PERIODICUM BIOLOGORUM
VOL. 126, No 1–2, 97–103, 2024
DOI: 10.18054/pb.v126i1-2.30627

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Short communication

Senolytic activity of alpha-Mangostin through *in vitro* TGF-β signaling inhibition

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Abbreviations
HUVEC – human umbilical vein endothelial cells
hMSCs – human mesenchymal stem cells
IMR-90 – human lung fibroblasts, fetal
TGF-β – transforming growth factor β
SA-β-gal – senescence-associated beta galactosidase

Keywords:
α-Mangostin; senescence; TGF-β

Received April 10, 2024
Revised April 16, 2024
Accepted April 16, 2024

Abstract

Background and purpose: *TGF-β is a multifunctional cytokine involved in various cellular processes, including cell growth, differentiation, apoptosis and immune regulation. Depending on the cellular context, it plays an important role in aging, mostly through inducing and/or maintaining cellular senescence. Senescent cells contribute to the pathogenesis and progression of various diseases by secreting pro-inflammatory factors and altering tissue environment, thereby accelerating aging and promoting age-related disorders. TGF-β pathway suppression is considered a plausible strategy for senescent cell eradication. Small molecules, especially of natural origin, are gaining attention as effective modulators of senescence-associated pathways, including TGF-β.*

Materials and methods: *In search of TGF-β modulators with potential senolytic activity, we performed an initial screen of TGF-β modulation on 480 naturally occurring compounds. Top inhibitors were re-tested for TGF-β signaling inhibition and α-Mangostin was selected based on its safety and availability profile. We used the flow cytometry-based SA-β-gal activity assay to determine the ability of α-Mangostin to remove etoposide-induced senescent cells in vitro. Potential cytotoxicity was assessed by MTT test.*

Results: *In our model system, α-Mangostin at 10μM literally abolished the myostatin-induced TGF-β signaling in HEK-293 cells. Furthermore, the compound showed a substantial potential for clearance of senescent cells in three primary cell lines of different origin (Imr-90, HUVEC and hMSCs). At the same time, 10μM α-Mangostin did not affect cell viability in either cell line.*

Conclusion: *Our results suggest that α-Mangostin, as a naturally occurring compound, is a potential TGF-β modulating senolytic, and encourages further research in formulation development and targeted in vivo studies.*

INTRODUCTION

At the level of an organism, aging is the phenotypic outcome of a complex set of molecular events, leading to a time-dependent decrease in systemic functions. In 2013., nine 'hallmarks' of aging have been defined, with 3 more added in 2022 *(1, 2)*. Some of those intertwined phenomena include genomic instability, telomere attrition, chronic inflammation and cell senescence. First described in 1961 by Hayflick and Moorhead *(3)*, cellular senescence is the irreversible cell cycle arrest. Apart from replicative senescence due to telomere shortening, it can be induced by various stressors such as activation of oncogenes, oxidative stress, viral infection and mitochondrial dysfunction *(4, 5)*. Senescent cells exhibit a specific secretory profile or SASP (senescence-

associated secretory phenotype) which involves the production of pro-inflammatory cytokines, chemokines, growth factors, and matrix metalloproteinases. SASP enables senescent cells to affect their environment by modulating the local inflammation status, immune regulation, tissue remodeling and can also induce senescence in neighboring cells in paracrine manner. Senescent cells accumulate with age and contribute to tissue dysfunction, chronic inflammation, and the progression of age-related pathologies such as neurodegenerative and cardiovascular diseases, cancers, diabetes and kidney dysfunction *(6–10)*. It is therefore not surprising that the search for senolytics (compounds or biomolecules that selectively remove senescent cells) has been increasingly dynamic during the last 15 years. Transforming Growth Factor-beta (TGF-β) is a pleiotropic cytokine implicated in the regulation of many processes such as cell growth and differentiation, embryonic development, wound healing, inflammation, immune response modulation and tumorigenesis *(11–14)*. Importantly, TGF-β activity has been linked to cellular senescence *(15)*. Although the direction of this effect is context dependent, most studies have confirmed that TGF-β signaling positively correlates with senescence in various tissues and cell types, and that the activity of this pathway increases with age *(16)*. While TGF-β can induce senescence in response to various stress signals, it also plays a crucial role in maintaining the senescent phenotype and modulating the senescence-associated secretory phenotype (SASP) *(17)*. A potential TGF-β modulator with senolytic properties should preferably be a natural compound with an established and low-risk safety profile and a potential for feasible bioavailability optimization. Therefore, after a preliminary TGF-β inhibition screen of 480 naturally occurring compounds, we re-tested the top compounds, and the most promising candidate meeting the above mentioned criteria was evaluated on the basis of its senolytic activity and cytotoxicity.

