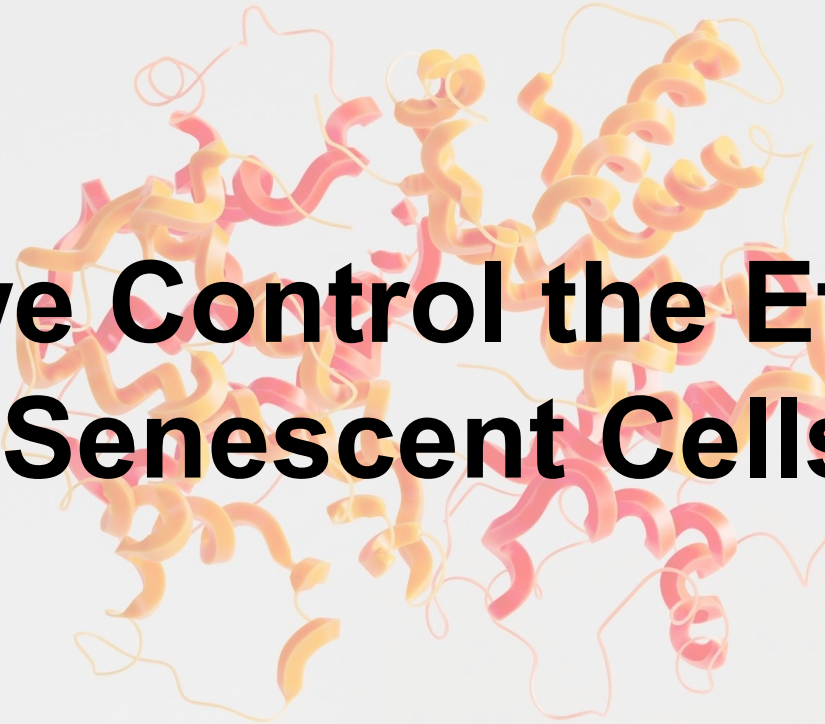
Xu, M. et al. Senolytics improve physical function and increase lifespan in old age. Nat Med 24, 1246–1256 (2018).

Looking Older than Chronological Age=More SCs and Greater Irregular Elastic Fibers

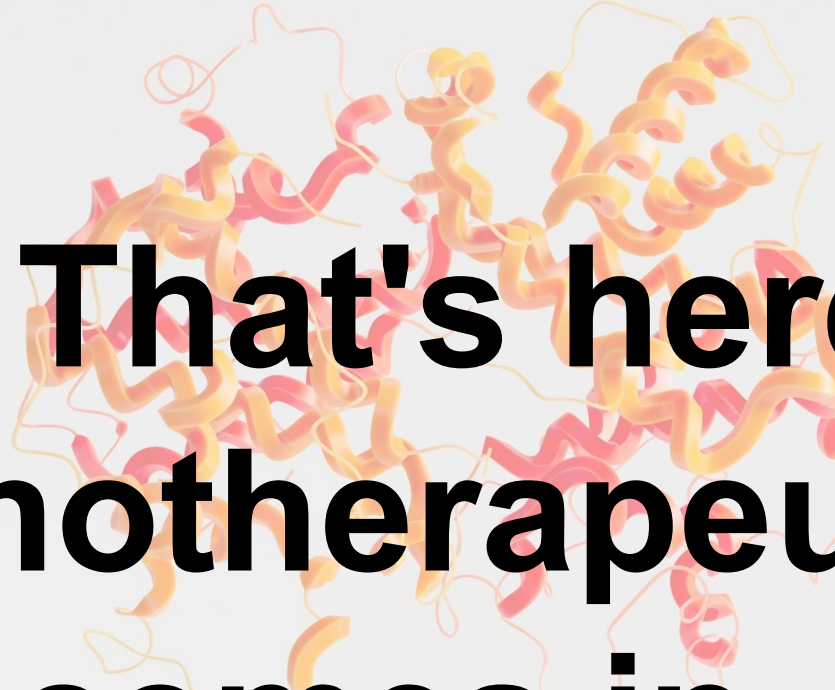


- **178 participants (aged 45–81 years)**
- **Subjective facial age and wrinkles were compared to p16INK4a counts, Local elastic fiber morphology in sun protected area**
- **p16INK4a positive cell numbers in sun-protected human arm skin are indicative of both local elastic fiber morphology and the extent of aging visible in the face.**

Mariëtte E. et al, P16INK4a Positive Cells in Human Skin Are Indicative of Local Elastic Fiber Morphology, Facial Wrinkling, and Perceived Age, The Journals of Gerontology: Series A, Volume 71, Issue 8, August 2016, Pages 1022–1028, https://doi.org/10.1093/gerona/glv114

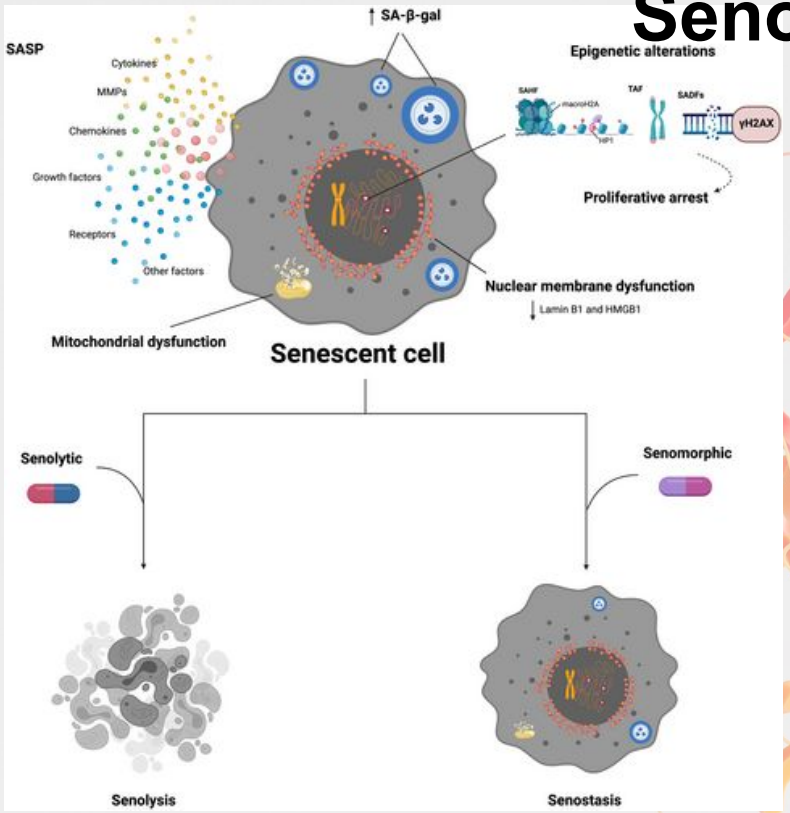


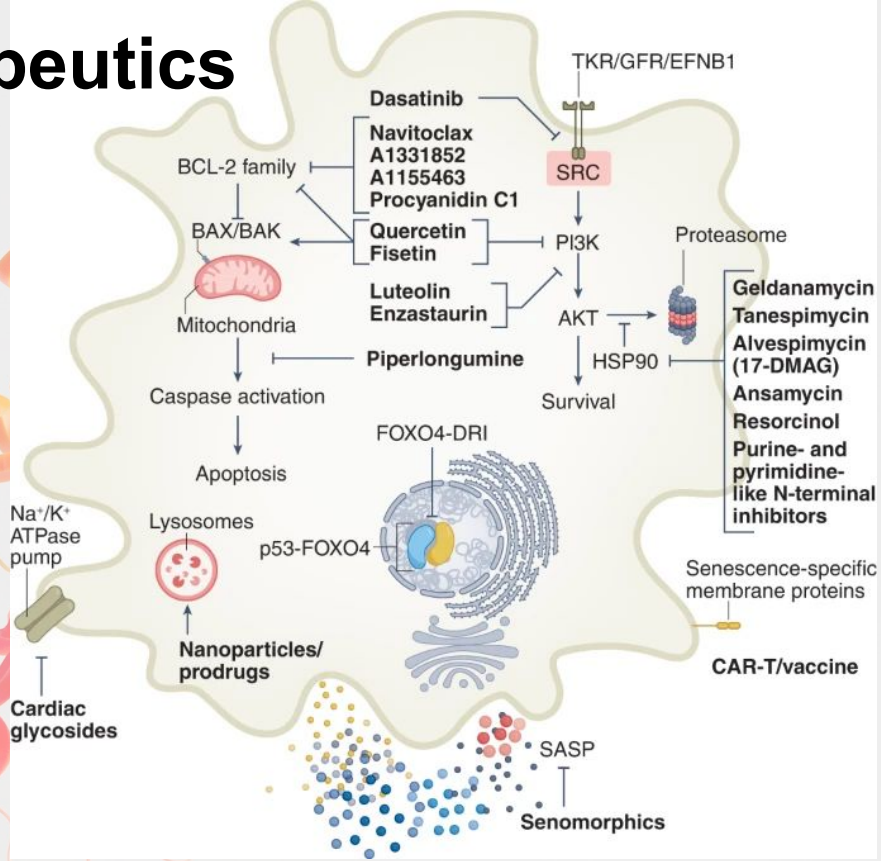
Can we Control the Effects of Senescent Cells?



That's here senotherapeutics comes in…

Senotherapeutics





Senotherapeutics are classified as: <u>senolytics</u> which kills SCs selectively; <u>senomorphics</u> which modulate functions and morphology of SCs to those of young cells, or delays the progression of young cells to SCs in tissues; and <u>immune-system mediators of the clearance of SCs</u>.

