

15th Alliance for Healthy Aging Conference

From mechanisms of age-associated diseases to healthy aging

October 11–13, 2026, Cologne, Germany

Abstract Booklet



Organisation:

CECAD (Cologne Cluster of Excellence on Aging and Aging-associated Diseases)



MAX PLANCK INSTITUTE
FOR BIOLOGY OF AGEING



Max Planck Institute
for Metabolism Research



Venue:

Max Planck Institute for Biology of Ageing &
CECAD Research Center, Cologne, Germany

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Program

Sunday, October 11th

CECAD Foyer and Lecture Hall

16:00 – 20:00	Registration
17:00 – 19:00	Trainee Round Table Event (Lecture Hall, by registration only)
19:00 – 21:00	Welcome Reception (by registration only)

Monday, October 12th

Max Planck Institute for Biology of Ageing – Foyer

8:00 – 18:30	Registration
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Max Planck Institute for Biology of Ageing – Lecture Hall

9:00 – 9:15	Welcome Statements: Björn Schumacher, CECAD, Cologne	
9:15 – 10:00	Keynote lecture: Emma Teeling, University College Dublin	Repair, remove, resist: How do bats achieve extended healthspans?

Session 1: Metabolism / Proteostasis / Nutrition

Chair: Björn Schumacher

10:00 – 10:20	Invited Speaker: Constantinos Demetriades, University Medical Center Groningen	Spatial Organization of Nutrient Signaling to mTORC1
10:20 – 10:35	Brecht Attema, ERIBA, University Medical Center Groningen	Elevated plasma bile acid levels are associated with cardiac alterations in mice and men
10:35 – 10:50	Joris Demmers, CECAD, Cologne	Transcription speed is controlled by mTOR via the super elongation complex
10:50 – 11:05	Kazuto Kawamura, Max Planck Institute for Biology of Ageing, Cologne	Restoration of biological age during prolonged fasting–refeeding mediated by a fasting-responsive linker histone
11:05 – 11:20	Ingrid van der Pluijm, Erasmus University Medical Center, Rotterdam	XPG deficiency accelerates vascular aging via VSMC phenotypic switching and metabolic dysfunction, which is mitigated by dietary restriction
11:20 – 11:35	Seda Koyuncu, CECAD, Cologne	The aging factor EPS8 induces disease-related protein aggregation through RAC signaling hyperactivation
11:35 – 11:50	Jonas Martin Schmidt, Goethe University Frankfurt	Spatiotemporal Proteostasis Rewiring in Senescence Progression Revealed by Translatome & Mitochondrial Importome Analyses

11:50 – 12:05	Kathrin Ulrich, Saarland University, Saarbrücken	A redox-regulated chaperone in inter-tissue proteostasis regulation maintains a functional proteome and promotes healthy aging
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12:05 – 13:15 LUNCH

Session 2: Senescence

Chair: Gavin Richardson

13:15 – 13:35	Invited Speaker: Marco Demaria, University Medical Center Groningen	Purinergic signaling reinforces an inflammatory SASP and promotes senescence-associated dysfunctions
13:35 – 13:50	Daniela Costa, Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester	Tomatidine is a senotherapeutic compound that improves cognitive function and reduces cellular senescence in aged mice
13:50 – 14:05	Helene Martini, Mayo Clinic, Rochester	Mitochondrial metabolism and epigenetic crosstalk in senescent cells
14:05 – 14:20	Allancer Nunes, University of Minnesota	Agonists of extra-oral bitter taste receptors suppress cellular senescence and enhance GLP-1 secretion
14:20 – 14:35	Lilian Sales Gomez, Mayo Clinic, Rochester	Cellular Senescence in the Aging Liver Contributes to Cognitive Decline – Crosstalk between Liver and Brain
14:35 – 14:55	Invited Speaker: Joao Passos, Mayo Clinic, Rochester	Mitochondrial nucleic acids: drivers of Inflammaging
14:55 – 15:10	Markus Schosserer, Medical University of Vienna	Exploring ribosomal RNA modifications and corresponding snoRNAs in human skin cell senescence
15:10 – 15:25	Boshi Wang, ERIBA, University Medical Center Groningen	Sexually dimorphic expression of FABP5 favors tissue persistence of chemotherapy-induced senescence in male mice
15:25 – 15:45	Invited Speaker: Xavier Revelo, University of Minnesota	Cellular Senescence in Macrophages: Implications for Aging and Disease

15:45 – 16:15 COFFEE BREAK

Session 3: DNA

Chair: Lene Juel Rasmussen

16:15 – 16:30	Arturo Bujarrabal-Dueso , Institute for Genome Stability in Aging and Disease, CECAD, Cologne	A Novel H2A.Z-Dependent Mechanism Underlies DREAM Complex Regulation of the DNA Damage Response
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16:30 – 16:45	Taiyu Ge, Institute for Genome Stability in Aging and Disease, CECAD, Cologne	Vitamin B12 boosts H3K4me2 deposition to promote transcription recovery following DNA damage
16:45 – 17:00	Isabela Santos Valentim, Leibniz-Institut für Alternsforschung- Fritz-Lipmann-Institut, Jena	DREAMing of Healthy Aging: Deciphering the Molecular Links Between Sleep, Circadian Rhythms, and Cellular Repair
17:00 – 17:15	Ilze Skujina, University of Galway, University College Dublin	Rapid DNA double-strand break repair as a candidate mechanism of exceptional longevity in the bat <i>Myotis myotis</i>
17:15 – 17:30	Seunghwa Woo, Mayo Clinic, Rochester	Telo-CUT: A Telomere-Specific DNA Damage Model Reveals Drivers of Aging
17:30 – 17:50	Morten Scheibye-Knudsen, University of Copenhagen	Data driven interventions in human aging

17:50 – 18:15 BREAK

CECAD Research Center, Foyers and 1st floor

18:15 – 22:00 Poster Session (with finger food and drinks)

18:30 – ca. 19:25 Poster Flash Talks (4 minutes each)

18:30 – 18:34	Maarouf Baghdadi , Max Planck Institute for Biology of Ageing, Cologne	The combined treatment with rapamycin and trametinib partially recapitulates the in vitro molecular effects of genetic variants identified in exceptionally long-lived individuals
18:35 – 18:39	Mengyuan Cai , Erasmus MC, Rotterdam	Epigenetic Aging Indicators are Associated with Bone Mineral Density in a Population-Based Setting
18:40 – 18:44	Ariel Eraso , Senckenberg, Translational Biodiversity Genomics, Frankfurt	Aggregate Lifespan Encyclopaedia (ALE) – A new way to record animal lifespans
18:45 – 18:49	Ana Catarina Franco , Mayo Clinic, Rochester	A Spatial Multi-Omic Atlas of Cellular Senescence in Mouse and Human Skin
18:50 – 18:54	Angelina Job Kolady , CECAD, Cologne	Unravelling the role of H3K4me2 in response to DNA damage and transcription stress during development and ageing
18:55 – 18:59	Sandeep Kumar , Chulalongkorn University, Bangkok	Hesperidin and Melatonin Synergistically Enhance Longevity and Mitigate Amyloid- α Toxicity Through ACR-16-Dependent Activation of DAF-16/NRF2 Driven Autophag
19:00 – 19:04	Joelina Lienau , University of Copenhagen	Old muscle, new rules: muscle derived FGF21 drives systemic resistance to protect failing muscle
19:05 – 19:09	Christos Mavrommatis , University of Cologne / Mayo Clinic, Rochester	Methylation-associated Gene Expression (MaGE) proxies of cellular senescence: scalable systemic senescence biomarkers in relation to disease severity and 174 incident disease outcomes

19:10 – 19:14	Ayesha Sanober , IMB gGmbH, Mainz	Accelerated aging is a determinant of arterial thrombosis risk in individuals with low genetic and cardiovascular risk factor burden
19:15 – 19:19	Attila Sebe , University of Oulu	Lower blood cell mitochondrial DNA amount and inflammation associates with higher all-cause mortality and aging: a 30-year prospective epidemiological study
19:20 – 19:24	Feixiang Yan , Mayo Clinic, Rochester	A ttntv cardiomyopathy model in African Turquoise Killifish enables identification of aging hallmark-associated gerotherapeutic strategies

Tuesday, October 13th

Max Planck Institute for Biology of Ageing – Foyer

8:00 – 18:30 Registration/Information

Max Planck Institute for Biology of Ageing – Lecture Hall

Chair: Paul Robbins

9:00 – 09:45	Keynote lecture: Guido Kroemer , Université de Paris Cité	ACBP/DBI: a pro-aging danger signal
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Session 4: Immunology

9:45 – 10:05	Christina Camell , University of Minnesota	Chronic inflammation and organ dysfunction driven by an ageing immune system
10:05 – 10:20	Drake Alton, Robert and Arlene Kogod Center on Aging, Mayo Clinic, Roches- ter	Immune CD38 Overexpression Drives Systemic NAD Depletion and Physiologic Decline
10:20 – 10:35	Claudia Chini, Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester	Inducible Chronic NAD Deficiency in Mice drives Inflammation and Cellular Senescence
10:35 – 10:50	Mauro Corrado, University of Cologne / CECAD	Suppression of T Cell–Driven Type I Interferons in Ageing Reveals Gut-Specific Vulnerability
10:50 – 11:05	David Achudhan, Mayo Clinic, Roches- ter	MMP12 drives senescence-related bone loss via the cGAS–STING pro-inflammatory pathway
11:05 – 11:20	Kavya Gupta, Mayo Clinic, Rochester	Rejuvenating the Aging Bone Marrow Niche to Mitigate Clonal Hematopoiesis via GLP-1 Mediated HSPCs Regeneration

11:20–12:45 LUNCH

Session 5: Neuroscience

Chair: Folkert Kuipers

12:45 – 13:05	Anne Schäfer, Max Planck Institute for Biology of Ageing, Cologne	Immunoregulatory Microglia in Alzheimer's Disease
13:05 – 13:20	Alessandro Gialluisi, LUM University, Ca- samassima & IRCCS Neuromed, Italy	Independent influence of multidomain aging clocks on cardio- and cerebro-vascular risk: evidence from an Italian population cohort
13:20 – 13:35	Sara Graves, Mayo Clinic, Rochester	Sex-specific benefits of p21 knockout in PS19 tauopathy female, but not male, mice
13:35 – 13:50	Amanda Latham, Mayo Clinic, Rochester	Characterizing Cellular Drivers of Cognitive Decline Across the Aging Mouse Hippocampus
13:50 – 14:05	David H. Meyer, Uni- versity of Cologne, CECAD	Aging clocks delineate neuron types vulnerable or resilient to neurodegeneration and identify neuroprotec- tive interventions
14:05 – 14:20	Allyson Palmer, Mayo Clinic; Rochester	Delirium and Long-Term Cognitive Trajectories in Cognitively Normal Older Adults: Insights from the Mayo Clinic Study of Aging
14:20 – 14:35	Yossawat Suwanlikit, Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester	DNA Methylation-Based Clonal Hematopoiesis Burden Associates with Structural Brain Atrophy