MATERIALS AND METHODS

Compounds

GreenPharma Natural Compound Libraries (GPNCL v001 and v003) containing a total of 480 compounds were used for an initial TGF-β signaling screen (GreenPharma S.A.S., Orléans, France, unpublished). Compounds showing at least 70% inhibition of luciferase signal were re-tested using the same protocol as described below. All compounds were supplied as DMSO stock solutions.

Luciferase-based TGF-β assay

Hek293-(CAGA)12 cell line was a kind gift of Dr. Tom Thompson (Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati). This cell line contains a luciferase reporter gene under the control of a promoter containing a Smad binding element (SBE) (CAGA (12)), and robustly responds to signaling by activin and TGF-β class ligands. 1 day before treatment, cells were seeded into 96-well plates at 20000 cells per well in DMEM (high glucose) supplemented with 10% FBS and 100 µg/ml G418. After 24h, media was aspirated and replaced with 150 µl fresh DMEM (no FBS) containing 20 ng/ml growth/differentiation factor-8 (GDF-8, myostatin, Abcam ab269163) to activate the TGF-β cascade. At the same time, compounds were added at final concentration of 10µM. All treatments were performed in triplicates, and the equivalent volume of DMSO was used for controls. 24h after treatment, the medium was removed and 100 µl of 1:1 (DMEM: OneGlo Luciferase substrate) mixture was added to lyse the cells. Plates were gently vortexed for 2 minutes, and readings were taken on Tecan Infinite M200 microplate reader after 10 minutes.

Cell lines, flow cytometry and MTT test

Imr-90 (healthy fetal lung fibroblasts), HUVEC (human umbilical vein endothelial cells) and hMSC (bone-marrow-derived mesenchymal stem cells) were purchased from ATCC (CCL-186, PCS-100-010 and PCS-500-012, respectively) and were cultured following the supplier's instructions. Senescence was induced by 10 µM etoposide (Sigma Aldrich E1383) for 24 hours. 2 days after etoposide removal, cells were treated with selected compounds at 10 µM for 48 hours. Percentage of senescent cells was measured using the CellEvent™ Senescence Green Flow Cytometry Assay Kit (Abcam C10840). Briefly, after treatment and trypsinization, cells were fixed with 2% paraformaldehyde in PBS for 10 minutes at room temperature and washed twice with PBS/1% BSA. Pellets were resuspended in 1:750 dilution of CellEvent Green Senescence Probe in the supplied buffer, incubated for 1h at 37°C without CO_2, washed twice with PBS and analyzed on BD LSR II flow cytometer. At least 10000 cells were used for analyses. For cytotoxicity assessment, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; tetrazolium dye) assay was performed. Briefly, 5×10^3 cells were seeded in a 96-well plate; 24 h later, cells were treated with different concentrations of α-Mangostin and further processed as described in *(18)*.

RESULTS

Selection of TGF-β signaling inhibitors

After the initial screen, top 70 compounds were re-tested on Hek293-(CAGA)12 cells. A total of 63 compounds decreased the luciferase signal to less than 30% when compared to control. Complete list of compounds is available in Supplemental data. Out of 12 compounds that completely abolished luciferase induction, 11 were

Table 1. *Top 10 TGF-beta signaling inhibitors selected after the re-evaluation of 70-compound GPNCL library screen. Known toxins and antibiotics are excluded. Luciferase signal indicates the percent of remaining TGF-beta signaling activity.*