Senolytics

- By blocking antiapoptotic, pro-survival pathways to avoid self-destruction, resistant/senescent cells die.
  - Senescent cell anti-apoptotic pathways (SCAPs), like that found in cancer, is upregulated in senescent cells.
  - Transiently disabling SCAP pathways results in apoptosis of the senescent cells with a tissue-destructive SASP, while non-senescent cells or those senescent cells with a pro-growth, non-apoptotic SASP remain viable
  - In some types of senescent cells, SCAPs can be redundant, so that targeting a single SCAP may not eliminate such cells—but combination treatment targeting multiple SCAPS may be effective.
- over 20 clinical trials of senolytic therapies are completed, ongoing or planned
- Removal of SC has shown to increase healthspan and lifespan in animal models

Senolytic Examples

- **Dasatinib (D)--Multi-Tyrosine Kinase Inhibitor**
  - senescent human fat cell progenitors (preadipocytes or mesenchymal stromal cells) are sensitive to D but not Q or F
- **Quercetin (Q)--Flavonoid**
  - senescent human umbilical vein endothelial cells are sensitive to Q or F but not D
  - flavonoid that targets BCL-2/BCL-XL, PI3K/AKT, and p53/ p21/serpine SCAPs
- **FOXO4 Peptide**
  - forkhead box O4 (FOXO4) retains p53 in the nucleus, so peptides interfering with this interaction can lead to p53-mediated apoptosis in some types of senescent cells
- **HSP90 Inhibitor**
  - HSP90 prevents proteasomal degradation of AKT, hence inhibiting HSP90 disables pro-survival signaling
- **D+Q**
  - senescent mesenchymal embryonic fibroblasts from Ercc1−/− mice and bone marrow mesenchymal progenitors from old mice are not eliminated by either D or Q alone, but are eliminated by the combination
- **Fisetin (F)--Flavonoid**
- **Chimeric antigen receptor (CAR) T cells**
- **Glycoprotein nonmetastatic melanoma protein B (GPNMB) Vaccine**

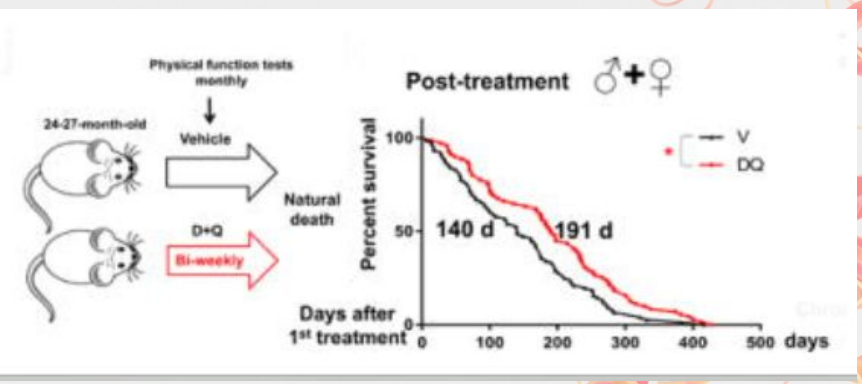
“Hit-and-run” treatment with senolytics can reduce human senescent cell burdon

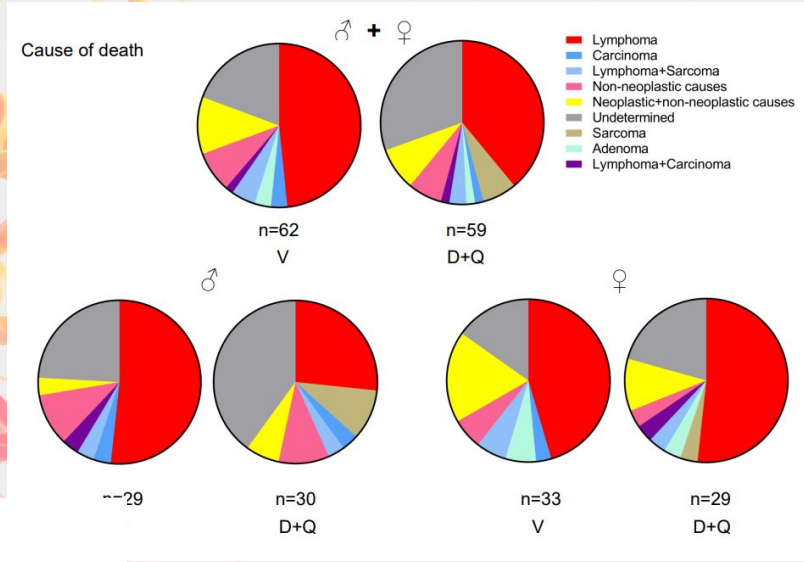
- Open label Phase 1 pilot study
- 3 days of oral D 100 mg and Q 1000 mg to subjects with diabetic kidney disease
- Adipose tissue, skin biopsies, and blood were collected for senescent cells and circulating SASP factors
- D + Q reduced adipose tissue senescent cell burden within 11 days
- Skin epidermal p16INK4A+ and p21CIP1+ cells were reduced
- Circulating SASP factors were reduced

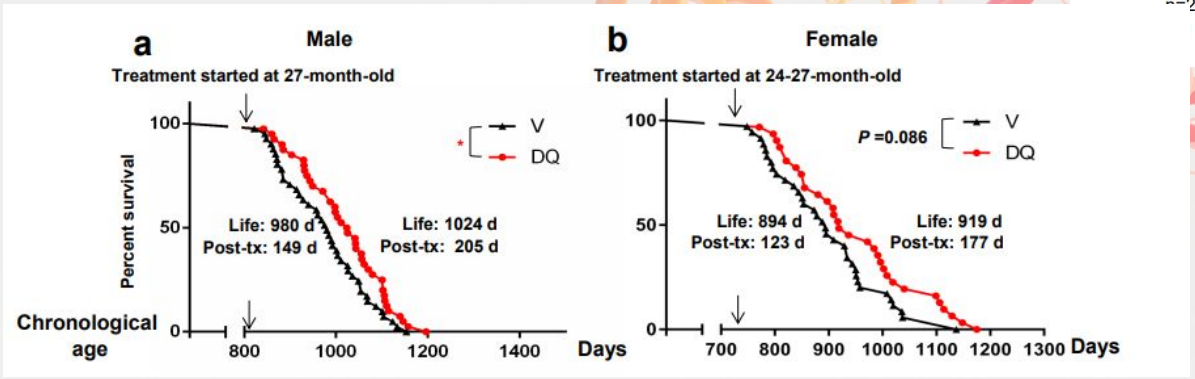
Hickson LJ,et al. Senolytics decrease senescent cells in humans: Preliminary report from a clinical trial of Dasatinib plus Quercetin in individuals with diabetic kidney disease. EBioMedicine. 2019 Sep;47:446-456. doi: 10.1016/j.ebiom.2019.08.069. Epub 2019 Sep 18. Erratum in: EBioMedicine. 2020 Feb;52:102595.

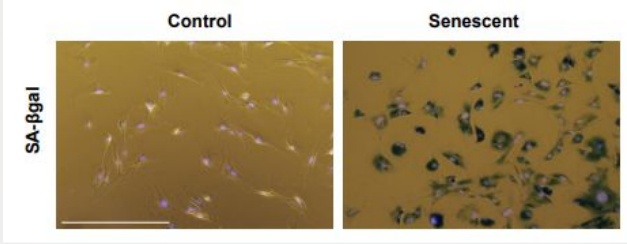
Senolytic Increase lifespan in Mice by 36%

D+Q starting at 24-27 months of age (equivalent to age 75-90 years in humans)









Senolytics: Bcl-2 inhibitors on Skin Fibroblasts

- Primary human dermal fibroblasts (HDFs) were induced to senescence by long-term passaging, UV irradiation, and H2O2 treatment.
- Cell viability was measured after treatment of ABT-263 and ABT-737
- Young and aged hairless mice were intradermally injected with drugs or vehicle on the dorsal skin for 10 days.
- RESULT:
  - In vitro: ABT-263 and ABT-737 induced selective clearance of senescent dermal fibroblasts, regardless of the method of senescence induction.
  - In vivo: Aged mouse treated with ABT-263 or ABT-737 showed increased collagen density, epidermal thickness, and proliferation of keratinocytes, as well as decreased senescence-associated secretory phenotypes, such as MMP-1 and IL-6.

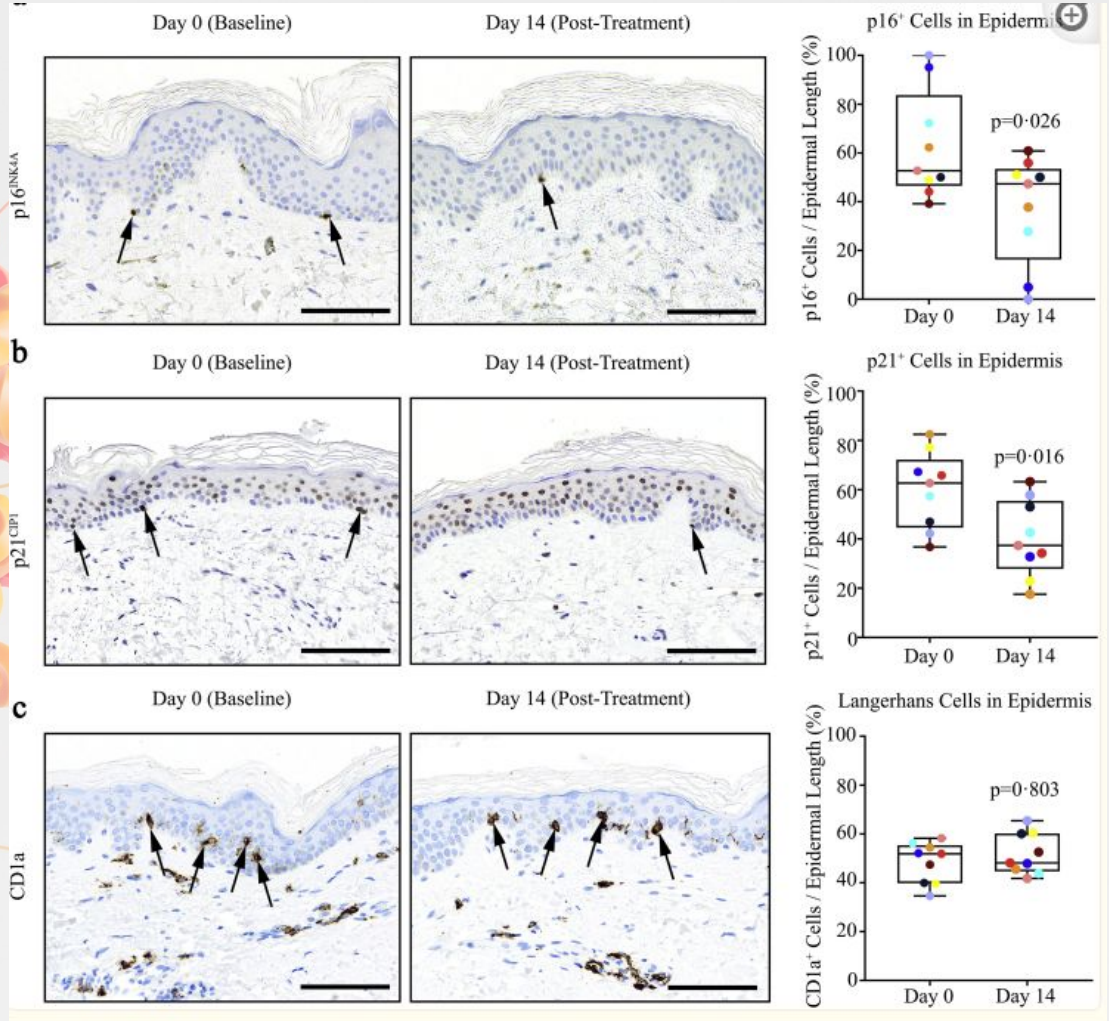
Kim, H., Jang, J., Song, M.J., Kim, G., Park, C.-.-H., Lee, D.H., Lee, S.-.-H. and Chung, J.H. (2022), Attenuation of intrinsic ageing of the skin via elimination of senescent dermal fibroblasts with senolytic drugs. J Eur Acad Dermatol Venereol, 36: 1125-1135. https://doi.org/10.1111/jdv.18051