14:35 – 15:15 **Coffee Break**

Session 6: Physiology

Chair: Nathan LeBrasseur

15:15 – 15:35	Invited Speaker: Simon Tual-Chalot, Newcastle University	ADAR2 drives atherosclerosis by controlling metabolic inflammation
15:35 – 15:55	Invited Speaker: Kristina Kirschner, Mayo Clinic, Rochester	One clone, many diseases: blood mutations and the ageing body
15:55 – 16:10	Andrea Heinz, Univer- sity of Copenhagen	Elastin remodeling and elastokine signaling in aging tissues
16:10 – 16:25	Eduardo Chini, Mayo Clinic, Rochester	NAD ⁺ as a Therapeutic Lever for Renal Resilience in Perioperative Acute Kidney Injury
16:25 – 16:40	Walter Sandt, CECAD, Cologne	Evolutionary conserved diapause mechanisms of slowed aging and rejuvenation
16:40 – 17:00	Invited Speaker: Fabian Finger, University Copenhagen	Mitochondrial membrane lipid cardiolipin controls fiber- type adaptations in aging muscle via ERR γ
17:00 – 17:20	Invited Speaker: Jane Reznick, CECAD, Cologne	Young at Heart: neoteny and regeneration in the naked mole-rat

17:20 -17:35 Concluding remarks:
Nathan LeBrasseur, Mayo Clinic, Rochester

Cologne Brauhaus (by registration only)

19:00 – 22:00 Conference Dinner

Invited and keynote speakers



Names in alphabetic order:
Marco Demaria, Groningen
Christina Camell,
Minnesota
Constantinos Demetriades,
Groningen,
Fabian Finger, Groningen
Kristina Kirschner,
Rochester
Guido Kroemer, Paris

Joao Passos, Rochester
Xavier Revelo, Minnesota
Jane Reznick, Cologne
Anne Schäfer, Cologne
Morten Scheibye-Knudsen,
Copenhagen
Emma Teeling, Dublin
Simon Tual-Chalot,
Newcastle

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Immune CD38 Overexpression Drives Systemic NAD Depletion and Physiologic Decline

Drake Alton^{1,2}; Gustavo Souza M.S.³; Gina Ciccio M.S.³; Katrina Bueser³; Shangcheng Yu Ph.D.³; Laura Coleman Ph.D.³; Yasmin Jahan-mihan³; Bing Han M.D., Ph.D.³; Sonu Kashyap Ph.D.³; Claudia C.S. Chini, Ph.D.^{3,4}; Eduardo N. Chini, M.D., Ph.D.^{3,4,5}

¹ Mayo Clinic Alix School of Medicine, FL, USA

² Mayo Clinic Graduate School of Biomedical Sciences, FL, USA

³ Mayo Clinic Department of Anesthesiology, FL, USA

⁴ Mayo Clinic Department of Molecular and Pharmacology and Experimental Therapeutics, MN, USA

⁵ Kogod Center on Aging at Mayo Clinic, MN, USA

CD38 is a major driver of age-associated NAD decline and a key regulator of metabolic health. Although immune-derived CD38 is closely linked to hallmarks of aging, including senescence, whether CD38 can causally drive systemic age-like decline remains unknown. Here, we generated a hematopoietic CD38 overexpression mouse using a Vav-iCre system and observed 50–90% NAD depletion in every organ tested. CD38 OE mice were viable, but displayed reduced exercise capacity, metabolic rewiring, increased frailty, and decreased survival. High-parameter immunophenotyping revealed broad immune remodeling, including multiple changes that mimic the aged immune system, while histological analysis showed striking increases in fibrosis and inflammation. Transcriptional profiling further demonstrated tissue adaptation to chronic NAD depletion, including upregulation of the mitochondrial NAD transporter SLC25A51. Together, these findings establish immune-derived CD38 as sufficient to drive organism-wide NAD depletion and systemic decline and further defines its relationship with the hallmarks of aging.

Elevated plasma bile acid levels are associated with cardiac alterations in mice and men

Attema, B.¹; Eijgenraam, T.R.^{2,3}; Yntema, T.²; de Vries, H.D.^{2,4}; Kloosterhuis, N.J.²; Koster, M.H.²; Hovingh, M.V.²; Mulder, N.L.²; de Bruyn, K.⁵; de Boer, J.F.^{2,5}; & Kuipers, F.^{1,5}

¹ *European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;*

² *Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;*

³ *Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;*

⁴ *Faculty Campus Fryslân, University of Groningen, Leeuwarden, The Netherlands;*

⁵ *Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*

Ageing increases the risk of cardiovascular disease (CVD), the leading cause of death in elderly. CVD frequently co-occurs with liver disease, and elevated circulating bile acids (BAs) have been implicated in cardiovascular dysfunction, but the underlying mechanisms are unresolved. This study examined the relationship between circulating BAs and heart disease. Female Cyp2c70^{-/-} mice, exhibiting a human-like BA composition with cholangiopathic liver disease, fibrosis, and hypercholanemia, were studied at 6 and 12 months to assess heart function. Plasma BAs were >10-fold higher compared to controls and predominantly consisted of hydrophobic BAs. Cardiac assessment revealed contractile dysfunction with increased cardiac volumes and output, together with cardiac inflammation and metabolic dysfunction. In patients with chronic heart failure (HF), plasma BAs, but not liver damage markers, were positively correlated with the HF marker NT-proBNP. Sulfated lithocholic acids showed the strongest association. All in all, findings identify BAs as mediators of hepato-cardiac interactions and implicate them as potential modulators of CVD during ageing.

A Novel H2A.Z-Dependent Mechanism Underlies DREAM Complex Regulation of the DNA Damage Response

Bujarrabal-Dueso, A.^{1,2}; Schumacher, B.^{1,2}.

¹ *Institute for Genome Stability in Aging and Disease, Medical Faculty, University and University Hospital of Cologne, Cologne, Germany*

² *Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany*

The DREAM complex is a conserved transcriptional repressor of cell cycle genes that promotes quiescence. Using *C. elegans* we found that DREAM binds and represses multiple genes involved in the DNA damage response. DREAM-complex-deficient mutants show a remarkable resistance to, and improved repair of various DNA-damage types. The mechanisms of DREAM complex regulation are not fully understood, but emerging evidence shows that this complex interacts with epigenetic modulators that could be driving its repressive function.

Using a forward genetic screen in *C. elegans* we found that a mutation for the gene *ssl-1* (ortholog of SRCAP/EP400), involved in the maintenance and deposition of H2A.Z, confers severe sensitivity to DNA damage. Employing RNA-seq and ChIP-seq analysis on these mutants we observe a broad downregulation of DREAM-associated genes, including repair genes. Repressed genes in *ssl-1* mutants are characterized by a marked reduction of H2A.Z around the promoter area, suggesting a novel function for H2A.Z deposition that regulates the normal expression of DNA repair genes. Furthermore, the capacity to deposit H2A.Z is also required for the DNA damage resistance phenotype of DREAM deficient organisms.

Inducible Chronic NAD Deficiency in Mice drives Inflammation and Cellular Senescence

Claudia C. S. Chini¹, Andres Benitez-Rosendo¹, Morgan Feuz², Gina L. Ciccio Lopez¹, Gustavo H. Souza¹, Laura Colman¹, Katrina Rae Bueser¹, Sonu Kashyap¹, Ralph Meyer²; Mirella Meyer-Ficca², and Eduardo N. Chini¹.

¹Metabolism and Molecular Nutrition Laboratory, Kogod Center on Aging, Department of Anesthesiology and Perioperative Medicine, Mayo Clinic College of Medicine, Jacksonville, Florida, USA.

²Department of Veterinary Clinical and Life Sciences, College of Veterinary Medicine, Utah State University, Logan, UT, USA.

Nicotinamide adenine dinucleotide (NAD) is a critical metabolite that declines in vitamin B3 (niacin) deficiency, disease states, and aging. However, it is unclear whether NAD deficiency can directly drive inflammation and cellular senescence in vivo. We characterized an inducible transgenic mouse model of acquired niacin dependency (ANDY), that when placed on a niacin-free diet (NFD) develop multisystem tissue NAD decline. Male and female ANDY mice fed NFD showed increased inflammation, as evidenced by increased hepatic mRNA expression of inflammatory markers, immune cell infiltration, and oxidative stress. In addition, NFD-fed mice showed increased senescence-associated β -galactosidase (SA- β -gal) activity, as well as increased expression of cell cycle arrest genes in several tissues, indicating a higher senescence burden. Importantly, dietary niacin supplementation restored NAD levels and reversed many of these senescence-related phenotypes. Together, these findings demonstrate that chronic NAD deficiency is sufficient to drive inflammation and cellular senescence in vivo, and that these processes are at least partially reversible.

NAD⁺ as a Therapeutic Lever for Renal Resilience in Perioperative Acute Kidney Injury

Kashyap, S.¹; Kanamori, K.S.²; Chini, C.C.¹; Ciccio Lopez, G.L.¹; and **Eduardo N. Chini**¹

¹ *Signal Transduction and Molecular Nutrition Laboratory, Kogod Aging Center, Department of Anesthesiology and Perioperative Medicine, Mayo Clinic College of Medicine, Jacksonville, FL. 32224, USA.*

² *Department of Surgery, Division of Vascular Surgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California 90095, USA.*

NAD⁺ is essential for kidney energy metabolism, supporting mitochondrial respiration, ATP production, and cellular repair in this highly metabolic organ. To test whether perioperative NAD⁺ augmentation reduces surgery-associated AKI, we conducted a randomized, double-blind, placebo-controlled trial in 52 patients undergoing high-risk cardiovascular or vascular surgery. Participants received placebo (N=26) or Basis™ (N=26), containing nicotinamide riboside and pterostilbene, starting two weeks before surgery and continuing for six weeks after surgery. Basis™ reduced AKI incidence by RIFLE criteria and significantly lowered the rate of >30% eGFR decline (4.8% vs 30.4%; P=0.048) and postoperative BUN increase >50% (4.8% vs 34.8%; P=0.023). Further, placebo-treated patients meeting RIFLE AKI criteria showed greater increase in creatinine, BUN, cystatin C, and decline in eGFR compared to Basis™. Basis™ also significantly increased PBMC NAD⁺ levels at hospital discharge. These findings support perioperative NAD⁺ augmentation as a promising metabolic strategy to preserve renal function and improve resilience to surgical ischemic stress.