Compound	Description	Luciferase signal (% control)
α-Mangostin	Xanthonoid (*Garcinia mangostana)*	0
piplartine	amide alkaloid from long pepper plant (*Piper longum*)	0.8
menadione	provitamin K3, vitamin K2 precursor	6.25
wedelolactone	coumestan (*Eclipta alba*)	8.1
valtrate	iridoid (*Valeriana jatamansi*)	13.8
primin	quinone(*Primula obconica)*	15.97
corilagin	ellagitannin (various plant species)	19.03
tanshinone IIA	diterpenoid (*Salvia miltiorrhiza*)	20.19
humulone	α-lupulic acid (*Humulus lupulus*)	20.47
reynosin	sesquiterpene lactone (Magnolia grandiflora)	20.64

discarded as antibiotics and known toxins, which was expected. For those compounds, the decrease in luciferase is due to their cytotoxicity and not the specific TGF-*β* signaling inhibition. Top 10 candidates, based on toxicity and general safety data, are listed in Table 1. The most potent compound is α-Mangostin, a xanthonoid isolated from *Garcinia mangostana* (mangosteen tree). α-Mangostin is commonly consumed as part of the fruit and has also been investigated for its potential health benefits in traditional medicine and scientific research. Based on this, we decided to proceed with cytotoxicity assays and assessment of α-Mangostin senolytic potential.

Cytotoxicity assay

Using the MTT test, we assessed the cytotoxicity of different α-Mangostin concentrations after 24h treatment. Hek293-(CAGA)12 cells were the most resistant to α-Mangostin treatment. Furthermore, no significant effect of cell viability was detected at 10μM for either cell line. This suggests that the decrease in myostatin-induced TGF-*β* signaling is a specific effect of Mangostin and not its cytotoxicity. The results are in line with previous studies *(19– 21)* which were, however, focused on cancer cell lines. Here we show that, in different non-cancer cell

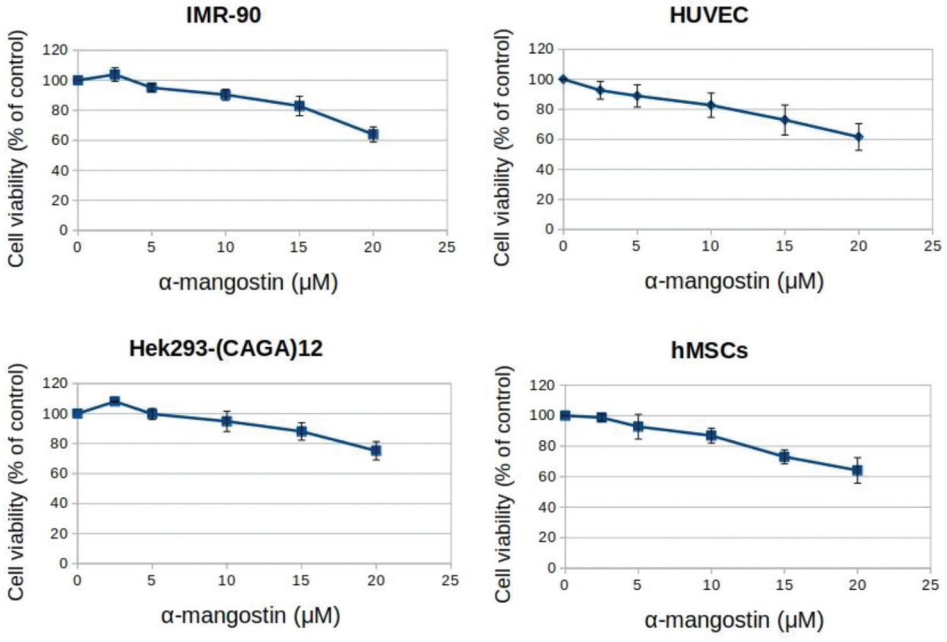

Figure 1. *The results of cell viability (MTT) assay on different cell lines after treatment with 10μM Mangostin for 24h. Imr-90, human lung fibroblasts; Hek293-(CAGA)12, model cell line for TGF-β signaling assay; HUVEC, human umbilical vein endothelial cells; hMSCs, human bone-marrow-derived mesenchymal stem cells.*

lines, α-Mangostin does not significantly reduce cell viability at a concentration sufficient to completely abolish TGF-*β* signaling.