1st human study with senolytics for disease: Idiopathic Pulmonary Fibrosis

- A two-center, open-label study of intermittent DQ (D:100 mg/day, Q:1250 mg/day, three-days/week over three-weeks)
- Subjects with idiopathic pulmonary fibrosis, a cellular senescence-driven fatal disease, showed significantly improved walking endurance, gait speed, chair rise test performance, and scores
- Correlations were observed between change in function and change in SASP-related matrix-remodeling proteins, microRNAs, and pro-inflammatory cytokines

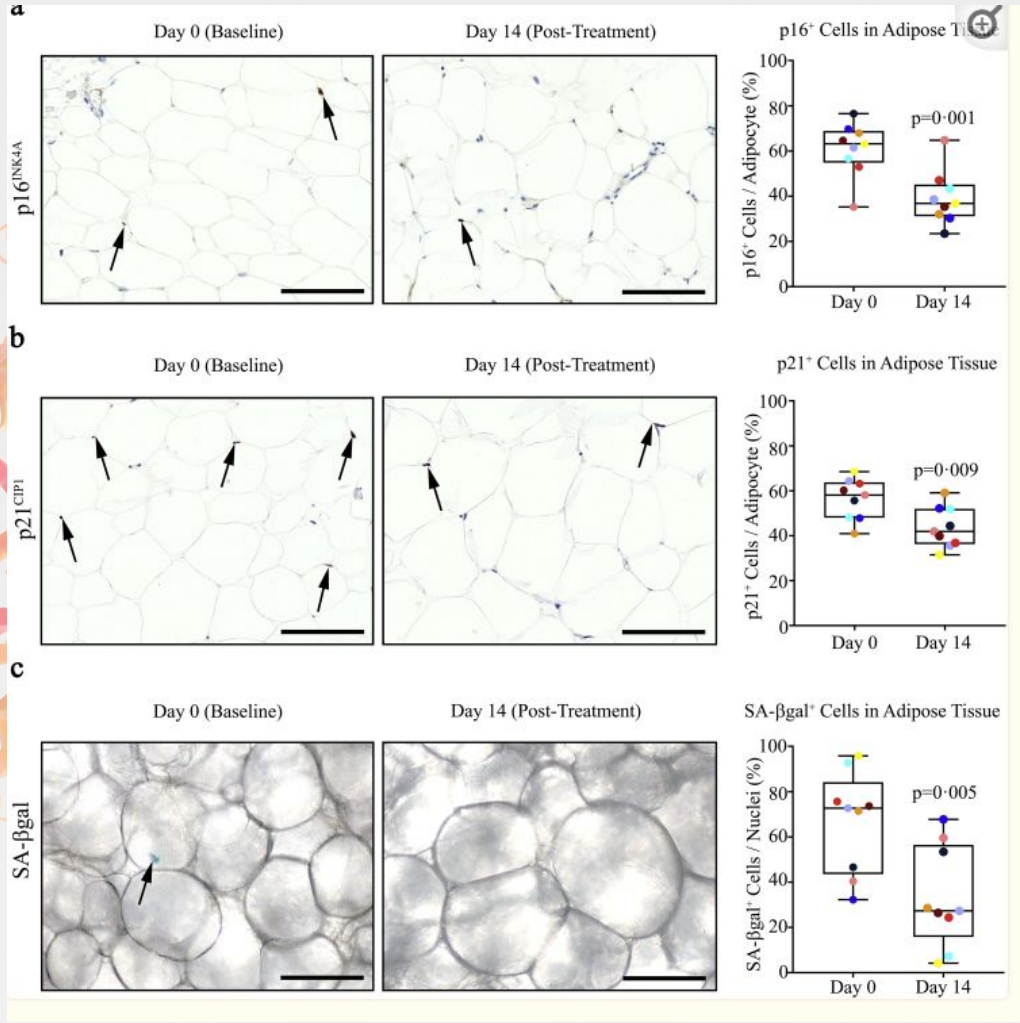
Justice J.N., Nambiar A.M., Tchkonia T., LeBrasseur N.K., Pascual R., Hashmi S.K. Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. EBioMedicine. 2019;40:554–563. doi: 10.1016/j.ebiom.2018.12.052

- **After 2 weeks, senescent cells were reduced in the epidermis while normal cells (LC) numbers did not change.**
- **Epidermal but not dermal p16INK4A+ cells have been associated with cardiovascular disease risk and “biological ageing”**



Justice J.N., Nambiar A.M., Tchkonia T., LeBrasseur N.K., Pascual R., Hashmi S.K. Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. EBioMedicine. 2019;40:554–563. doi: 10.1016/j.ebiom.2018.12.052

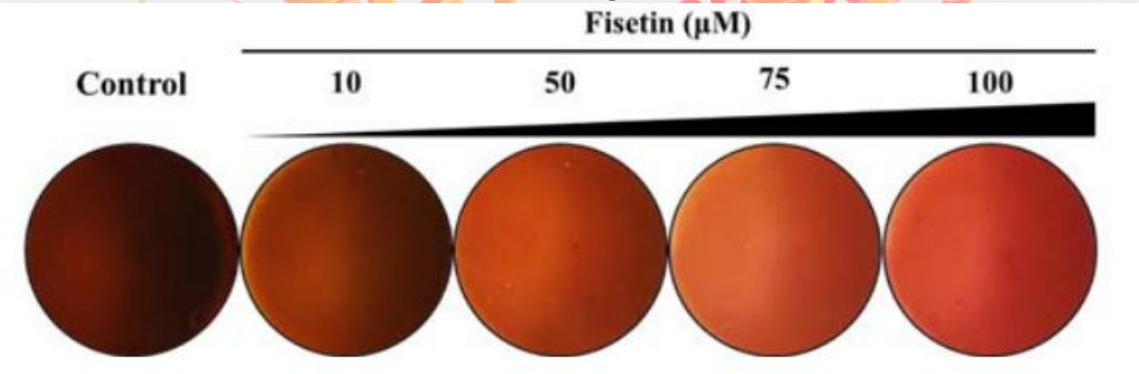
Senolytics decreased senescent adipocytes in humans



Justice J.N., Nambiar A.M., Tchkonia T., LeBrasseur N.K., Pascual R., Hashmi S.K. Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. EBioMedicine. 2019;40:554–563. doi: 10.1016/j.ebiom.2018.12.052

SENOLYTIC: Fisetin in Skin Health

- Fisetin is the most potent senolytic flavonoid on numerous organs, including fat
- A novel function of fisetin in skin health via down-regulation of melanosis and adipogenesis, and up-regulation of skin fibril-related genes was observed.
- Fisetin treatment inhibits melanin synthesis in B16F10 melanoma cells



Shon MS, Kim RH, Kwon OJ, Roh SS, Kim GN. Beneficial role and function of fisetin in skin health via regulation of the CCN2/TGF-β signaling pathway. Food Sci Biotechnol. 2016 Mar 31;25(Suppl 1):133-141. doi: 10.1007/s10068-016-0110-y.

Senolytic: Clinical anti-aging study

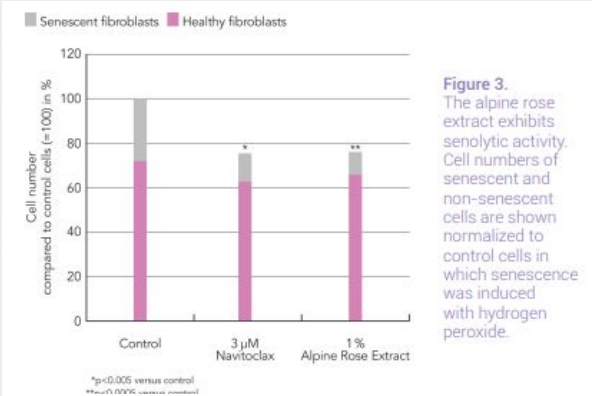


Figure 3. The alpine rose extract exhibits senolytic activity. Cell numbers of senescent and non-senescent cells are shown normalized to control cells in which senescence was induced with hydrogen peroxide.