Suppression of T Cell–Driven Type I Interferons in Ageing Reveals Gut-Specific Vulnerability

Corrado, M.^{1,2,3}

¹ Institute for Genetics, Faculty of Mathematics and Natural Sciences, University of Cologne, Cologne, DE

² Cologne Excellence Cluster on Aging and Aging-associated Diseases (CECAD), University of Cologne, Cologne, DE

³ Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, DE

Ageing is associated with chronic sterile inflammation (inflammageing), progressive metabolic fitness impairment and immune function decline. Leveraging transcriptional, proteomic and metabolic profiling of human and mouse T cells isolated from young and elderly donors, we found a prominent Type I interferon (Type I IFN) signature and mitochondrial alterations in the elderly cohort (more prominent in males than females). Notably, mitochondrial deficiencies have been linked to Type I IFN response through a mechanism dependent on mitochondrial DNA (mtDNA) release and its activation of the cGAS–STING pathway. This is commonly considered a targetable pathway to reduce inflammation and improve healthspan in ageing. Nevertheless, the mechanism of mtDNA release in T cells as well as the physiological impact on gut homeostasis of inhibiting Type I IFNs during ageing has not been clarified. We found that T cells during ageing present mitochondrial alterations with reduction of cardiolipin content and that cardiolipin deficiency alone phenocopies T cell ageing with mtDNA release via BAX/BAK pores and cGAS/STING-mediated Type I IFN response activation. While we confirmed this pathway is genetically and pharmacologically treatable, its genetic inhibition in mice with T cell specific cardiolipin deficiency resulted in additional deterioration of gut phenotype with more severe colitis and immune infiltration, leading to a more premature death in mice. This study shows the sex- and tissue-specific evolutionary conserved functional heterogeneity of Type I IFN response in human and mice in different tissues. While Type I IFNs suppression in non-barrier sites could benefit healthspan during ageing, its abrogation at barrier sites impairs control of microbiota thereby accelerating age-associated tissue dysfunction.

Tomatidine is a senotherapeutic compound that improves cognitive function and reduces cellular senescence in aged mice

Costa, Daniela G.^{1,2,3,4*}; Gee, Lucy M.^{1,2*}; Lee, Gung^{1,2}; Sales Gomez, Lilian^{1,2}; Franco, Ana Catarina^{1,2}; Vizioli, Maria Grazia^{1,2}; Valdivieso, Karla^{1,2}; Zhang, Lei J.^{5,6}; Pirius^{1,2}; Martini, Helene^{1,2}; Porritt, Rebecca A.⁷; Saul, Dominik⁸; Cavadas, Claudia^{3,4}; Ebert, Scott⁹; LeBrasseur, Nathan K.^{1,2}; Khosla, Sundeep^{1,2,9}; Passos, João F.^{1,2}; Adams, Christopher M.⁹; Robbins, Paul D.⁶; Jurk, Diana^{1,2}

¹ Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN 55905, USA

² Robert and Arlene Kogod Center on Aging, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

³ Center for Neuroscience and Cell Biology (CNC-UC), University of Coimbra, Portugal; Center for Innovation in Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal.

⁴ Faculty of Pharmacy, University of Coimbra, Portugal.

⁵ Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA, USA

⁶ Masonic Institute on the Biology of Aging and Metabolism, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA.

⁷ Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

⁸ Robert Bosch Center for Tumor Diseases, Stuttgart

⁹ Division of Endocrinology, Diabetes, Metabolism and Nutrition, Mayo Clinic, Rochester, MN, USA.

*Contributed equally

Cellular senescence is a key driver of aging and age-related dysfunction across tissues, including the brain. Through a high-content phenotypic screen of natural compounds in senescent cells, we identified tomatidine, a tomato-derived aglycone of tomatine, as a previously unrecognized senotherapeutic agent. In vitro, tomatidine selectively suppressed senescence-associated secretory phenotype (SASP) expression in senescent human brain microvascular endothelial cells and fibroblasts without altering p16Ink4a or p21Cip1 levels, consistent with a senomorphic mechanism. In aged mice, tomatidine treatment reduced frailty and improved motor coordination and cognitive performance. These functional improvements were accompanied by decreased markers of senescence (p16Ink4a, p21Cip1, and telomere-associated DNA damage foci) in multiple tissues, including liver, skin, and hippocampal neurons. In the brain, tomatidine reduced endothelial cell senescence, increased tight junction protein expression, and attenuated neuroinflammation and microglial activation, suggesting improved blood-brain barrier integrity. Together, these findings identify tomatidine as a promising senescence-targeting compound with systemic and neuroprotective effects in aging, supporting translational development for age-related disorders.

MMP12 drives senescence-related bone loss via the cGAS-STING pro-inflammatory pathway

David Achudhan^{1,2}; Susan Law²; Robert J. Pignolo^{1,2,4*}; Abhishek Chandra^{1,2,5*}

¹ Department of Physiology and Biomedical Engineering,

² Robert and Arlene Kogod Center on Aging,

³ Endocrine Research Unit,

⁴ Department of Medicine, ⁵Department of Biochemistry and Molecular Biology

*To whom correspondence should be addressed.

chandra.abhishek1@mayo.edu and pignolo.robert@mayo.edu

Cellular senescence is a major cause of tissue aging and multiple other acute and chronic conditions, mostly driven by an irreversible, apoptosis-resistant, non-proliferative state, and accompanied by mitochondrial dysfunction and secretion of pro-inflammatory proteins collectively called the senescence associated secretory phenotype (SASP). One such SASP factor, matrix metalloproteinase 12 (MMP12), has high expression in senescence-related conditions, but its exact roles in the pathophysiology of osteoporosis is unclear. Here, we characterized the effects of MMP12 abrogation on the regulation of senescence-associated production of SASP in vitro and in vivo, and senescence-associated bone loss. Downregulation of MMP12 function, either by a specific MMP12 inhibitor or an siRNA against MMP12, suppressed the transcription of several SASP genes and prevented mitochondrial abnormalities in senescent cells. We utilized a mouse model of Mmp12 deletion and demonstrated that both mmp12^{+/-} and mmp12^{-/-} mice prevented detrimental radiation effects on bone and promoted improved bone architecture as compared to irradiated wildtype mice. Interestingly, cellular senescence, the SASP, adipocyte marker genes and osteoclast-associated genes seen in irradiated bones of wild-type (WT) mice were diminished in irradiated bones of mmp12^{-/-} mice. Furthermore, we demonstrated that pharmacological inhibition of Mmp12 also suppressed aging and radiation-induced bone loss in WT mice. Mechanistically, MMP12 inhibition suppresses DNA damage, mitochondrial hypertrophy and activation of the cGAS-STING pathway, which regulates the SASP. These data clearly support that MMP12 is a key factor in senescence-associated bone loss, thus providing a novel therapeutic target.

Transcription speed is controlled by mTOR via the super elongation complex

Joris J.P.G. Demmers^{1,5}; Jiang Chang¹; Jose M. Heredia-Genestar¹; Barbara Steurer¹; Ziqin Tang²; Sander Barnhoorn¹; Ping Gao¹; Renata M.C. Brandt¹; Y. van Loon¹; Jeroen A.A. Demmers³; Altaf H. Sarker⁴; Serena T. Bruens¹; Arjan F. Theil¹; Priscilla K. Cooper⁴; Jurgen A. Marteijn¹; Jan H.J. Hoeijmakers^{1,2,5}; Joris Pothof¹

¹ Department of Molecular Genetics, Erasmus MC Cancer Institute, Erasmus Medical Center, 3000 CA, Rotterdam, The Netherlands

² Princess Maxima Center for Pediatric Oncology, Oncode Institute, Heidelberglaan 25, 3584 CS, Utrecht, The Netherlands

³ Proteomics Center, Erasmus Medical Center, 3000 CA, Rotterdam, The Netherlands

⁴ Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720, USA

⁵ University of Cologne, Faculty of Medicine, Cluster of Excellence for Aging Research, Institute for Genome Stability in Ageing and Disease, Joseph Stelzmann Strasse 26, 50931 Cologne, Germany

The mechanistic target of rapamycin (mTOR), a kinase whose activity inversely correlates with lifespan 1–3, is a central regulator of cell growth in response to nutrients and growth factors 3,4. Active mTOR promotes general translation 5,6 and synthesis of ribosomal and transfer RNA 7–9 to support protein translation. As transcription precedes translation, we wondered whether mTOR might also affect general transcription of mRNAs. Here, we demonstrate that mTOR regulates transcriptional output by genome-wide control of RNA polymerase II transcription elongation speed via the super elongation complex factor AFF1, a novel mTOR target that is phosphorylated by mTOR in the cytoplasm and subsequently translocates to the nucleus. This transcriptional regulation is mechanistically distinct from mTOR's role in translation. Inhibition of mTOR decreased transcription speed, reducing transcriptional output and cellular mRNA levels in both cells and mice, while simultaneously improving transcriptional accuracy, and reducing R-loops and DNA-damage-driven transcription stress, a recently discovered pro-aging phenotype across organs and species 10. These findings extend mTOR's functional role to include genome-wide RNAPII transcriptional control, thereby tightly balancing mRNA levels and translation, while also enhancing pro-aging transcription-associated phenotypes.

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Vitamin B12 boosts H3K4me2 deposition to promote transcription recovery following DNA damage

Taiyu Ge^{1,2}; Georgia Chatzinikolaou^{3,4}; Evi Goulielmaki^{3,4}; George A. Garinis^{3,4}; Siyao Wang^{1,2,5}; Björn Schumacher^{1,2,3}

¹ *Institute for Genome Stability in Aging and Disease, Medical Faculty, University and University Hospital of Cologne, Cologne, Germany*

² *Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*

³ *Department of Biology, University of Crete, Heraklion, Greece*

⁴ *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece*

⁵ *Institute of Molecular Biology (IMB), Mainz, Germany*

DNA lesions impair transcription and contribute to aging, but how nutrient state regulates recovery from this stress is unclear. Here we show that dietary vitamin B12 rescues developmental delay and lifespan shortening amid ultraviolet-induced DNA damage in *Caenorhabditis elegans*. Mechanistically, vitamin B12 benefits upon UV require the methionine cycle, whereas methionine supplementation is sufficient to recapitulate B12 effects. We further demonstrate that vitamin B12 promotes transcription recovery after DNA damage through increased H3K4me2 levels, independently of nucleotide excision repair. Consistently, methionine supplementation in human cells suppresses UV-induced apoptosis and promotes transcription recovery after DNA damage through depositing H3K4me2. Moreover, vitamin B12 supplementation extends lifespan, maintains body weight and improves liver transcription activity with an elevated H3K4me2 in progeroid mice. Together, our study reveals transcription recovery after DNA damage as metabolically regulated and identifies a conserved nutrient-epigenetic axis that promotes organismal resilience to genotoxic stress.

Independent influence of multidomain aging clocks on cardio- and cerebro-vascular risk: evidence from an Italian population cohort

Morelli, M.¹; Pepe, A.¹; Di Castelnuovo, A.¹; Costanzo, S.^{1,3}; Licastro, D.⁴; dal Monego, S.⁴; Panzera, T.¹; Magnacca, S.¹; De Curtis, A.¹; Cerletti, C.¹; Donati, M.B.¹; de Gaetano, G.¹; Iacoviello, L.^{1,2}; **Gialluisi, A.**^{1,2}

¹Epidemiology and Prevention Research Unit, IRCCS Neuromed, Pozzilli, Italy

²Department of Medicine and Surgery, LUM University, Casamassima, Italy

³Research Center in Epidemiology and Preventive Medicine (EPIMED), Department of Medicine and Surgery, University of Insubria, Varese, Italy

⁴Area Science Park, Trieste, Italy

Objective: To investigate whether different aging clocks can predict incident cardiovascular (CVD), coronary heart (CHD) and cerebrovascular events (CVE).