α-Mangostin effectively removes etoposide-induced senescent Imr-90, HUVEC and hMS cells

Compared to classical immunohistochemistry, flow cytometry–adapted fluorescent beta-galactosidase substrates offer higher sensitivity, dynamic range and single cell resolution. Therefore, we chose this approach to estimate the ability of α-Mangostin to reduce the senescent cell burden in culture. In all four cell lines, 24h etoposide treatment resulted in a significant increase in the percentage of SA-*β*-gal positive cells after a 48h recovery period. α-Mangostin significantly reduced the proportion of senescent cells in Imr-90 and HUVEC lines (Figure 2).

After setting an arbitrary threshold of FL1 intensity at 10^{-2}, we could estimate and compare the percentage of SA-*β*-gal positive cells (Table 2). Due to differences in cell size/morphology, staining efficiency and sensitivity to topoisomerase inhibition by etoposide, the comparison of absolute values between cell lines is not applicable. However, relative values point to the same direction. Imr-90 cells are more sensitive to senescence induction by etoposide than HUVEC, but also a greater proportion of Imr-90 fibroblasts are removed by α-Mangostin.

Table 2. *Effect of 10 μM α-Mangostin treatment (24h) on Imr-90 and HUVEC senescent cell burden in vitro.*

	% of SA-*β*-gal positive cells	
	Imr-90	HUVEC
Control	7.25	7.54
Etoposide	29.7	19.5
α-Mangostin	10.8	12.4

In hMSCs, the senolytic effect of α-Mangostin was even more pronounced (Figure 3).

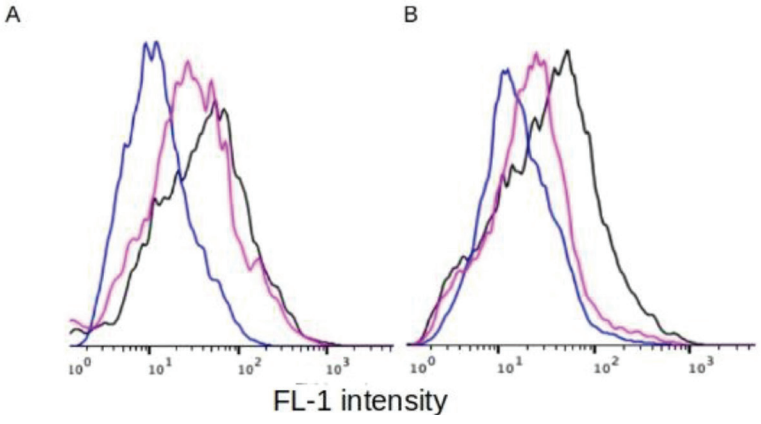

Figure 2. *SA-β-gal activity (FL1 intensity) distribution in HUVEC (A) and Imr-90 (B) cell lines. Blue, control; black, etoposide-treated cells; purple, etoposide treatment followed by 24h of 10μM α-Mangostin.*