- Double-blind, placebo-controlled clinical study
- 44 Caucasian women aged between 40 and 65 years (mean age: 55 years) with redness on the cheeks were split into two groups
- 2% alpine rose extract cream vs placebo
  - Rhododendron ferrugineum L. (Ericaceae), commonly named Alpine rose, is an evergreen subalpine shrub growing throughout the European mountains
- Entire face and neck twice daily for 28 days.
- Skin color was measured using a Spectrocolorimeter CM700-d (Konica Minolta, Japan) and skin elasticity was determined with a Cutometer MPA 580 (Courage + Khazaka, Germany)

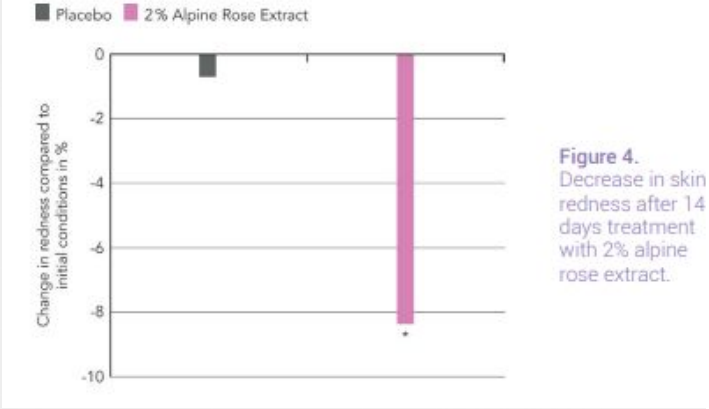


Figure 4. Decrease in skin redness after 14 days treatment with 2% alpine rose extract.

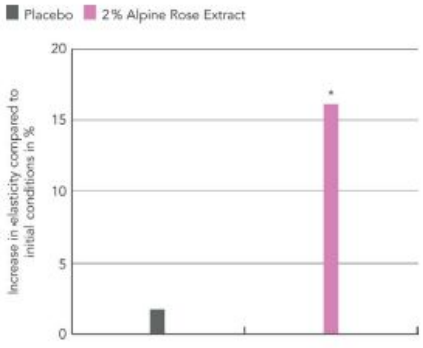


Figure 6. Increase in skin elasticit after 28 day treatment w 2% alpine ro extract.

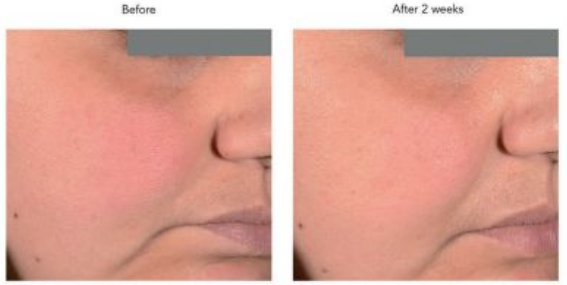


Figure 5. Before and after picture taken of a volunteer who applied 2% alpine rose extract twice daily for 14 days.

Wandry F, et al. Senolytics: eliminating “zombie cells” in the skin. Skin Care.15(5):2020.

Also reduced scalp erythema

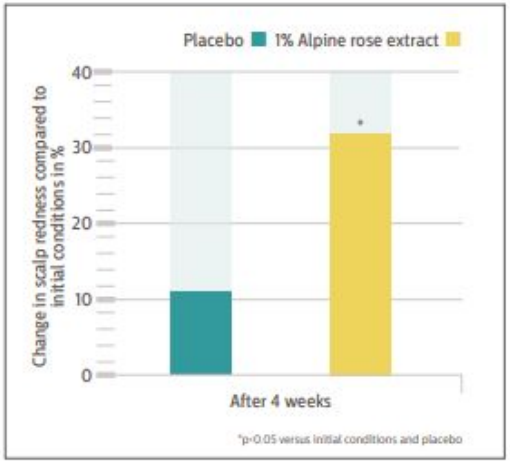


Figure 5: Quantified reduction in scalp redness
Scalp treatment with 1% Alpine rose extract compared to baseline and placebo control after 4 weeks

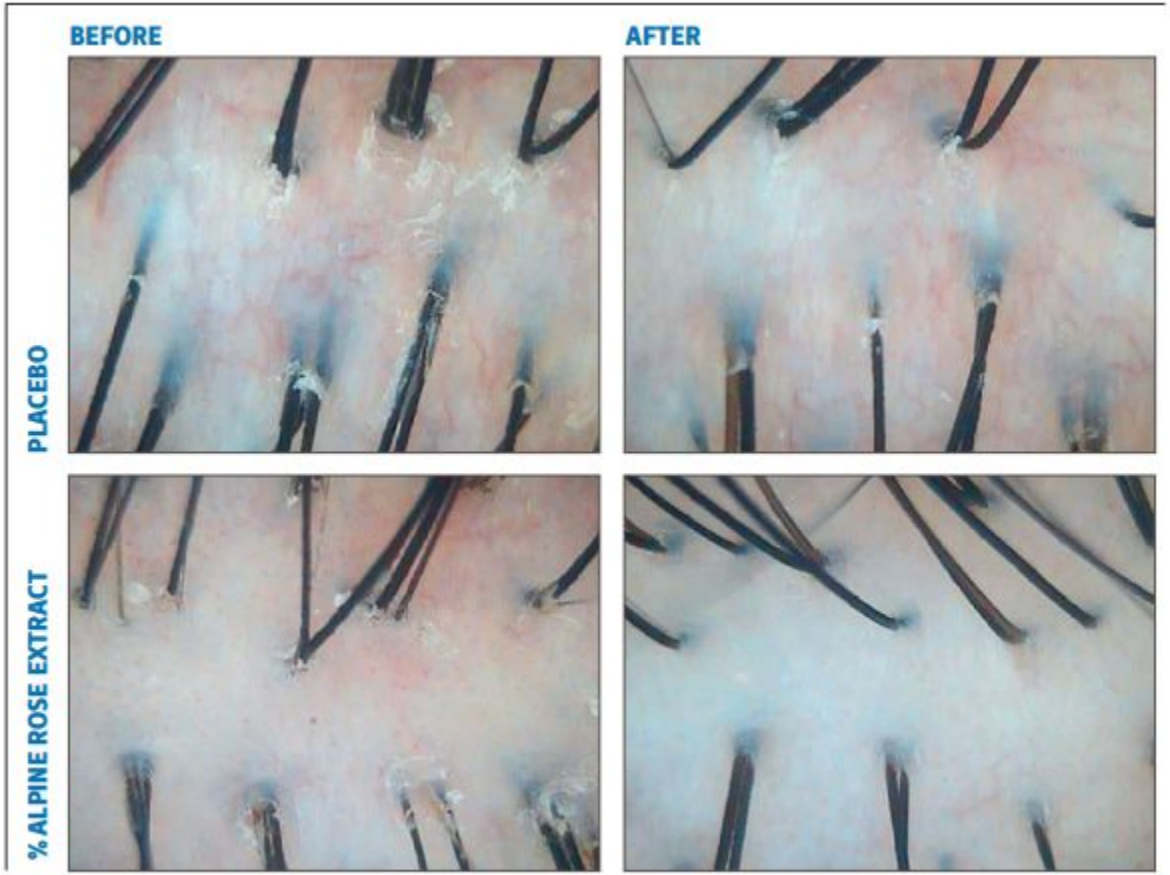
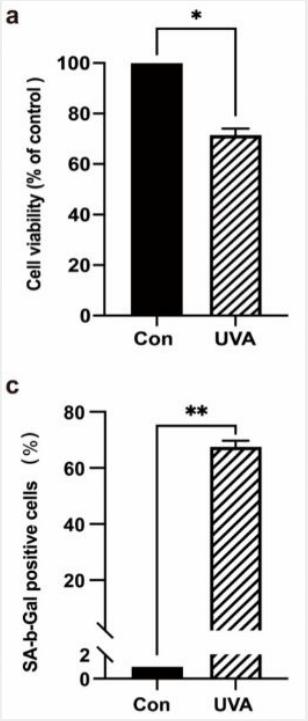


Figure 4: Volunteer before & after applying 1% Alpine rose extract serum once daily after hair wash for four weeks

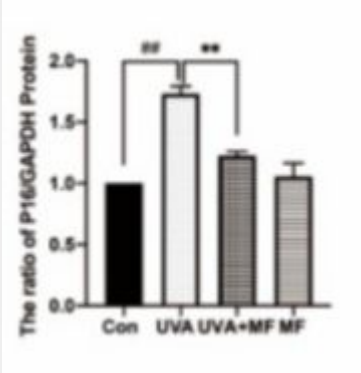
Senomorphics

- **Senomorphics is a wide range of agents that can modulate the phenotypes of SCs to those of young cells through interfering with inflammaging, senescence-related signal pathways, and SASP, without induction of SC apoptosis**
- **Typical Senomorphic agents:**
  - **Rapamycin: Induce autophagy by mTOR inhibition**
  - **Metformin: Induce autophagy by mTOR inhibition and stimulates phosphorylation of AMPK**
  - **Jak Inhibitors: Block JAK/STAT pathway**