Methods: In the Moli-sani cohort (≥ 35 years; 53% women) we tested associations for clocks based on clinical circulating biomarkers (BloodAgeAcc and PhenoAgeAcc; N=22,315) and on DNA methylation (DNAm: Horvath, Hannum, DNAmPhenoAge, GrimAge, DunedinPACE; N=2,055).

Results: PhenoAgeAcc and BloodAgeAcc were independently associated with higher CVD hazard (1,510 incident cases): 3.4 (95% CI: 2.4–4.4)% and 2.9 (2.0–3.8)%, per 1-year biological age acceleration. Similar evidence was observed for CHD and CVE. DNAm clocks revealed further independent associations, with GrimAge (13.5 (4.6–23.2)% increased CVD risk), and a global improvement in the prediction of CHD risk (ΔC -index [95% CI]: 0.008 [0.001–0.025]).

Conclusions: Multidomain aging clocks are useful predictors of cardiovascular events, explaining partially independent fractions of risk. Their incremental value for CVD risk prediction, especially beyond established clinical risk factors, warrants further evaluation.

Sex-specific benefits of p21 knockout in PS19 tauopathy female, but not male, mice.

Sara I. Graves¹; Karthik B. Jeganathan¹; and Darren J. Baker^{1,2,3,4}

¹ Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN, USA

² Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA

³ Paul F. Glenn Center for Biology of Aging Research, Mayo Clinic, Rochester, MN, USA

⁴ The Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, USA

The protein encoded by the *Cdkn1a* locus, p21^{waf1/cip1}, plays important roles in cell cycle inhibition, regulation of apoptosis, and DNA damage response. Upregulation of p21^{waf1/cip1} is also one mechanism by which senescent cells enter and maintain a state of stable growth arrest. Accumulation of p21^{waf1/cip1}-positive senescent cells is observed in aging and age-related diseases, including neurodegenerative diseases like Alzheimer's disease. We have previously shown that preventing senescent cell accumulation via p16^{ink4a}-based manipulations largely prevents tau pathology in a mouse model of Alzheimer's disease. However, direct evidence for p21^{waf1/cip1} upregulation as a driver of tau pathology in either senescence-dependent or senescence-independent contexts does not yet exist. This study tests the necessity of p21 expression for neurodegenerative disease phenotypes by genetically ablating p21 in a mouse model of tauopathy. We report sexually dimorphic attenuation of senescence-associated gene expression, neuroinflammation, neurovascular abnormalities, phosphorylated tau burden, and neurodegeneration with knockout of p21 in females, but not males. These results provide interesting clues into the sex-specific biology of tau-dependent disease.

Rejuvenating the Aging Bone Marrow Niche to Mitigate Clonal Hematopoiesis via GLP-1 Mediated HSPCs Regeneration

Gupta, K.^{1,2*}; Arif, N.^{1,2*}; Robertson, N. A.^{1,2}; Rowsey, J. L.^{2,3}; Thompson, K. L.²; Monroe, D.G.^{2,3}; LeBrasseur, N. K.²; Khosla, S.^{2,3,4}; Robbins, P. D.^{5,6}; Jurk, D.^{2,4,7}; Passos, J.^{2,4}; Chandra, T.^{2,8,9}; Kirschner, K.^{1,2,8}

¹Department Internal Medicine, Division of Hematology, Mayo Clinic, Rochester, MN, USA

²Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, USA

³Division of Endocrinology, Mayo Clinic, Rochester, MN, USA

⁴Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA

⁵Masonic Institute on the Biology of Aging and Metabolism, University of Minnesota, Twin Cities, Minneapolis, MN 55455, USA

⁶Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Twin Cities, Minneapolis, MN 55455, USA

⁷Department of Neurology, Mayo Clinic, Rochester, MN, USA

⁸Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, USA

⁹Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN, USA

*Contributed equally

Hematopoietic aging is driven by inflammatory remodeling of the bone marrow niche, impairing hematopoietic stem and progenitor cell (HSPC) function and promoting expansion of Clonal Hematopoiesis of Indeterminate Potential (CHIP). While intrinsic stem cell defects are well studied, how age-associated stromal dysfunction regulates stem cell fitness and clonal selection remains unclear. We investigate whether activation of Glucagon-Like Peptide-1 (GLP-1) signaling can rejuvenate the aged hematopoietic microenvironment. We hypothesize that semaglutide suppresses niche-derived inflammaging, restores stromal function, and enhances wild-type HSPC fitness. Using young, middle-aged, and aged mice, we combine stromal secretome profiling, colony-forming and serial replating assays, flow cytometry, and single-cell transcriptomics to define mechanisms linking metabolic signaling, niche aging, and hematopoietic dysfunction. These studies establish GLP-1 receptor agonism as a clinically translatable strategy to preserve hematopoietic healthspan and limit progression to hematologic disease.

Elastin remodeling and elastokine signaling in aging tissues

Heinz, A¹

¹Department of Pharmacy, University of Copenhagen, Denmark

Elastin is a critical extracellular matrix protein that provides elasticity and resilience to large arteries and cardiovascular tissues. With aging, elastin fibers undergo fragmentation and degradation, leading to the release of bioactive elastin-derived peptides, known as elastokines¹. These peptides exert significant biological effects through interactions with elastin receptor complexes, promoting inflammation, oxidative stress, vascular remodeling, and calcification. Accumulating evidence suggests that elastokines contribute to endothelial dysfunction, arterial stiffness, hypertension, and progression of atherosclerosis, thereby linking extracellular matrix aging to cardiovascular disease. Understanding the molecular mechanisms underlying elastin degradation and elastokine signaling may reveal novel biomarkers and therapeutic targets for age-associated cardiovascular disorders and vascular aging². This presentation highlights current advances in understanding elastin degradation and its impact on cardiovascular aging and age-related pathological processes.

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Restoration of biological age during prolonged fasting–refeeding mediated by a fasting–responsive linker histone

Kazuto Kawamura¹; Anna R. Diederich¹; Birgit Gerisch¹; Roberto Ripa¹; Christian Latza¹; Joachim D. Steiner^{1,4}; Stephanie Fernandes²; Filippo Artoni²; David H. Meyer³; Damini Sant¹; Simon Oehm⁴; Franziska Grundmann⁴; Roman–Ulrich Müller⁴; Constantinos Demetriades²; Adam Antebi¹

¹ *Molecular Genetics of Ageing Department, Max Planck Institute for Biology of Ageing, Cologne, Germany*

² *Cell Growth Control in Health and Age-related Disease Research Group, Max Planck Institute for Biology of Ageing, Cologne, Germany*

³ *Institute for Genome Stability in Aging and Disease, University of Cologne, Cologne, Germany*

⁴ *Department II of Internal Medicine and Center for Molecular Medicine Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany*

Reversal of biological age can occur in the immortal germline during early embryogenesis or during Yamanaka factor reprogramming. Whether there are other contexts that promote restoration of biological age, especially in an adult organism, is largely unknown. Here we describe an experimental paradigm in which we observe remarkable somatic restoration and significant reduction of predicted biological age in an aged adult organism. We observe progressive degeneration and increases in predicted biological age during prolonged periods in fasting-induced adult reproductive diapause of *Caenorhabditis elegans*. When recovery is initiated by refeeding, germline regeneration, somatic repair and regrowth occur, accompanied by a reduction in predicted biological age. Interestingly, transcriptomic changes that ensue during refeeding-induced recovery are inversely correlated with ad libitum age-related transcriptomic changes, suggesting restoration of the transcriptome to a more youthful state. Through an RNAi screen we identify the linker histone *hil-1* as a key mediator of resilience during fasting and restoration during refeeding. *hil-1* mutation leads to reduced lifespan during adult reproductive diapause while having no effect on ad libitum lifespan. During refeeding, repression of *hil-1* facilitates somatic regrowth and recovery of locomotor function. Accordingly, HIL-1 is a fasting–responsive linker histone whose expression is strongly upregulated during adult reproductive diapause and downregulated during refeeding, suggesting chromatin plasticity is important for starvation survival and restoration. Induction of HIL-1 is dependent on DAF-16/FOXO and HLH-30/TFEB, two key transcription factors that promote longevity. Regulation of HIL-1/H1.0 may be evolutionarily conserved as we also observe an increase in H1.0 expression in response to amino acid starvation in human cell culture. Furthermore, H1.0 knockout cells are less resilient to prolonged amino acid starvation. Our study reveals refeeding-induced recovery from adult reproductive diapause as a time window of biological age plasticity in adult tissues, and identifies *hil-1*/H1.0 as part of an epigenetic switch of resilience and restoration during prolonged fasting–refeeding.

The aging factor EPS8 induces disease-related protein aggregation through RAC signaling hyperactivation

Koyuncu, S.^{*1,2}; Dominguez-Canterla, Y.^{3,4}; Alis, R.^{3,4}; Salarzai, N.^{1,2}; Petrovic, D.^{1,2}; Flames, N.^{3,4}; Vilchez, D.^{*1,2,5,6}

¹*Institute for Integrated Stress Response Signaling, Faculty of Medicine, University Hospital Cologne, Cologne, Germany*

²*Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*

³*Developmental Neurobiology Unit, Instituto de Biomedicina de Valencia IBV-CSIC, Valencia, Spain*

⁴*Valencia Biomedical Research Foundation, Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to the Instituto de Biomedicina de Valencia (IBV), Valencia, Spain*

⁵*Institute for Genetics, University of Cologne, Cologne, Germany*

⁶*Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany*

Aging is a major risk factor for neurodegenerative diseases associated with protein aggregation, including Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). Although these diseases involve different aggregation-prone proteins, their common late onset suggests a link to converging changes resulting from aging. Here, we found that age-associated hyperactivation of EPS8/RAC signaling in *C. elegans* promotes aggregation of HD-related polyglutamine repeats and ALS-associated mutant FUS and TDP-43. Conversely, knockdown of *eps-8* or RAC orthologs prevents protein aggregation and subsequent deficits in neuronal function during aging. Similarly, inhibiting EPS8 signaling reduces protein aggregation and neurodegeneration in human cell models. We further identify the deubiquitinating enzyme USP4 as a regulator of EPS8 ubiquitination and degradation. Notably, reducing USP-4 upregulation during aging prevents EPS-8 accumulation, extends longevity and attenuates disease-related changes. Our findings suggest that targeting EPS8 and its regulatory mechanisms could provide therapeutic strategies for age-related diseases.

Characterizing Cellular Drivers of Cognitive Decline Across the Aging Mouse Hippocampus

Amanda Latham^{1,2}; Sonia Rodriguez^{1,2}; Ethan Leitschuh^{1,2}; Marissa Schafer^{1,2,3,4}

¹Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA

²Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, USA

³Department of Neurology, Mayo Clinic, Rochester, MN, USA

⁴Department of Neuroscience, Mayo Clinic, Rochester, MN, USA

As global life expectancy increases, the prevalence of age-related cognitive impairment and dementia continues to rise, affecting more than 50 million people worldwide. The hippocampal formation and adjacent corpus callosum and fimbria-fornix white matter tracts are particularly vulnerable to age-associated structural and functional decline linked to cognitive dysfunction. Emerging evidence suggests that inflammatory cell populations contribute to brain aging; however, the cellular and molecular mechanisms driving hippocampal dysfunction remain incompletely understood. To define the cellular niches associated with brain aging and cognitive decline, we performed comprehensive behavioral phenotyping and brain analyses across the murine lifespan in male and female C57Bl/6N mice aged 6 – 29 months. Across multiple cognitive and behavioral domains, aging was associated with impairments in spatial memory, recognition memory, executive function, exploratory behavior, and anxiety-related behaviors. To define the cellular states, transcriptional changes, and microenvironment-specific interactions linked to domain-specific cognitive dysfunction with age, we generated spatial transcriptomic and whole-transcriptome single-nucleus RNA sequencing datasets from the hippocampal formation and surrounding white matter. Our analyses reveal the emergence of senescent and inflammatory immune and glial populations with age, particularly within white matter-associated microenvironments. By leveraging these datasets, we aim to provide mechanistic insight into the cell-mediated processes underlying cognitive decline in the aging brain.