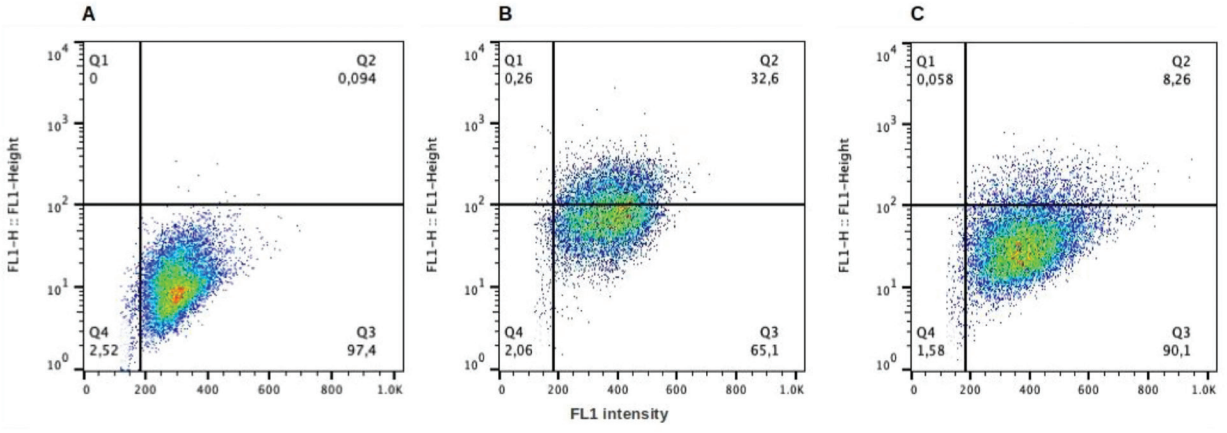

Figure 3. *Flow cytometry profiles of untreated hMSCs (A), etoposide-treated hMSCs (B) and etoposide-treated hMSCs after 24h of α-Mangostin treatment (C). Q2 quadrant corresponds to SA-β-gal positive cells. Percentages of cells are shown for each quadrant.*

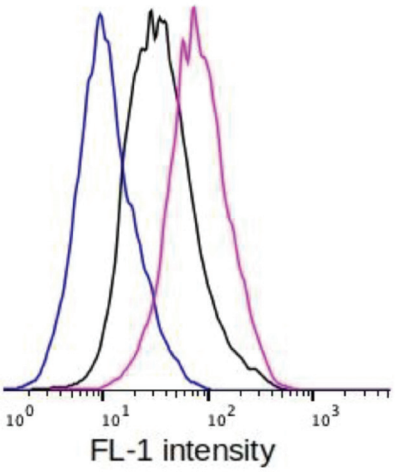

Figure 4. *SA-β-gal activity (FL1 intensity) distribution in human bone-marrow-derived mesenchymal stem cells (hMSCs). Blue, control; purple, etoposide-treated cells; black, etoposide treatment followed by 24h of 10μM α-Mangostin.*

In hMSCs, α-Mangostin reduced the senescent cell content to approx. 25% in 24 hours, with no apparent cytotoxicity (Figure 4).

DISCUSSION

Aging can be considered as a biological process implicated in the progressive decline of physiological function and increased disease susceptibility of an organism with advancing age. At the tissue level, cellular senescence contributes variably to the aging phenotype and chronic disease development *(22, 23)*. Senescent cells, while ceasing to proliferate, adopt a Senescence-Associated Secretory Phenotype (SASP), secreting a cocktail of pro-inflammatory cytokines, chemokines, growth factors, and proteases that modulate the tissue microenvironment. This state contributes to aging and the progression of age-related diseases by promoting inflammation and tissue dysfunction. On the other hand, it simultaneously benefits the organism by suppressing potential tumor formation and aiding in wound healing. However, senescence is also considered as one of the hallmarks of cancer, as SASP and 'transitory senescence' can promote tumor development and resistance to therapy *(24)*. Thus, the secretion of factors by senescent cells can enhance immune surveillance against precancerous cells, reducing cancer risk, and promote tissue repair processes, facilitating the healing of wounds but their role in carcinogenesis is of dual nature *(25, 26)*. Transforming Growth Factor-beta (TGF-β) is a pleiotropic cytokine implicated in a myriad of cellular processes, including cell growth, differentiation, apoptosis, and the immune response *(27)*. In context of cellular senescence, TGF-β signaling exerts context-dependent effects on cellular fate. TGF-β can both induce senescence in response to various stressors and also maintain the senescent phenotype and modulate SASP. This dual role makes TGF-β a target of interest for modulating senescence and, by extension, aging and related pathologies *(28, 29)*.