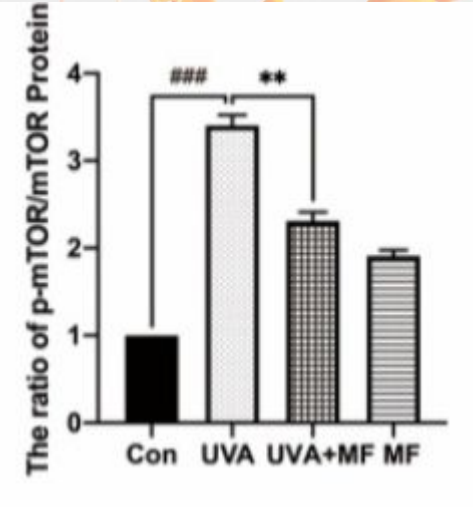
Metformin and Photoaging



Human foreskin fibroblasts



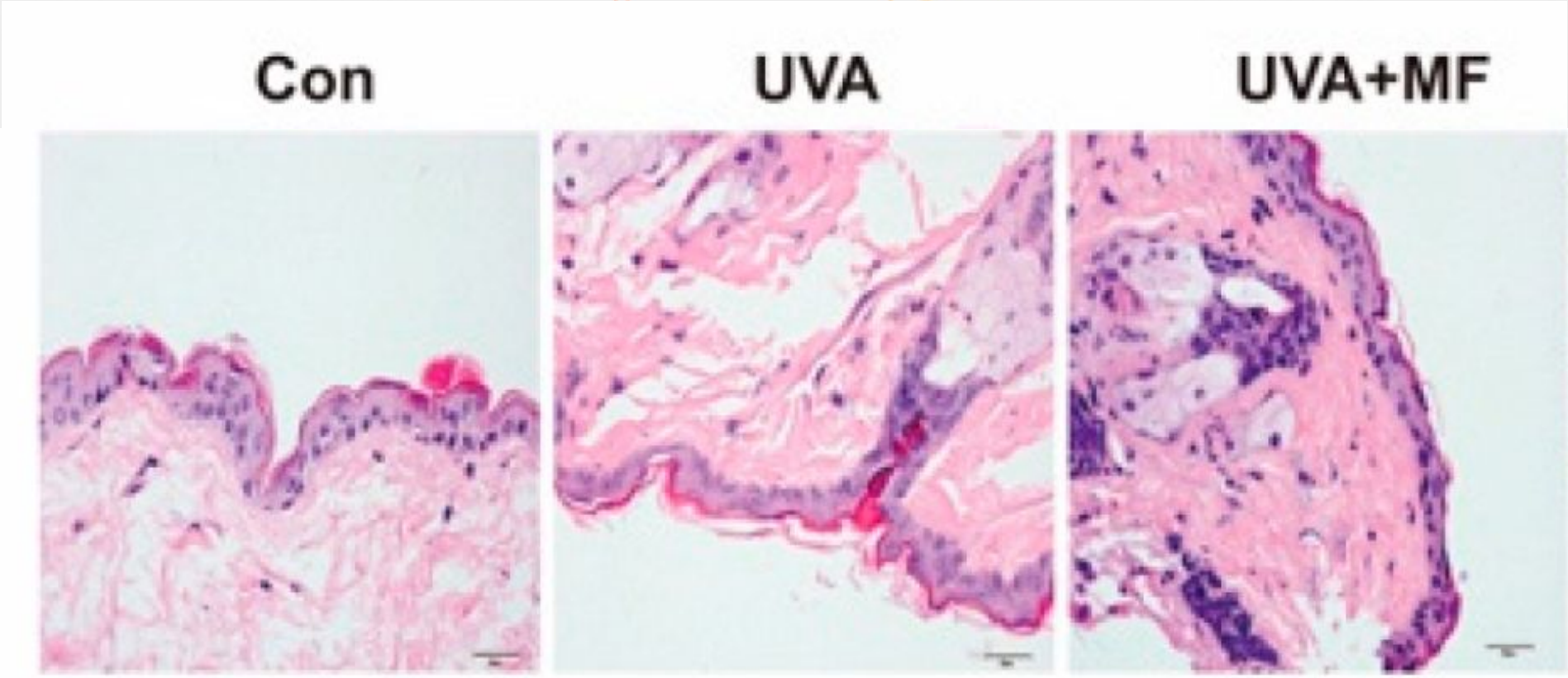
Reduced senescence with Metformin



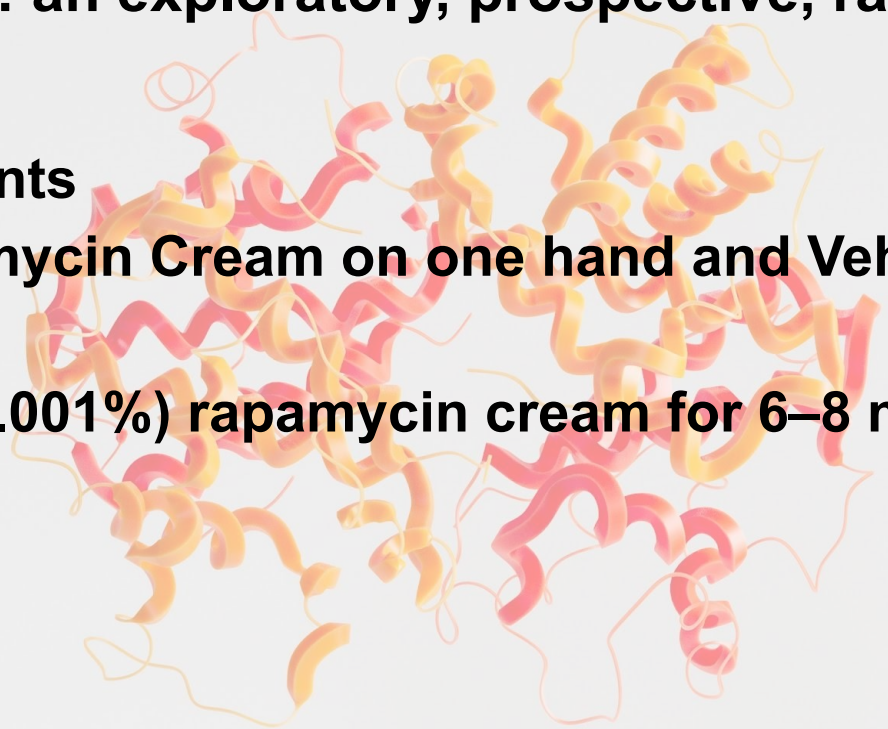
Induced Autophagy

Chen Q, Zhang H, Yang Y, Zhang S, Wang J, Zhang D, Yu H. Metformin Attenuates UVA-Induced Skin Photoaging by Suppressing Mitophagy and the PI3K/AKT/mTOR Pathway. Int J Mol Sci. 2022 Jun 23;23(13):6960. doi: 10.3390/ijms23136960.

Metformin can improve UVA-induced skin photoaging in mice



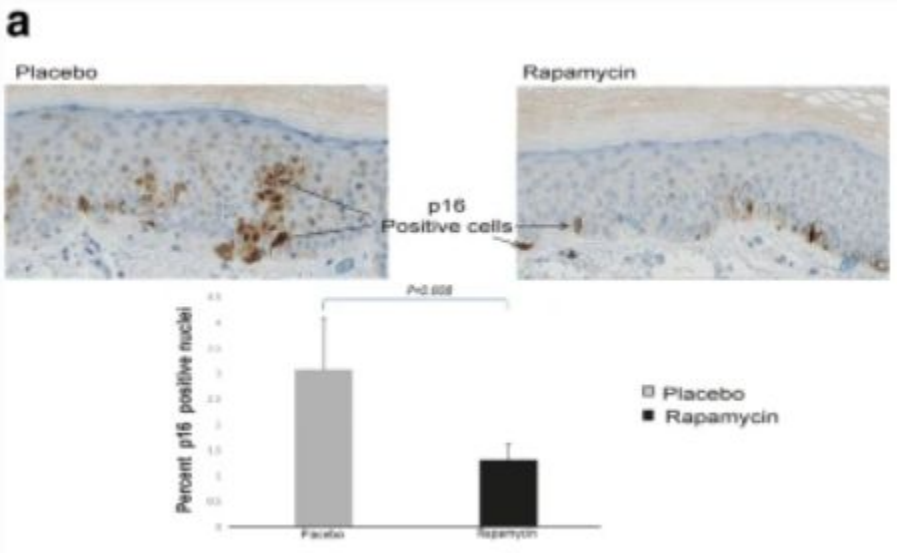
Topical rapamycin reduces markers of senescence and aging in human skin: an exploratory, prospective, randomized trial: Human Study



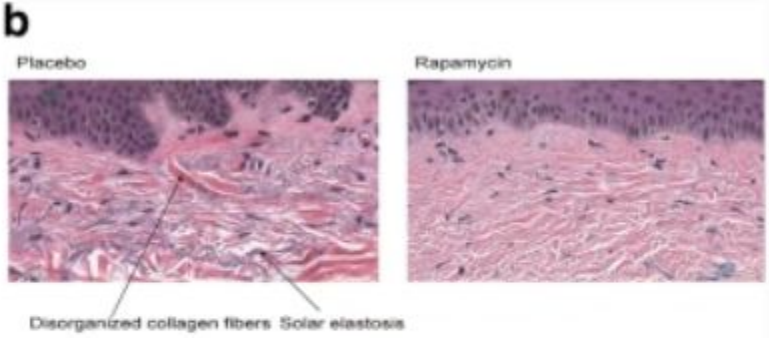
- 17 participants
- Used Rapamycin Cream on one hand and Vehicle on the other QD
- (10 μM, or 0.001%) rapamycin cream for 6–8 months.

Chung, C.L.*et al.* Topical rapamycin reduces markers of senescence and aging in human skin: an exploratory, prospective, randomized trial. *GeroScience* 41, 861–869 (2019). https://doi.org/10.1007/s11357-019-00113-y