Mitochondrial metabolism and epigenetic crosstalk in senescent cells.

Martini, Helene^{1,2}; Birch, Jodie³; Marques, Francisco⁴; Victorelli, Stella^{1,2}; Lagnado, Anthony^{1,2}; Pirius, Nicholas^{1,2}; Franco, Ana Catarina^{1,2}; Lee, Gung^{1,2}; Han, Yaeun^{1,2}; Rowsey, Jennifer²; Gaspar-Maia, Alexander⁵; Ismail, Mohammed Wazim⁵; Mazzone, Amelia⁵; Hitosugi, Taro⁶; Espe, Tianna M⁶; Li, Ya⁶; Washington, Alexander⁷; Kostalari, Enis⁷; Havas, Aaron⁸; Murad, Rabi⁸; Lei, Xue⁸; Porritt, Rebecca⁸; Maddocks, Oliver⁹; Jurk, Diana^{1,2}; Khosla, Sundeep²; Adams, Peter D.⁸; Passos, Joao F.^{1,2}

¹Physio&Biomed Engineering Mayo Clinic MN

²Robert&Arlene Kogod Center Aging Mayo Clinic MN

³LMS&ICS Faculty of Medicine Imperial College London UK

⁴Biochemistry Albert Einstein College of Medicine NY

⁵Dpt of Lab Medicine and Pathology & Epigenomics programs Mayo Clinic MN

⁶Dpt Oncology, Division of Oncology Research Mayo Clinic MN

⁷Gastroenterology and Hepatology, Mayo Clinic MN

⁸Sanford Burnham Prebys Medical Discovery Inst CA

⁹School of Cancer Sciences University of Glasgow UK

Chronic low-grade inflammation is a hallmark of aging and a major risk factor for morbidity and mortality in older adults. Senescent cells (SCs) promote tissue dysfunction and organismal aging in part through the senescence-associated secretory phenotype (SASP). However, the mechanisms contributing to the SASP are not fully elucidated. Our team has shown that mitochondrial dysfunction plays a central role in aging and SASP production. Understanding how mitochondrial dysfunction drives the SASP is essential for identifying new therapeutic targets to counteract the detrimental impact of SCs during aging.

Here, we demonstrate that mitochondrial metabolism is a key regulator of the epigenetic regulation of the SASP, where mitochondria-derived metabolites, such as citrate and acetyl-CoA, fuel histone acetylation at SASP gene loci, promoting their expression and inflammation in SCs. Interfering with mitochondrial metabolism, by inhibiting SLC25A1 and citrate export, reduces histone acetylation and SASP expression. Moreover, we show that mtDNA/cGAS/STING signaling cooperates with this metabolic-epigenetic pathway to enable full SASP activation and inflammation. Finally, single-cell multiome analysis in muscle demonstrates that pharmacological inhibition of SLC25A1 in vivo reduces chromatin accessibility at SASP genes, decreases inflammation, and is associated with improved healthspan in aged mice, underscoring its therapeutic potential. Our data support the concept that the SASP is driven by crosstalk between mitochondrial metabolism and epigenetic changes and identify new targets to combat the pro-aging effects of SCs.

Aging clocks delineate neuron types vulnerable or resilient to neurodegeneration and identify neuroprotective interventions

Gallrein, C.^{1,2,3,5,6}; **Meyer, D.H.**^{1,2,5,6}; Woitzat, Y.^{3,4}; Ramirez–Ramirez, V.^{1,2}; Vuong–Brender, T.^{1,2}; Kirstein, J.^{3,4} and Schumacher, B.^{1,2,6}

¹*Institute for Genome Stability in Aging and Disease, Medical Faculty, University and University Hospital of Cologne, Cologne, Germany*

²*Cologne Excellence Cluster for Cellular Stress Responses in Aging–Associated Diseases (CECAD), University of Cologne, Cologne, Germany*

³*Leibniz Institute on Aging – Fritz–Lipmann–Institute (FLI), Jena, Germany*

⁴*Friedrich Schiller University, Institute for Biochemistry and Biophysics, Jena, Germany*

⁵*These authors contributed equally*

⁶*Corresponding authors*

Different neuron types show distinct susceptibility to age-dependent degeneration, yet the underlying mechanisms are poorly understood. Here we applied aging clocks to single neuron types in *C. elegans* and found that distinct neurons differ in their biological age. Ciliated sensory neurons with high neuropeptide and protein biosynthesis gene expression show accelerated aging and degeneration, correlating with loss of function, which could be prevented by pharmacological inhibition of translation. We show that the *C. elegans* neuronal aging transcriptomes correlate with human brain aging patterns and anticorrelate with geroprotective interventions. We performed an in silico drug screen to identify potentially neuroprotective small molecules. We show that the plant metabolite syringic acid and the piperazine derivative vanoxerine delay neuronal degeneration, and propose these compounds as neuroprotective interventions. Furthermore, we identify neurotoxins that accelerate neurodegeneration, indicating that distinguishing aging trajectories between neuron types can inform on protective interventions as well as risk factors.

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Agonists of extra-oral bitter taste receptors suppress cellular senescence and enhance GLP-1 secretion

Allancer D.C. Nunes¹; Anna Carey¹; Aidan Josephs¹; Anandsai Kothapalli¹; J. Michael French²; Maik Behrens³; Christina D. Camell¹ and Paul D. Robbins¹

¹*Masonic Institute on the Biology of Aging and Metabolism and Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA.*

²*Amaro Therapeutics, Inc.*

³*Leibniz Institute for Food Systems Biology at the Technical University of Munich, Freising, Germany.*

Genetic variation in bitter taste-sensing type 2 receptors (TAS2Rs), can modulate food preferences, absorption and metabolic processing, potentially modulating biological pathways affecting the aging process. In adult tissues, the accumulation of senescent cells contributes to tissue dysfunction, accelerated aging, and the pathogenesis of various age-related diseases. Additionally, aging is also associated with a decline in the levels and function of GLP-1 and GLP-1 receptor expression. Given that TAS2Rs are activated by certain senotherapeutics (e.g., the bitter flavonoids fisetin and quercetin) and their activation potentially stimulates GLP-1 secretion by intestinal L-cells, we investigated the dual senotherapeutic and GLP-1 secretagogue effects of TAS2Rs agonists. We demonstrated that treating senescent cells with the TAS2R1/Tas2r108 agonist, KDT-501, the TAS2R46 agonist Berberine, or the TAS2R1/TAS2R14/TAS2R40 agonist Xanthohumol exerts a senotherapeutic effect, suppressing senescence markers and the inflammatory SASP. Furthermore, all three compounds significantly elevated active GLP-1 levels in the conditioned media of intestinal cells. Long-term treatment of aged mice with KDT-501 significantly downregulated markers of senescence and SASPs in visceral adipose tissue and small intestines, improved glucose tolerance as well as increased the circulating level of active GLP-1. Importantly, we also demonstrated that the well-established senolytic Fisetin signals via Tas2r108/Tas2r135 and Tas2r121 in murine intestinal STC-1 cells to suppress senescence and stimulate GLP-1 secretion. This secretagogue effect was abrogated by siRNA knockdown of G α -gustducin, the primarily taste-specific G protein alpha-subunit, as well as the pharmacological inhibition of PLC β 2 and IP3R. Overall, these results suggest that in addition to senotherapeutic properties, TAS2R agonists exerts a GLP-1 secretagogue effect, providing an attractive alternative to clinical GLP-1 mimetics.

Delirium and Long-Term Cognitive Trajectories in Cognitively Normal Older Adults: Insights from the Mayo Clinic Study of Aging

Allyson K. Palmer MD^{1,2}, PhD; Aidan F. Mullan MA³; Jonathan Graff-Radford MD⁴; Heling Jia⁵; Sunghwan Sohn PhD⁵; Sunyang Fu, PhD⁶; Jennifer St. Sauver, PhD⁷; Marissa Schafer, PhD^{2,4,8,9}; Fernanda Bellolio MD MS^{2,10,11}

¹Department of Medicine, Division of Hospital Internal Medicine, Mayo Clinic, Rochester, MN.

²Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN

³Department of Quantitative Health Sciences, Division of Biostatistics and Informatics, Mayo Clinic, Rochester, MN.

⁴Department of Neurology, Mayo Clinic, Rochester, MN.

⁵Department of Artificial Intelligence and Informatics, Mayo Clinic, Rochester, MN

⁶Department of Clinical and Health Informatics, McWilliams School of Biomedical Informatics at UTHealth Houston, Houston, TX

⁷Department of Quantitative Health Sciences, Division of Epidemiology, Mayo Clinic, Rochester, MN

⁸Department of Neuroscience, Mayo Clinic, Rochester, MN

⁹Department of Biomedical Engineering, Mayo Clinic, Rochester, MN

¹⁰Emergency Medicine, Mayo Clinic, Rochester, MN.

¹¹Department of Medicine, Division of Community Internal Medicine, Geriatric Medicine and Palliative Care, Section of Senior Services and Geriatric Medicine, Mayo Clinic, Rochester, MN.

Delirium is a state of acute confusion characterized by deficits in attention that occurs commonly in hospitalized older adults and is associated with adverse outcomes. However, the impact of delirium on longitudinal cognitive trajectories remains incompletely understood. This cohort study utilized data from the Mayo Clinic Study on Aging (MCSA), a prospective, population-based longitudinal study of cognitive aging among residents of Olmsted County, Minnesota, to evaluate the association of delirium during hospitalization with subsequent development of mild cognitive impairment (MCI) and dementia. Adults enrolled in MCSA who experienced at least one hospitalization between 2004 and 2023 were included, with follow-up continuing until development of MCI or dementia, last MCSA visit, or end of study. Delirium was identified using a validated natural language processing algorithm applied to hospitalization notes and documentation within 30 days and modeled as a time-dependent exposure. Primary outcomes were incident MCI and dementia identified through consensus diagnoses during comprehensive MCSA visits performed every 15 months. Associations were assessed using Cox proportional hazards regression, adjusted for age, sex, education level, and APOE $\epsilon 4$ allele status. Among 3,501 individuals who were cognitively unimpaired at baseline (median age 74.1 years; 52.1% male; median education 14 years; 26.0% APOE $\epsilon 4$ carriers), 31.2% developed MCI and 6.6% developed dementia over a median follow-up of 7.2 years. Delirium was strongly associated with increased risk of both MCI (adjusted hazard ratio 3.67; 95% CI 2.19–6.15) and dementia (adjusted hazard ratio 5.39; 95% CI 2.94–9.85). Longitudinal cognitive testing performance declined more rapidly among patients who developed delirium. These findings suggest that delirium may represent a critical risk factor or trigger for long-term cognitive deterioration and highlight the importance of delirium prevention, early detection, and post-hospitalization cognitive surveillance.