The search for agents that can remove senescent cells through TGF-β signaling inhibition, we have identified α-Mangostin, a natural xanthone compound found in the mangosteen fruit, as a promising candidate. α-Mangostin has been shown to have antioxidant, anti-inflammatory, and anticancer properties, making it relevant for targeting aging and cellular senescence *(30)*. Here we show that α-Mangostin abolishes GDF-8-induced TGF-β signaling in 3 different cell lines: IMR-90 (fetal), HUVEC (newborn) and hMSCs (adult donor). Also, we have demonstrated α-Mangostin's capability to reduce the burden of senescent cells in these cell lines through flow cytometry. Finally, and importantly, we confirmed that in our experimental model, the compound is not toxic to cells at effective concentration. Different cell lines used in this study point to different implications of α-Mangostin action in aging and disease research. Thus, the effect of α-Mangostin on senescent IMR-90 cell lines points towards potential applications in addressing pulmonary diseases. Lung fibroblasts becoming senescent contribute to pathologies like fibrosis and chronic obstructive pulmonary disease (COPD) *(31, 32)*, where the removal of such cells could mitigate tissue dysfunction and inflammation, suggesting a therapeutic pathway for improving lung health. In this experimental setup HUVEC cell line represents the vascular system, where the accumulation of senescent endothelial cells leads to cardiovascular diseases *(33)*. α-Mangostin's capacity to clear these cells may offer strategies to prevent or ameliorate conditions such as atherosclerosis and hypertension, thereby preserving cardiovascular health. Similarly, the impact of α-Mangostin on hMSCs highlights its potential in regenerative medicine. The senescence of mesenchymal stem cells impairs tissue repair and regeneration, crucial in conditions like osteoporosis and arthritis *(34, 35)*. By reducing senescence in these cells, α-Mangostin could enhance tissue regeneration and function, offering new therapeutic avenues.

Taken together, our data show that α-Mangostin, a readily available and basically a safe compound, is a feasible candidate for future in vivo and mechanistic studies of senescent cell removal from various tissues. The potential of its application spans from pulmonary and cardiovascular diseases to regenerative medicine.

Supplementary material is available on-line at https://hrcak.srce.hr/pb

REFERENCES

1. LÓPEZ-OTÍN C, BLASCO MA, PARTRIDGE L, SERRANO M, KROEMER G 2013 The hallmarks of aging. Cell 153: 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