Results

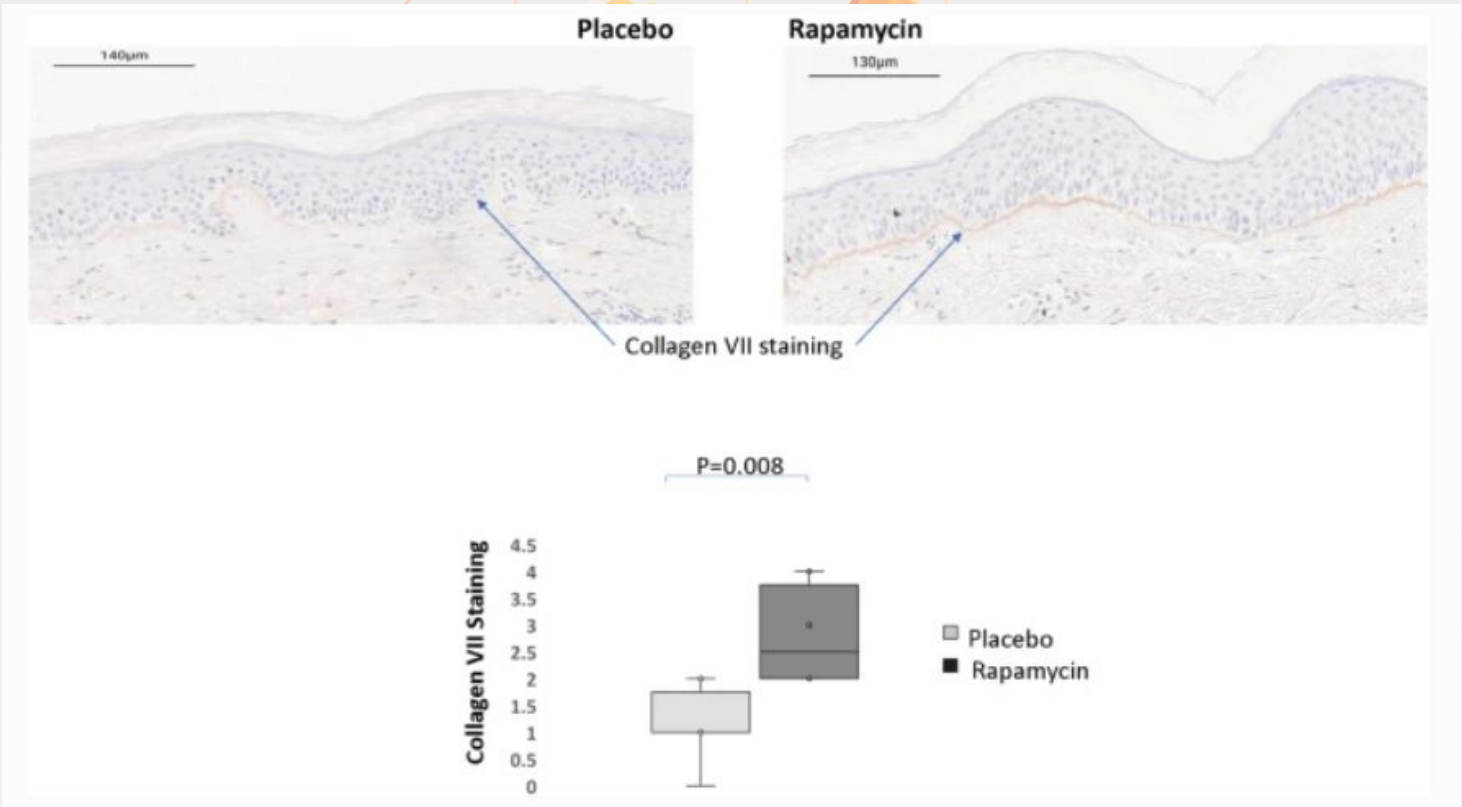


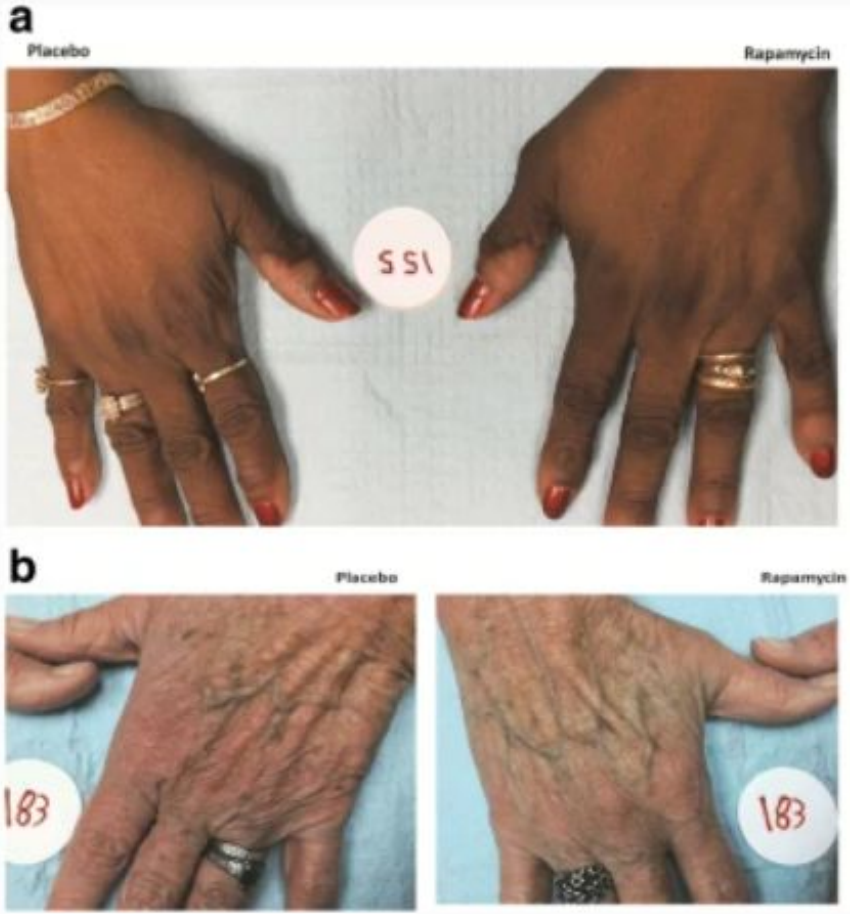
Rapamycin reduced senescent cells on hand skin



Histologic evidence of photoaging- solar elastosis is indicated with an arrow. A reduction in the presence of these histologic markers of age-damaged skin was noted in multiple patient biopsies treated with rapamycin.

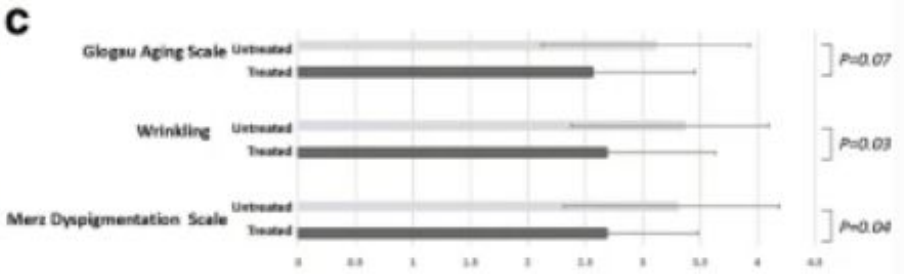
Topical rapamycin increases collagen VII in the basement membrane of human skin





Patients noticed:

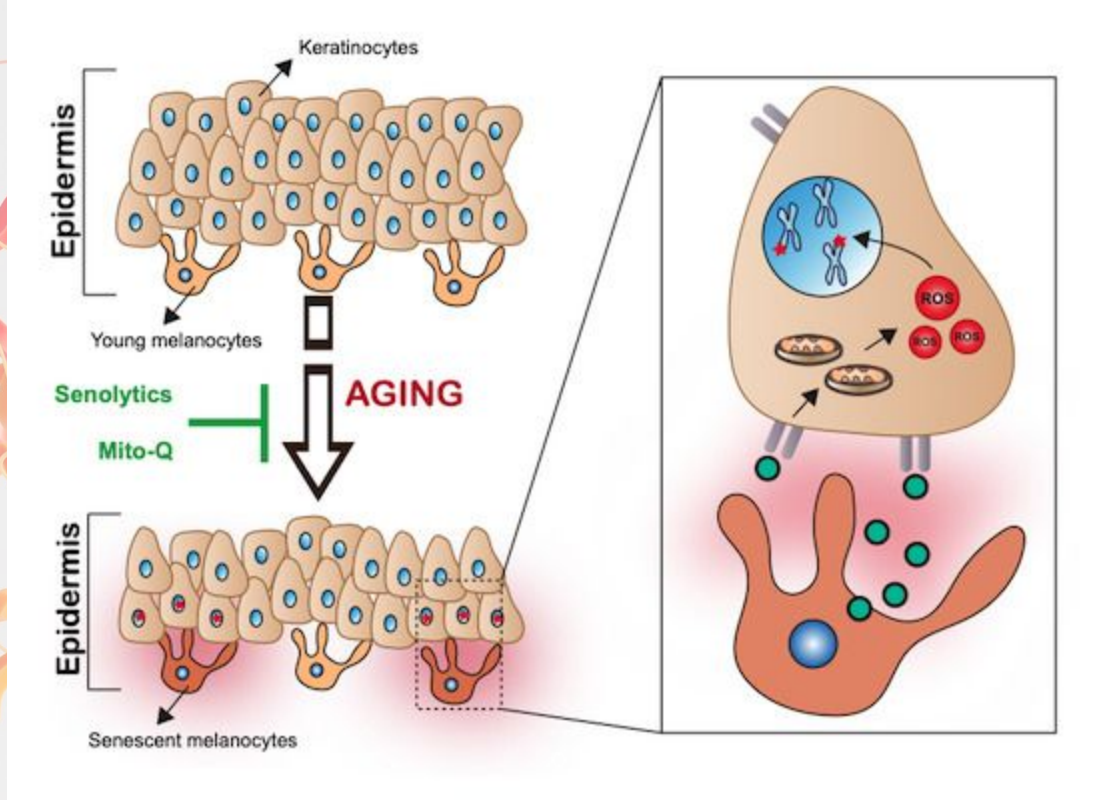
- decrease in fine wrinkles
- increase in dermal volume
- brighter and more even skin tone
- reduced sagging of the skin



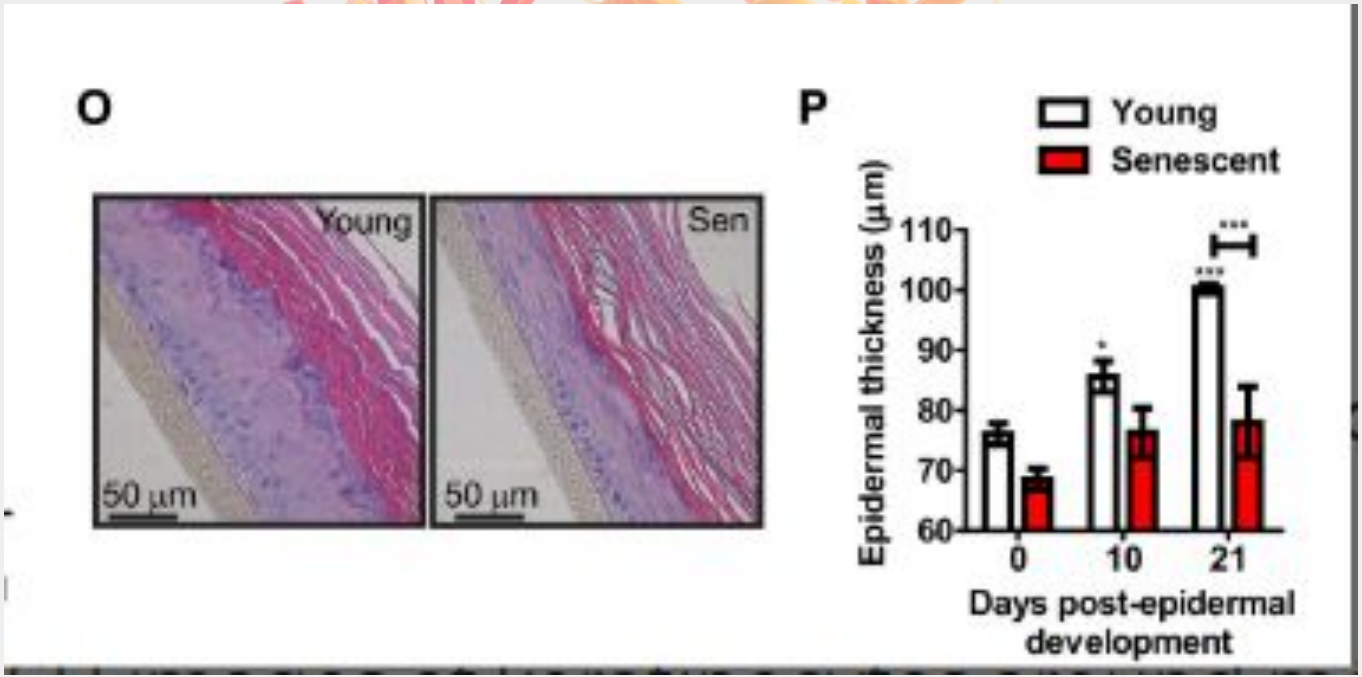
These changes were evident approximately 4 months following the initiation of treatment, and continued improvement was noted upon subsequent visits.