Cellular Senescence in the Aging Liver Contributes to Cognitive Decline Crosstalk between Liver and Brain

Lilian Gomez¹; Gung Lee¹; Ana Catarina Franco¹; Seunghwa Woo¹; Sundeep Khosla¹, Diana Jurk¹

¹ *Mayo Clinic, Department of Physiology and Biomedical Engineering, Robert and Arlene Kogod Center on Aging, Rochester, MN USA*

Aging is characterized by the accumulation of senescent cells in multiple tissues, including liver and brain. Previous work from our group showed that clearance of p16⁺ senescent cells alleviates brain inflammation, improves cognition, and reduces liver steatosis in aging and obesity. Clinical studies further link liver diseases with cognitive decline, suggesting a liver–brain axis, although the underlying mechanisms remain unclear. We hypothesize that hepatic senescence accelerates brain dysfunction during aging. Using p16-LOX-ATTAC;Alb-Cre mice, we show that aged mice exhibit memory impairment, while targeted elimination of senescent hepatocytes improves learning and memory. This is accompanied by reduced inflammatory markers in liver, brain, and plasma, as well as decreased hippocampal senescence. Our data suggest the existence of a liver–brain senescence signaling axis mediated by a secreted factor. Finally, we use the mouse model TRF1-FokI to demonstrate that hepatocyte telomere dysfunction contributes to learning and memory decline. Overall, these findings support a functional liver–brain crosstalk in aging, where senescent hepatocytes have a key role in cognitive outcomes, although further studies are needed to define the mechanisms involved.

Evolutionary conserved diapause mechanisms of slowed aging and rejuvenation

Sandt, Walter; Meyer, David; Schumacher, Björn

¹ *Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*

Aging is associated with a general decrease in physiological functionality, susceptibility to injury and increased risk of mortality as a function of time. Different species show a distinct plasticity of lifespan and ability to reset their biological age depending on their evolutionary adaptations. Diapause stages can be assumed in invertebrate and vertebrate species to allow individuals an extended survival under adverse environmental conditions. Here, we determine evolutionary conserved patterns of the slowed aging process in diapause and the rejuvenating exit programs. We show that Diapause is not simply youthful stasis but instead is characterized by a general co-expression-relationship between diapause and adult aging across species, as indicated by aging clocks and gene expression signatures that include the attenuation of metabolic processes. However, diapause entry triggers distinctive stress response programs that might explain the slowed aging trajectory. Diapause exit reactivates the metabolic processes and induces repair programs that might support the re-setting of the biological age. Our findings reveal conserved gene expression programs that in distinct species ranging from nematodes to mammals could slow aging and naturally rejuvenate cells and even organisms during diapause exit.

DREAMing of Healthy Aging: Deciphering the Molecular Links Between Sleep, Circadian Rhythms, and Cellular Repair

Valentim, I. S.¹; Romero-Expósito, F. J.²; Schwab, K.¹; Bayar, M.¹; Riege, K.¹; Lange, L.³; Engmann, O.³; Fisher, M.¹; Olmedo, M.²; Hoffmann, S.¹; & Ermolaeva, M. A.¹

¹*Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Beutenbergstrasse 11, 07745 Jena, Germany*

²*Department of Genetics University of Sevilla, Av. Reina Mercedes s/n, 41012 Sevilla, Spain*

³*Friedrich-Schiller-Universität Jena, Hans-Knöll-Str. 2, 07745 Jena, Germany*

Chronic sleep restriction and circadian disruption contribute to metabolic, cardiovascular, and neurocognitive decline. Using multi-omics, molecular, biochemical, and pharmacological approaches in *C. elegans*, human retinal cells, and mouse brain tissue, we identified the DREAM complex as a conserved regulator of sleep-dependent homeostasis. DREAM levels oscillate with circadian time and increase during wakefulness, promoting chromatin compaction that limits DNA damage. Sustained DREAM activation suppresses translation, oxidative phosphorylation, and stress responses, whereas sleep-associated DREAM decline enables chromatin relaxation and repair. Chronic sleep or circadian disruption maintained elevated DREAM activity, causing persistent chromatin closure, impaired repair, mitochondrial dysfunction, reduced resilience, and accelerated aging. DREAM inhibition restored repair pathways and improved cellular function despite continued circadian disruption. These findings identify DREAM as a molecular link between sleep, circadian rhythms, and aging, and a potential sleep-mimetic therapeutic target.

Spatiotemporal Proteostasis Rewiring In Senescence Progression Revealed By Translatome & Mitochondrial Importome Analyses

Rebecca George Tharyan¹; **Jonas Martin Schmidt^{1*}**; Nikita Verheyden¹; Denada Arifaj²; Koraljka Husnjak⁴; Shady Amr¹; Stefanie Dimmeler^{2,5,6} and Christian Münch^{1,2,3}

¹*Institute of Molecular Systems Medicine, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany.*

²*Cardio-Pulmonary Institute, Goethe University, Frankfurt am Main, Germany.*

³*SCALE, Goethe University, Frankfurt am Main, Germany.*

⁴*Institute of Biochemistry II, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany.*

⁵*Institute of Cardiovascular Regeneration, Goethe University, Frankfurt am Main, Germany.*

⁶*German Center for Cardiovascular Research (DZHK), Germany.*

**These authors contributed equally.*

Cellular proteostasis declines with age. Yet, its direct role in senescence and which proteins need active translation to maintain the senescent state remain elusive. Leveraging multiple proteomics methods, we mapped the temporal dynamics of the translatome, mitochondrial protein importome, phosphoproteome, and whole-cell proteome across three distinct human primary cellular senescence models to obtain spatiotemporal insight into senescence progression. We uncover a translational signature of senescence marked by a global decline in translation and altered proteostasis – most notably remodelling of ubiquitin–proteasome system – while selectively preserving pathways required to maintain the senescent state. Concurrently we observe impaired mitochondrial protein import driven by reduced synthesis of mitochondrial proteins and their import machinery. Integrating translatome proteomics with a genome-wide CRISPR/Cas9 screen, we uncovered novel potential senotherapeutic targets that are also preserved in general aging. Our findings advance the understanding of proteostasis dysfunction during senescence progression and open new avenues for therapeutic interventions to attenuate aging-related phenotypes.

Exploring ribosomal RNA modifications and corresponding snoRNAs in human skin cell senescence

Wagner-Schrittwieser, A.¹; Lopez Galisteo, R.^{1,2}; Schmid-Siegel, M.^{1,2}; Hammer, L.^{1,2}; Blaskovits, V.¹; Kremslehner, C.^{2,3}; Martic, I.⁴; Tessier, A.^{2,5}; Gendronneau, G.^{2,5}; Hengstschläger, M.¹; Cavinato, M.⁴; Gruber, F.^{2,3}; **Schossere, M.**^{1,2,6}

¹*Institute of Medical Genetics, Center for Pathobiochemistry and Genetics, Medical University of Vienna, Vienna, Austria*

²*Christian Doppler Laboratory for Skin Multimodal Imaging of Aging and Senescence, Vienna, Austria*

³*Department of Dermatology, Medical University of Vienna, Vienna, Austria*

⁴*Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck, Austria*

⁵*Innovation, Research and Development, CHANEL Parfums Beauté, France*

⁶*Max Planck Institute for Biology of Ageing, Cologne, Germany*

The accumulation of senescent cells contributes to skin ageing, but the lack of precise biomarkers complicates the development of interventions. This study investigates the role of ribosomal RNA modifications, specifically 2'-O-methylations and pseudouridines, in skin ageing. By inducing senescence in human keratinocytes and melanocytes, and using Direct RNA Nanopore sequencing, we identified consistent changes in rRNA modifications across different cell types. Functional studies by depleting SNORD90, which guides one of the identified senescence-associated rRNA modifications, reveal that senescence-associated rRNA modifications indeed influence cellular phenotypes in keratinocytes. Expression analysis of SNORD90 in young versus aged human skin showed a partial correlation with age, suggesting a role in skin ageing in vivo. Understanding these mechanisms in more detail could lead to novel anti-ageing strategies targeting RNA modifications in senescent cells.

Rapid DNA double-strand break repair as a candidate mechanism of exceptional longevity in the bat *Myotis myotis*

Ilze Skujina^{1,2}; Noel Lowndes¹; J. Luessing¹; Xuanjing Li²; Frédéric Touzalin²; Sebastien Riquier²; Asya Martirosyan³; Björn Schumacher³; Emma C. Teeling²

¹Centre for Chromosome Biology, University of Galway, Galway, Ireland

²Laboratory of Molecular Evolution and Mammalian Phylogenetics (BatLab), University College Dublin, Dublin, Ireland

³Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

DNA is continuously challenged by endogenous and environmental insults that generate DNA lesions, including highly cytotoxic DNA double-strand breaks (DSBs). Accumulation of DNA damage and the resulting genome instability are increasingly recognised as fundamental drivers of ageing and age-related disease. Bats represent a unique model for studying mechanisms of healthy ageing, as many species exhibit exceptional longevity despite the substantial metabolic demands associated with powered flight. Understanding how long-lived species maintain genome integrity may therefore provide important insights into the biology of ageing. We hypothesised that exceptionally long-lived bats possess enhanced DNA damage responses (DDRs) that promote efficient genome maintenance throughout life. To investigate this, we performed a comparative multi-omics analysis of DDR pathways in primary fibroblasts from the long-lived bat *Myotis myotis*, the shorter-lived bat *Rousettus aegyptiacus*, *Mus musculus* and *Homo sapiens* following ionising gamma radiation. Preliminary results reveal an exceptionally rapid response to DNA double-strand breaks in *M. myotis*, characterised by near-immediate DSB resolution following irradiation. Integrative analyses suggest that this phenotype may be driven by accelerated activation of non-homologous end joining (NHEJ), but exact mechanism still needs to be determined. These findings identify rapid DSB repair as a candidate mechanism contributing to exceptional longevity in bats and highlight early divergence in cellular stress-response and genome-maintenance programmes between long-lived and short-lived mammals.