2. LÓPEZ-OTÍN C, BLASCO MA, PARTRIDGE L, SERRANO M, KROEMER G 2023 Hallmarks of aging: An expanding universe. Cell 186: 243–278. https://doi.org/10.1016/j.cell.2022.11.001
3. HAYFLICK L, MOORHEAD PS 1961 The serial cultivation of human diploid cell strains. Exp Cell Res 25: 585–621. https://doi.org/10.1016/0014-4827(61)90192-6
4. GORGOULIS V, ADAMS PD, ALIMONTI A, BENNETT DC, BISCHOF O, BISHOP C, CAMPISI J, COLLADO M, EVANGELOU K, FERBEYRE G, GIL J, HARA E, KRIZHANOVSKY V, JURK D, MAIER AB, NARITA M, NIEDERNHOFER L, PASSOS JF, ROBBINS PD 2019 Cellular Senescence: Defining a Path Forward. Cell 179: 813–827. https://doi.org/10.1016/j.cell.2019.10.005
5. ZHANG L, PITCHER LE, YOUSEFZADEH MJ, NIEDERNHOFER LJ, ROBBINS PD, ZHU Y 2022 Cellular senescence: a key therapeutic target in aging and diseases. J Clin Invest 132. https://doi.org/10.1172/JCI158450
6. MEHDIZADEH M, AGUILAR M, THORIN E, FERBEYRE G, NATTEL S 2022 The role of cellular senescence in cardiac disease: basic biology and clinical relevance. Nat Rev Cardiol 19: 250—264. https://doi.org/10.1038/s41569-021-00624-2
7. MARTÍNEZ-CUÉ C, RUEDA N 2020 Cellular Senescence in Neurodegenerative Diseases. Front Cell Neurosci 14: 16. https://doi.org/10.3389/fncel.2020.00016
8. SCHMITT CA, WANG B, DEMARIA M 2022 Senescence and cancer - role and therapeutic opportunities. Nat Rev Clin Oncol 19: 619–636. https://doi.org/10.1038/s41571-022-00668-4
9. MURAKAMI T, INAGAKI N, KONDH H 2022 Cellular Senescence in Diabetes Mellitus: Distinct Senotherapeutic Strategies for Adipose Tissue and Pancreatic β Cells. Front Endocrinol 13: 869414. https://doi.org/10.3389/fendo.2022.869414
10. DOCHERTY MH, O'SULLIVAN ED, BONVENTRE JV, FERENBACH DA 2019 Cellular Senescence in the Kidney. J Am Soc Nephrol 30: 726–736. https://doi.org/10.1681/ASN.2018121251
11. TOMINAGA K, SUZUKI HI 2019 TGF-β Signaling in Cellular Senescence and Aging-Related Pathology. Int J Mol Sci 20. https://doi.org/10.3390/ijms20205002
12. MATSUDA S, REVANDKAR A, DUBASH TD, RAVI A, WITTNER BS, LIN M, MORRIS R, BURR R, GUO H, SEEGER K, SZABOLCS A, CHE D, NIEMAN L, GETZ GA, TING DT, LAWRENCE MS, GAINOR J, HABER DA, MAHESWARAN S 2023 TGF-β in the microenvironment induces a physiologically occurring immune-suppressive senescent state. Cell Rep 42(3), 112129. https://doi.org/10.1016/j.celrep.2023.112129
13. SIEGEL PM, MASSAGUÉ J 2003 Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer. Nat Rev Cancer 3: 807–821. https://doi.org/10.1038/nrc1208
14. BATLLE E, MASSAGUÉ J 2019 Transforming Growth Factor-β Signaling in Immunity and Cancer. Immunity 50: 924–940. https://doi.org/10.1016/j.immuni.2019.03.024
15. KRETOVA M, SABOVA L, HODNY Z, BARTEK J, KOLLAROVIC G, NELSON BD, et al. 2014 TGF-β/NF1/Smad4-mediated suppression of ANT2 contributes to oxidative stress in cellular senescence. Cell Signal 26: 2903–11. https://doi.org/10.1016/j.cellsig.2014.08.029
16. TAZAWA R, UCHIDA K, KENMOKU T, NAKAWAKI M, MUNESHIGE K, ISHII D, INOUE G, TAKASO M 2021 Increasing transforming growth factor-beta concentrations with age decrease apelin in the rat rotator cuff. J Orthop Surg Res 16: 539. https://doi.org/10.1186/s13018-021-02675-0
17. HAN X, LI W, HE X, LU X, ZHANG Y, LI Y, BI G, MA X, HUANG X, BAI R, ZHANG H 2023 Blockade of TGF-β signalling alleviates human adipose stem cell senescence induced by native ECM in obesity visceral white adipose tissue. Stem Cell Res Ther 14: 291. https://doi.org/10.1186/s13287-023-03525-y
18. PINTERIĆ M, PODGORSKI II, SOBOČANEC S, POPVIĆ HADŽIJA M, PARADŽIK M, DEKANIĆ A, MARINOVIĆ M, HALASZ M, BELUŽIĆ R, DAVIDOVIĆ G, AMBRIČ RISTOV A, BALOG T 2018 De novo expression of transfected sirtuin 3 enhances susceptibility of human MCF-7 breast cancer cells to hyperoxia treatment. Free Radic Res 52: 672–684. https://doi.org/10.1080/10715762.2018.1462495