Senescent human melanocytes drive skin ageing via paracrine telomere dysfunction

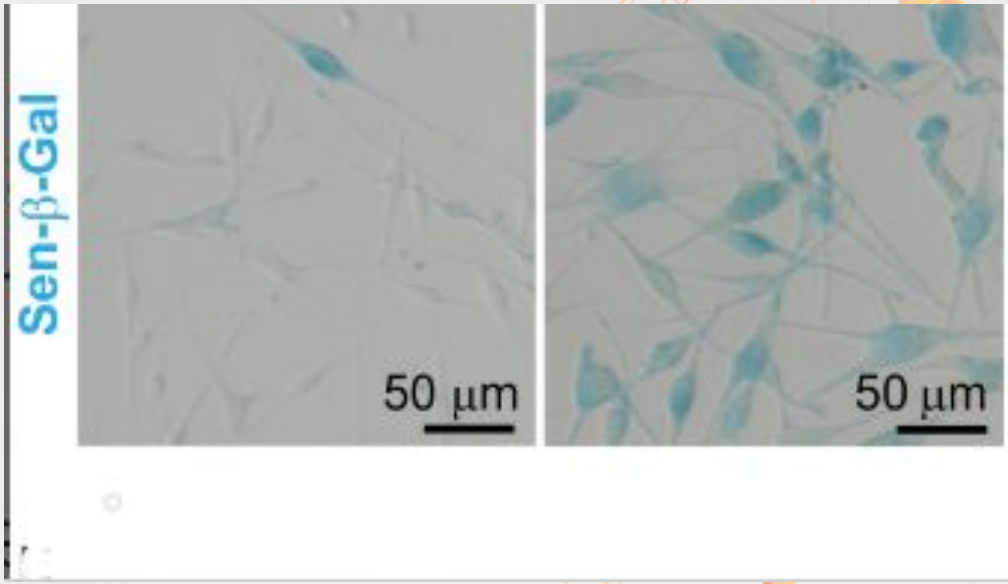
- **Senescent p16INK4-positive melanocytes accumulate in human skin with age.**
- **Senescent melanocytes induce telomere damage and senescence in surrounding cells in paracrine manner.**
- **Clearance of senescent melanocytes with a senolytic drug or ROS scavengers rescues epidermal atrophy in vitro using 3D human epidermal equivalents.**

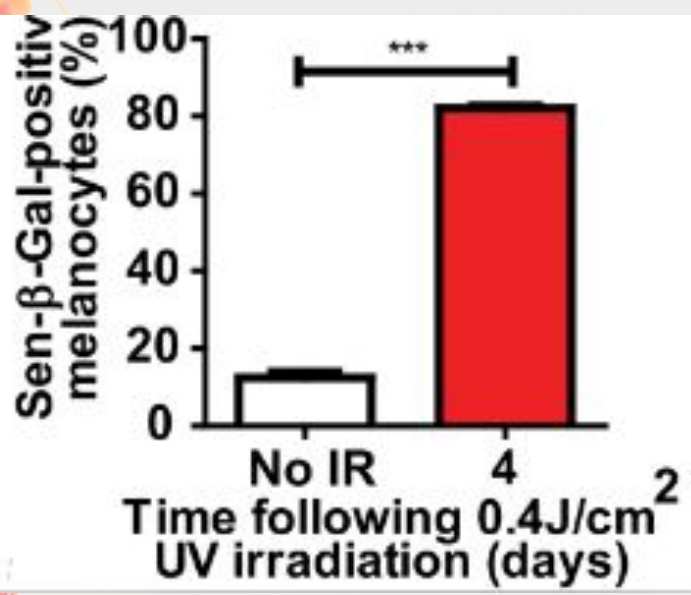


Senescent melanocytes induce paracrine senescence and contribute to epidermal atrophy in a 3D epidermal equivalent

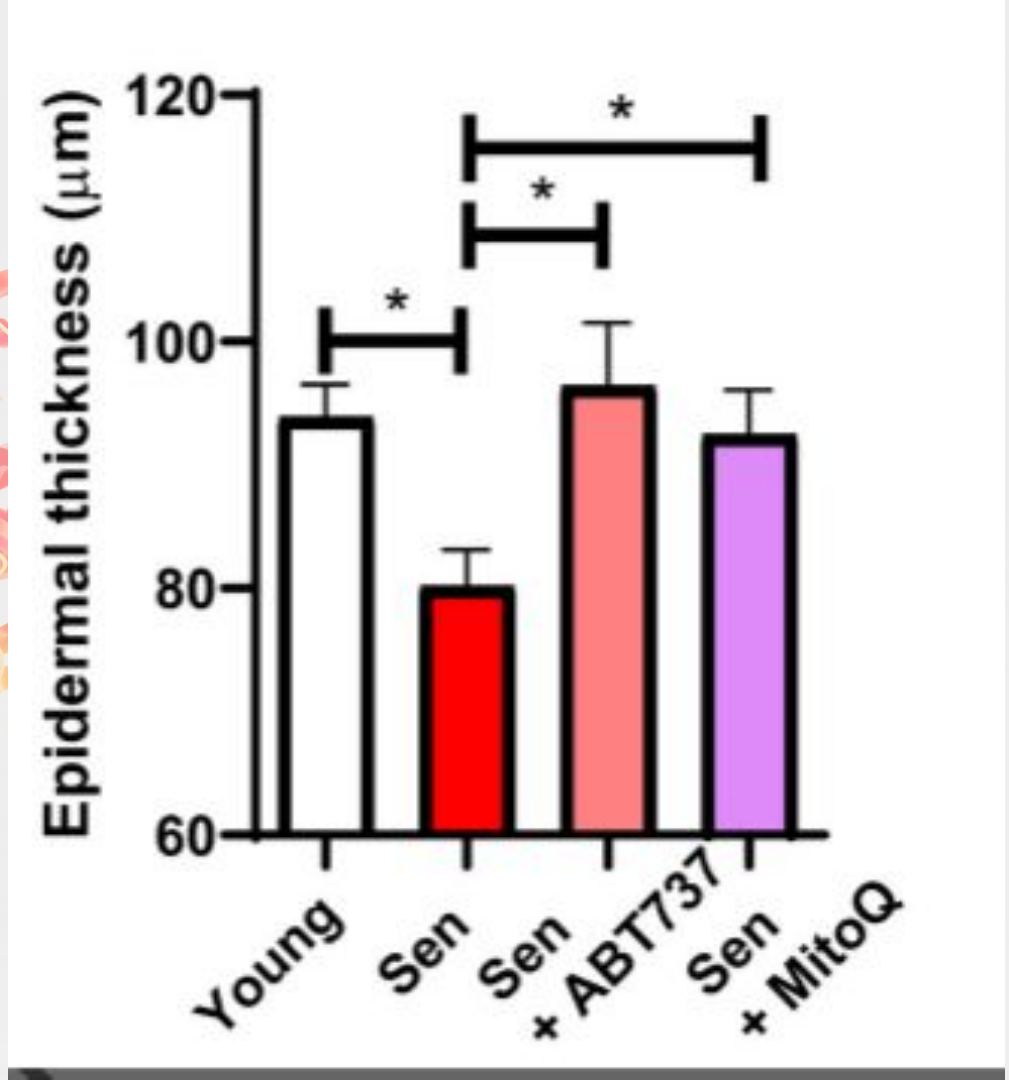


Repeated UVA+B exposure induces senescence in human epidermal melanocytes in vitro