DNA Methylation–Based Clonal Hematopoiesis Burden Associates with Structural Brain Atrophy

Suwanlikit, Y.^{1,2,3,*}; Crofts, S.J.^{1,2,4,*}; Cox, S.^{5,*}; Robertson, N.A.^{1,6}; Marioni, R.⁷; Tan, S.H.^{1,8}; Kirschner, K.^{1,9,#}; Chandra, T.^{1,6,10,#}

¹Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester MN, USA

²Mayo Clinic Graduate School of Biomedical Sciences, Rochester MN, USA

³Department of Pharmacology, Mahidol University, Bangkok, Thailand

⁴School of Biological Sciences, University of Edinburgh, Edinburgh, UK

⁵Lothian Birth Cohorts, Department of Psychology, University of Edinburgh, Edinburgh, UK

⁶Department of Quantitative Health Sciences, Mayo Clinic, Rochester MN, USA

⁷Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK

⁸Bioinformatics and Computational Biology, University of Minnesota, Minneapolis MN, USA

⁹Department of Hematology, Mayo Clinic, Rochester MN, USA

¹⁰Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester MN, USA

Clonal hematopoiesis (CH) effects on neurodegeneration remain poorly understood¹. Using COMET2, a methylation-based clonality predictor, we examined longitudinal associations between clonal burden and brain structure in the Lothian Birth Cohort 19363 (n = 1,091) across three time points (ages 73–79). Latent growth curve modeling revealed that rising clonality significantly predicted progressive gray matter loss ($\beta = -0.958$, $p < 0.01$), following a left-lateralized cortical atrophy pattern in medial temporal and parietal regions that spatially corresponded with an established Alzheimer's disease (AD) cortical map⁴ ($r = 0.344$, $p = 0.004$). Subcortical analyses showed relative caudate preservation and no significant hippocampal association. A complementary CH burden predictor derived from the EWAS study⁵, capturing systemic epigenetic remodeling across mutant and wild-type immune cells, was associated with global atrophy but showed divergent regional patterns. Together, these findings establish CH burden as a contributor to brain atrophy, with clonality specifically linked to AD-like cortical atrophy in cognitively healthy older adults.

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A redox-regulated chaperone in inter-tissue proteostasis regulation maintains a functional proteome and promotes healthy aging

Motiei, S.¹ & Ulrich, K.¹

¹ *Molecular and Cell Biology, Saarland University, Saarbruecken, Germany*

As organisms age, protein quality control and the response to unfolding stress decline. In addition, antioxidant defense diminishes leading to unspecific protein oxidation, unfolding and the formation of protein aggregates. Redox imbalance also causes a drop in ATP levels which hampers the function of ATP-dependent chaperones and proteases. Hence, the activation of ATP-independent chaperones becomes essential to prevent aggregation. Our work shows that thiol oxidation of the dual-function protein ASNA-1 (yeast Get3) induces a switch from its ATP-dependent targeting function for tail-anchored proteins into an ATP-independent chaperone. Depletion of ASNA-1 in *C. elegans* results in pathogenic protein aggregation, paralysis and early death. The effects are fully rescued by a targeting-deficient ASNA-1 mutant variant, highlighting the essential role of the chaperone function. We further found that intestinal ASNA-1 protects proteostasis in distal tissues and its overexpression causes lifespan extension. Our work defines the chaperone-mediated, cell non-autonomous function of a redox-regulated protein in organismal proteostasis.

XPG deficiency accelerates vascular aging via VSMC phenotypic switching and metabolic dysfunction, which is mitigated by dietary restriction.

Sanne J. M. Stefens¹; José María Heredia-Genestar¹; Martine de Boer²; Nicole van Vliet¹; Renata M. C. Brandt¹; Sander Barnhoorn¹; Merel Batenburg¹; Dirk J. Duncker²; Jan H. J. Hoeijmakers^{1,3,4,5}; Roland Kanaar^{1,3}; Jeroen Essers^{1,6,7}; **Ingrid van der Pluijm**^{1,6}

¹Department of Molecular Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands,

²Department of Cardiology, Erasmus University Medical Center, Rotterdam, The Netherlands,

³OncoCode Institute, Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, The Netherlands,

⁴Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands ,

⁵Institute for Genome Stability in Aging and Disease, Cologne Excellence Cluster for Cellular Stress Responses in Aging- Associated Diseases (CECAD), University of Cologne, Cologne, Germany,

⁶Department of Vascular Surgery, Cardiovascular Institute, Erasmus University Medical Center, Rotterdam, The Netherlands,

⁷Department of Radiotherapy, Erasmus University Medical Center, Rotterdam, The Netherlands

Aging is the strongest risk factor for cardiovascular disease, but mechanisms linking molecular damage to vascular decline remain unclear. Persistent DNA damage contributes to aging, yet its vascular impact is underexplored. We studied Xpg^{-/-} mice lacking the DNA repair enzyme XPG, resulting in DNA damage accumulation and premature aging. Their aortas showed elastin degradation, a shift in vascular smooth muscle cell (VSMC) identity from a contractile to osteogenic and macrophage-like phenotypes, and lipid droplet buildup. Bulk RNA sequencing revealed increased inflammation and reduced lipid and mitochondrial metabolism, demonstrating a link between genomic instability, immune activation, and metabolic disruption. In vitro, Xpg^{-/-} VSMCs exposed to the transcription-blocking DNA-damaging agent Illudin S accumulated lipid droplets, unlike wild-type cells. Dietary restriction restored metabolic gene expression, reduced inflammation, and improved vascular structure. These findings show that DNA damage drives vascular aging via VSMC re-programming and metabolic imbalance, while dietary restriction may counteract these effects.

Sexually dimorphic expression of FABP5 favors tissue persistence of chemotherapy-induced senescence in male mice

Boshi Wang¹; Abdullah Altulea¹; Tao Zhang¹; Jamil Nehme¹; Justina C. Wolters²; Ellen Nollen¹; Marco Demaria¹

¹European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen (UMCG), University of Groningen (RUG), Groningen, Netherlands

²Department of Pediatrics, University Medical Center Groningen (UMCG), University of Groningen (RUG), Groningen, Netherlands

Sex is still considered a confounding rather than a biological variable in oncology research, and differences between men and women in cancer incidence, progression and treatment are poorly understood. Sex chromosome-linked genes and sex-specific hormones play crucial roles in tumorigenesis, but their consequence on cancer-related cellular and molecular mechanisms remain only partially explored. As shown by us and other laboratories, senescent cells are prematurely induced by cancer chemotherapy treatments and contribute to cancer progression and therapy-related toxicities. We have recently observed that the temporal dynamics of therapy-induced senescent (TIS) cells turnover are distinct between males and females, with females showing a faster clearance. Unbiased proteomic analysis of male and female TIS mouse dermal fibroblasts (MDFs) revealed the fatty acid binding protein FABP5 as a top differentially expressed protein, higher in male senescent MDFs. Knock-down (KD) of *Fabp5* in male TIS MDFs switched their gene expression profiling, mitochondrial lipid content and mitochondrial functions to a female-like level. Importantly, using transplantation models, we observed that *Fabp5*-KD male TIS cells were removed with kinetics similar to female wild-type counterparts. In summary, our study shows a profound sexual dimorphism in how senescence persists upon exposure to cancer therapy, providing a novel sex-dependent mechanism that can be exploited to improve cancer prevention, diagnosis and treatment.

Telo-CUT: A Telomere-Specific DNA Damage Model Reveals Drivers of Aging

Seung-Hwa Woo^{1,2}; Anthony Lagnado^{1,2}; Diana Jurk^{1,2}; Nicholas Pirius^{1,2}; Yaeun Han^{1,2}; Helene Martini^{1,2}; Stacia P. A. Evert^{1,2}; Runqing Huang²; Carolyn M. Roos²; Thomas A. White^{1,2}; Jessica J. Brekke^{1,2}; Jenny L. Rowsey^{1,2}; Nathan K. LeBrasseur^{1,2}; Jordan D. Miller²; Sundeep Khosla^{1,2}; David G. Monroe^{1,2}; João F. Passos^{1,2}

¹Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, USA

²Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA

Telomere-associated DNA damage drives persistent DNA damage response (DDR) signaling and formation of telomere-associated foci (TAF), a hallmark of cellular senescence. However, whether chronic telomere-specific DDR is sufficient to promote organismal aging in vivo remains unclear. Here we develop TeloCUT, a tamoxifen-inducible mouse model in which telomeric double-strand breaks are generated through expression of a TRF1-FOKI fusion protein. Systemic activation of TeloCUT induced widespread TAF formation together with increased senescence and inflammatory signatures across multiple organs. Despite their young chronological age, TeloCUT mice developed progressive multi-system functional decline, reduced health span and shortened lifespan. Pharmacological inhibition of ATM signaling attenuated these effects, indicating that persistent telomeric DDR signaling through the ATM-p53 pathway is a key driver of the phenotype. These findings provide direct in vivo evidence that sustained DNA damage at telomeres is sufficient to drive systemic aging and establish a tractable model to investigate telomere-specific mechanisms and therapeutic targeting of persistent DDR.

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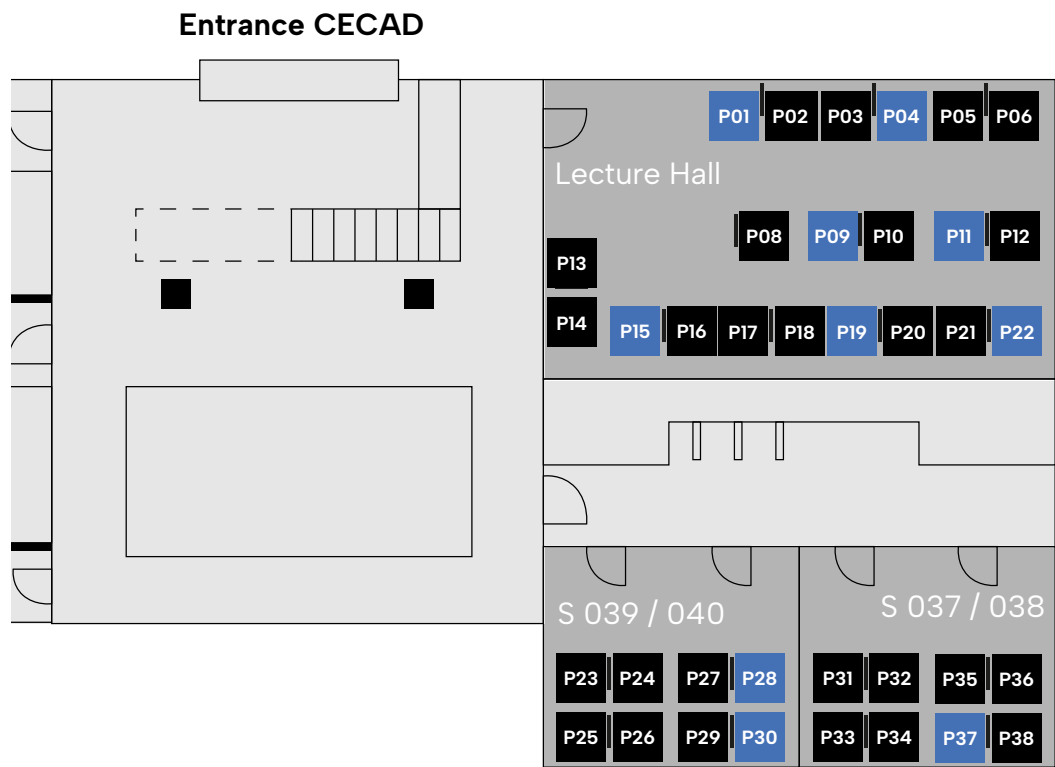
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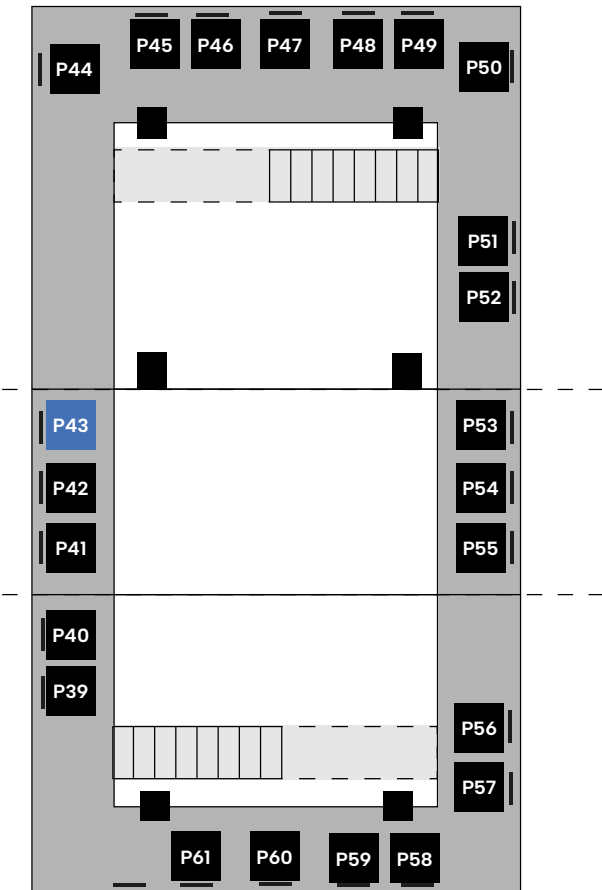
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Integrative Computational Analysis Reveals *H. pylori* GroEL as a Stabilizer of Neurotoxic Amyloid- β Oligomers