19. XU Q, MA J, LEI J, DUAN W, SHENG L, CHEN X, HU A, WANG Z, WU Z, WU E, MA Q, LI X 2014 α-mangostin suppresses the viability and epithelial-mesenchymal transition of pancreatic cancer cells by downregulating the PI3K/Akt pathway. BioMed Res Int 2014: 546353. https://doi.org/10.1155/2014/546353
20. CRUZ-GREGORIO A, ARANDA-RIVERA AK, APARICIO-TREJO OE, MEDINA-CAMPOS ON, SCIUTTO E, FRAGOSO G, PEDRAZA-CHAVERRI J 2023 α-mangostin induces oxidative damage, mitochondrial dysfunction, and apoptosis in a triple-negative breast cancer model. Phytother Res 37: 3394–3407. https://doi.org/10.1002/ptr.7812
21. ZHU X, LI J, NING H, YUAN Z, ZHONG Y, WU S, ZENG J-Z 2021 α-mangostin Induces Apoptosis and Inhibits Metastasis of Breast Cancer Cells via Regulating RXRα-AKT Signaling Pathway. Front Pharmacol 12: 739658. https://doi.org/10.3389/fphar.2021.739658
22. BAKER DJ, CHILDS BG, DURIK M, WIJERS ME, SIEBEN CJ, ZHONG J, SALTNESS RA, JEGANATHAN KB, VERZOSA GC, PEZESHKI A, KHAZAIE K, MILLER JD, VAN DEURSEN JM 2016 Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature 530: 184–189. https://doi.org/10.1038/nature16932
23. OGRODNIK M, ZHU Y, LANGHI LGP, TCHKONIA T, KRÜGER P, FIELDER E, VICTORELLI S, RUSWHANDI RA, GIORGADZE N, PIRTSKHALAVA T, PODGORNI O, ENIKOLOPOV G, JOHNSON KO, XU M, INMAN C, PALMER AK, SCHAFER M, WEIGL M, IKENO Y, JURK D 2019 Obesity-Induced Cellular Senescence Drives Anxiety and Impairs Neurogenesis. Cell Metab 29: 1061-1077.e8. https://doi.org/10.1016/j.cmet.2018.12.008
24. HANAHAN D 2022 Hallmarks of Cancer: New Dimensions. Cancer Discov 12: 31–46. https://doi.org/10.1158/2159-8290.cd-21-1059
25. COPPÉ J-P, DESPREZ P-Y, KRTOLICA A, CAMPISI J 2010 The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 5: 99–118. https://doi.org/10.1146/annurev-pathol-121808-102144
26. YANG J, LIU M, HONG D, ZENG M, ZHANG X 2021 The Paradoxical Role of Cellular Senescence in Cancer. Front Cell Dev Biol 9: 722205. https://doi.org/10.3389/fcell.2021.722205
27. MASSAGUÉ J, SHEPPARD D 2023 TGF-β signaling in health and disease. Cell 186: 4007–4037. https://doi.org/10.1016/j.cell.2023.07.036
28. LIU J, ZHANG J, LIN X, BOYCE BF, ZHANG H, XING L 2022 Age-associated callus senescent cells produce TGF-β1 that inhibits fracture healing in aged mice. J Clin Invest 132. https://doi.org/10.1172/JCI148073
29. UNTERGASSER G, GANDER R, RUMPOLD H, HEINRICH E, PLAS E, BERGER P 2003 TGF-β cytokines increase senescence-associated beta-galactosidase activity in human prostate basal cells by supporting differentiation processes, but not cellular senescence. Exp Gerontol 38: 1179–1188. https://doi.org/10.1016/j.exger.2003.08.008
30. GUTIERREZ-OROZCO F, CHITCHUMROONCHOKCHAI C, LESINSKI GB, SUKSAMRARN S, FAILLA ML 2013 α-mangostin: anti-inflammatory activity and metabolism by human

cells. J Agric Food Chem 61: 3891–3900. https://doi.org/10.1021/jf4004434

31. LIU R-M, LIU G 2020 Cell senescence and fibrotic lung diseases. Exp Gerontol 132: 110836. https://doi.org/10.1016/j.exger.2020.110836

32. HANSEL C, JENDROSSEK V, KLEIN D 2020 Cellular Senescence in the Lung: The Central Role of Senescent Epithelial Cells. Int J Mol Sci 21: 93279. https://doi.org/10.3390/ijms21093279

33. HU C, ZHANG X, TENG T, MA Z-G, TANG Q-Z 2022 Cellular Senescence in Cardiovascular Diseases: A Systematic Review. Aging Dis 13: 103–128. https://doi.org/10.14336/AD.2021.0927

34. LIU Y, CHEN Q 2020 Senescent Mesenchymal Stem Cells: Disease Mechanism and Treatment Strategy. Curr Mol Biol Rep 6: 173–182. https://doi.org/10.1007/s40610-020-00141-0

35. LI Y, WU Q, WANG Y, LI L, BU H, BAO J 2017 Senescence of mesenchymal stem cells (Review). Int J Mol Med 39: 775–782. https://doi.org/10.3892/ijmm.2017.2912