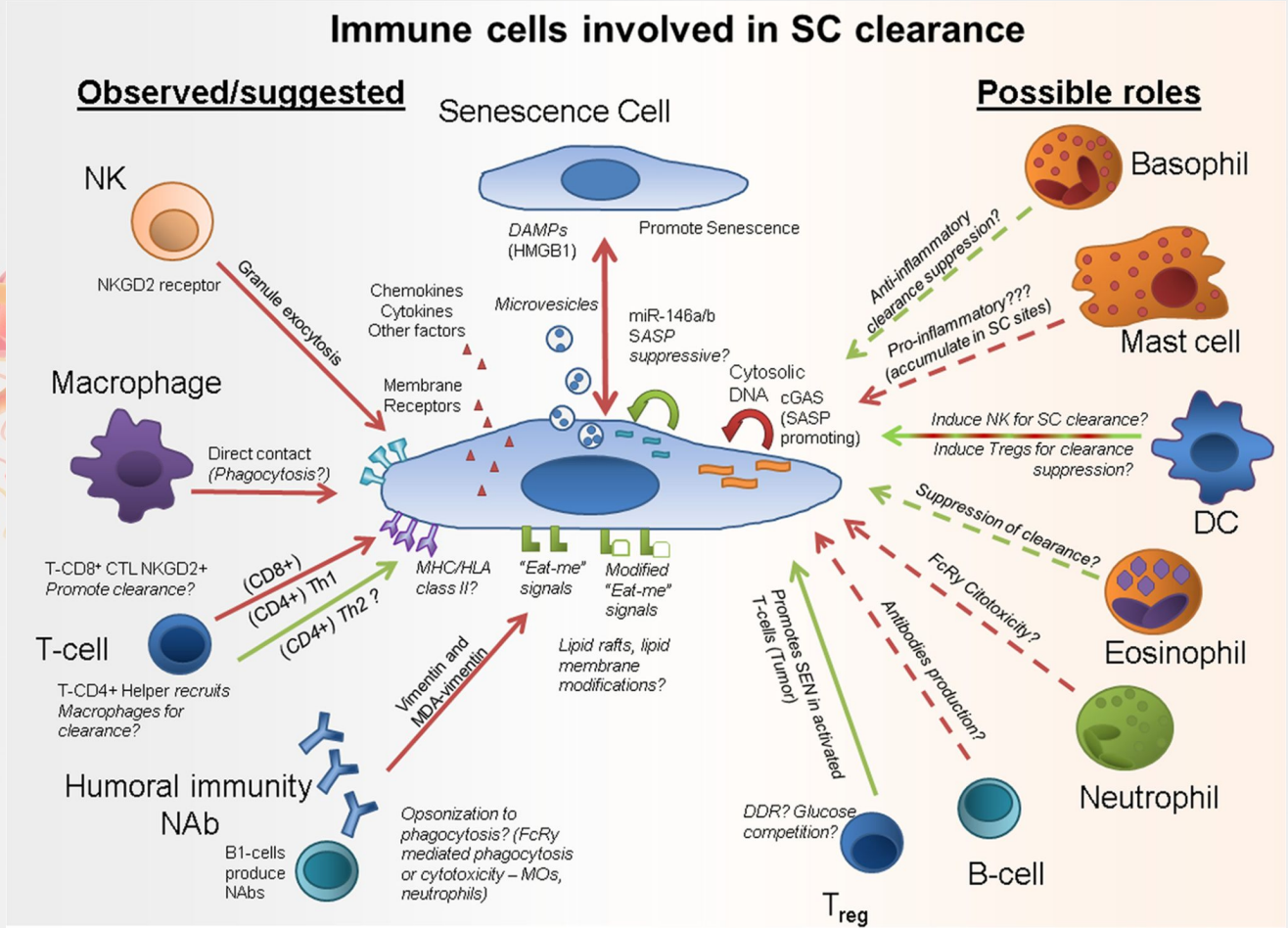


Clearance of senescent melanocytes or reducing mitochondrial ROS production rescues epidermal atrophy in 3D human epidermal equivalents



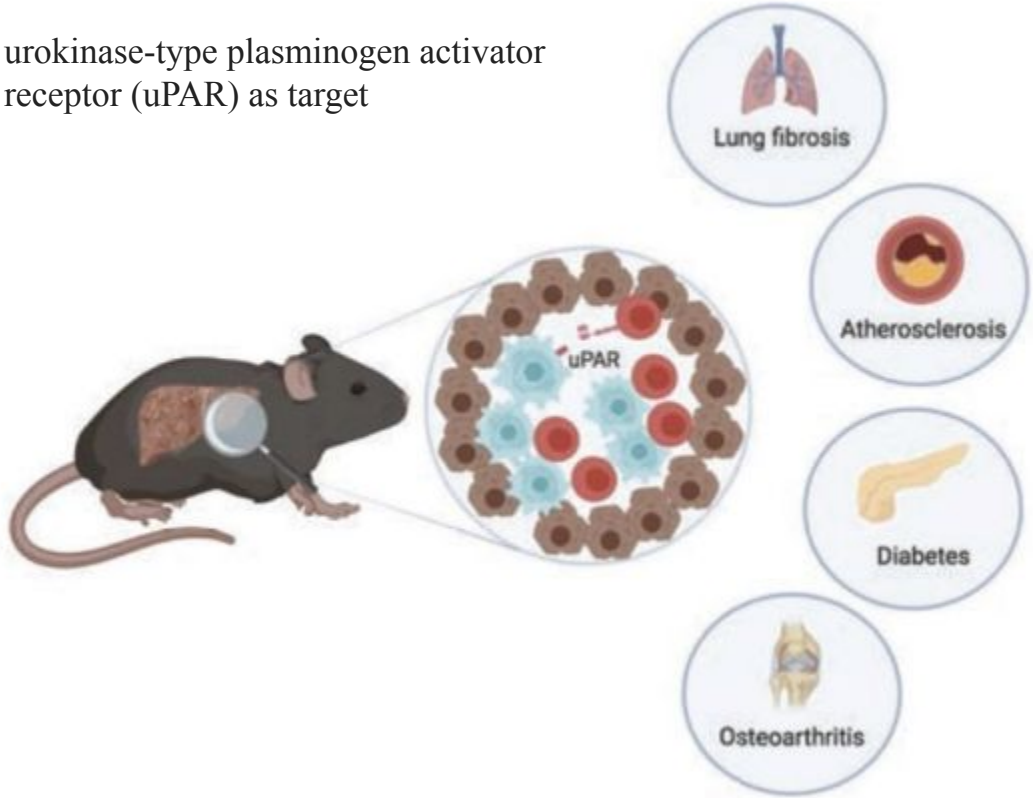
Immune-system mediators of the clearance senescent cells

- **Pretty much the entire immune system is geared to SC clearance through multiple mechanisms**
- **Inflammaging**
- **CAR-T therapy has been developed to be senolytic.**
- **Inflammaging**



Immune-system mediated clearance of SCs

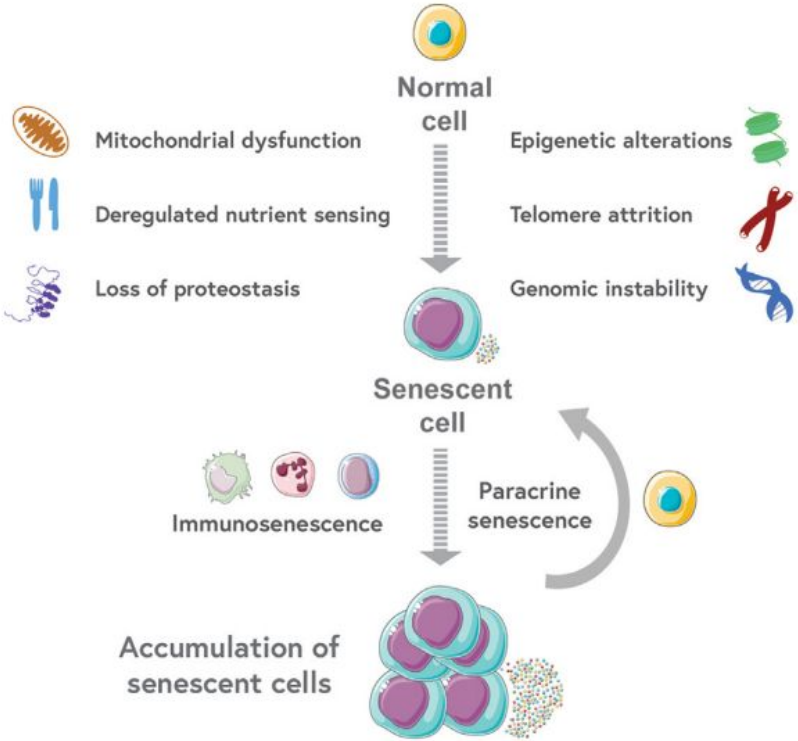
urokinase-type plasminogen activator receptor (uPAR) as target

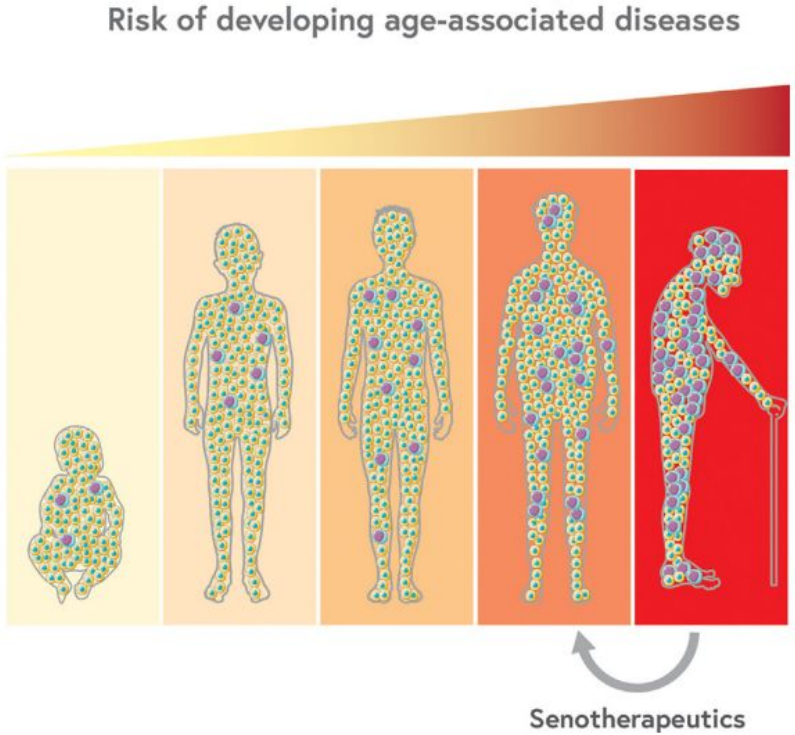


Senolytic CAR T cells reverse senescence-associated pathologies

Amor, C., Feucht, J., Leibold, J. et al. Senolytic CAR T cells reverse senescence-associated pathologies. Nature 583, 127–132 (2020). https://doi.org/10.1038/s41586-020-2403-9

Conclusion


Normal cell
Mitochondrial dysfunction
Deregulated nutrient sensing
Loss of proteostasis
Epigenetic alterations
Telomere attrition
Genomic instability
Senescent cell
Immunosenescence
Paracrine senescence
Accumulation of senescent cells


Risk of developing age-associated diseases
Senotherapeutics

- Senescent melanocytes induce skin phenotype of aging
- Removing senescent melanocytes from skin reverse phenotype of aging
- Removing and modulating the expression of senescent cells can improve hallmarks of aging in skin.
- Senescent cell anti-apoptotic pathways (SCAPs) need to be further studied in different cell types so as to target sinotherapeutics.