Wazeer Muhammed Abdullah^{1*}; Ishtiaq Ahmad^{1*}

¹ Dr Ikram ul Haq Institute of Industrial Biotechnology, GC University, Lahore, Pakistan

*To whom correspondence should be addressed: w.m.abdulla@gmail.com, ishtiaqahmad@gcu.edu.pk

Alzheimer's disease (AD) is characterized by amyloid- β (A β) aggregation, with soluble oligomers implicated as the most neurotoxic species. Recent evidence suggests microbial infections, including *Helicobacter pylori*, contribute to AD pathogenesis. This study investigates the role of *H. pylori* GroEL, a conserved chaperonin found in bacterial outer membrane vesicles (OMVs), in stabilizing toxic A β oligomers. A pan-genome analysis of 353 *H. pylori* strains identified GroEL as a highly conserved protein present in 83% of strains, which supported its widespread relevance. We structurally modelled a conserved 27-amino acid GroEL fragment and docked it against the A β (1-42) tetramer. Interaction analysis revealed stabilizing salt bridges, hydrogen bonds, and extensive non-bonded contacts within the GroEL-A β complex. Molecular dynamics simulations (50 ns) demonstrated that GroEL binding enhanced A β oligomer stability, evidenced by reduced structural deviations and a more extensive hydrogen bonding network compared to A β oligomer alone. These computational findings support a novel mechanism whereby *H. pylori* GroEL directly stabilizes soluble A β oligomers. We hypothesize that this stabilization inhibits their aggregation into plaques while paradoxically prolonging the lifetime of neurotoxic species, potentially increasing neurodegeneration through pathways distinct from canonical amyloid deposition. This highlights the complex role of bacterial proteins in AD and underscores the need for experimental validation of GroEL-A β interactions as a potential therapeutic target.

Keywords: *Helicobacter pylori*, Pan-genome Analysis, GroEL, Alzheimer's disease, Amyloid-beta oligomer, Molecular dynamics

Investigating the impact of microbiota and dietary intervention on mouse brain ageing

Alireza Ahmadi¹; Ward Nijen Twilhaar^{1,7}; Jen-Chien Chang^{2,7}; Tommy W. Terroate^{2,3,7}; Matteo Guerrini²; Hārūka Yabukami²; Sachi Kato²; Naoko Satoh-Takayama²; Nicola Hetherington^{1,2}; Prashanti Jeyamohan²; Hai Tao Wang⁴; Nils Rother⁵; Tomoko Kageyama²; Yan Jun Lan²; Shiori Maeda²; Miho Mochizuki²; Yasutaka Motomura²; Yumiko Nakanishi²; Natsuki Takeno²; Ko Tsutsui⁶; Marina Vilaseca Barcelo²; Chung Chau Hon²; Takeshi Matsui²; Hironobu Fujiwara⁶; Hiroshi Ohno²; Susan Nourshargh⁴; Sidonia Fagarasan²; Kazuyo Moro²; Aki Minoda^{1,2,8,9}

¹ Department of Human Biology, Radboud Institute for Molecular Life Sciences, Faculty of Science, Radboud University, Nijmegen, The Netherlands

² Center for Integrative Medical Sciences, RIKEN, Yokohama, Japan

³ Biology, Genomic and Bioinformatic Center, Brigham Young University, Provo, Utah, USA

⁴ Center for Microvascular Research, Queen Mary University of London, London, UK

⁵ Internal Medicine, Radboudumc, Nijmegen, The Netherlands

⁶ Center for Biosystems Dynamics Research, RIKEN, Kobe, Japan

⁷ These authors contributed equally, 8 Senior author. 9 Lead contact

Ageing is a multifaceted process influenced by both intrinsic factors – such as genomic instability – and extrinsic factors – such as diet, the microbiome and other environmental exposures. Little is known on how the microbiota affects tissue ageing trajectories. To gain insights into this, we have constructed an epigenome (scATAC-seq) and transcriptome (scRNA-seq) ageing atlas with ten mouse tissues (divided into two phases) of different ages reared under conventional or germ-free conditions (manuscript in biorXiv)

Beyond microbial influences, dietary interventions have emerged as important modulators of ageing. Among these, Caloric restriction has been proved by different studies to act as one of the most promising longevity interventions. However, the macronutrient composition of the diet also plays a critical role. To investigate the impact of protein levels on the aging process, mice were subjected to a low protein diet. After harvesting the tissues, both bulk and single-cell methods have been conducted on different tissues.

Building upon the findings from the first phase, the focus for the second phase consisting of snRNA-seq and snATAC-seq and bulk methods, currently under analyses, is to identify both global and region-specific age-associated alterations in mouse brain. In addition to that, we investigated the effect of diet and microbiota on ageing trajectory compared to normal ageing.

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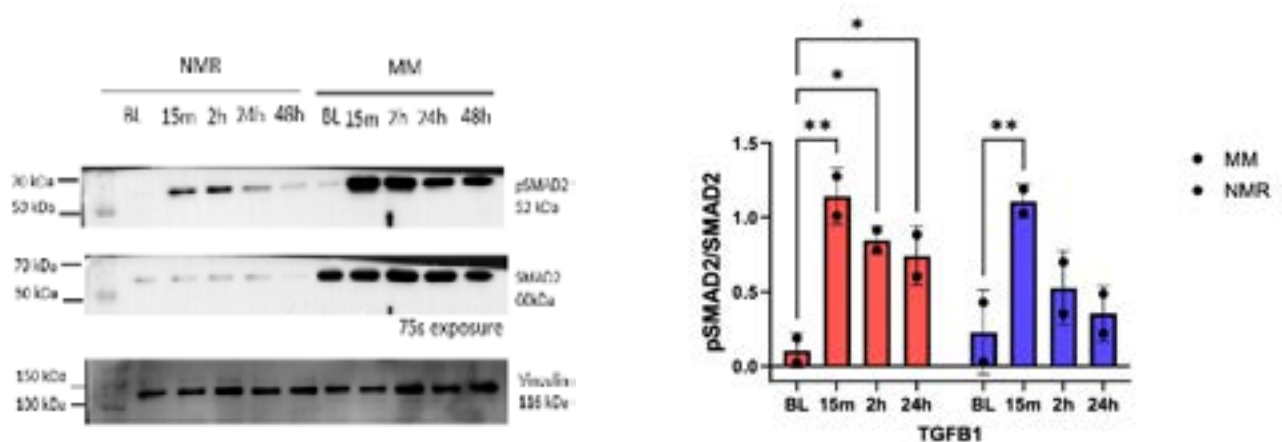
Effect of NMR ECM Composition on Maintaining Fetal Cardiac Profile and Regenerative Capacity

Asli Nur **Akaydin**¹; Jacome-Burbano, Maria Sol¹; Reznick, Jane¹

¹ *Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany*

Heart failure is the primary cause of death worldwide. Ischemic heart disease, the leading type of heart failure, leads to loss of cardiomyocytes (CM) in the heart muscle. Because of the limited regenerative capacity of the adult mammalian heart due to the loss of regenerative capacity 7 days after birth, the loss of CM is compensated by the formation of the collagen-rich scar tissue, which dampens the contractile function of the heart. Several fish and amphibian species can preserve their regeneration capacity throughout their lifespan. Naked mole-rat (NMR) is a subterranean eusocial animal that lives under harsh environmental conditions and as one of the adaptations against those conditions, our lab has shown that the NMR heart has fetal-like metabolic and physiologic characteristics that promote CM proliferation after injury, thereby promoting functional regeneration in an adult. Extracellular matrix (ECM) has been implicated in determining cell states and instructing regenerative versus fibrotic programs. The ECM of naked rat tissues is distinct and one prominent example is the large production of a high molecular-weight form of hyaluronan. We hypothesize that the distinct composition of the ECM in the naked mole-rat heart maintains its CMs in an immature and regeneration-permissive state. Furthermore, we have evidence that naked mole-rats secrete species-specific ECM upon myocardial infarction and we believe that distinct factors drive regenerative processes and avoid fibrotic healing. Given that fibroblasts are major producers of ECM and secreted immunoregulatory factors, we wanted to assess fibroblast activation in the regenerative naked mole-rat and compare it to the non-regenerative mouse fibroblasts. TGFβ1 stimulation revealed a species-specific response in naked mole-rat fibroblasts that may be linked to pro-regenerative outcomes we observe in vivo. Although activation of SMAD2 was equivalent to mouse, naked mole-rats express significantly less SMAD2 and 3 proteins, resulting in an overall lower content of activated SMAD. Expression of Serpinel, the direct target of pSMAD2/3, was robustly induced in NMR to similar levels as mouse; however, downstream processes, including expression of αSMA, Colla1 and Postn, were either significantly blunted or completely unchanged, suggesting that naked mole-rat is resistant to mounting the full spectrum of a fibrotic program, which we are exploring.

To assess whether cFB-derived ECM, including HA, maintains CMs in a more immature and regeneration-permissive state, we are co-culturing naked mole-rat and mouse CMs with cFB in a matched and cross-species paradigm and assessing CM proliferation, binucleation, as well as other markers of maturity. To specifically assess the contribution of HA, we also treated the cFB with 4-MU, an inhibitor of HA synthesis. Altogether, we aim to identify secreted ECM factors that can reprogram CMs to allow proliferation and can modulate the injured niche and steer the healing response to regenerative rather than fibrotic repair.



Establishing an Adeno-Associated Virus (AAV) Core Facility: Advancing Research at the University of Cologne

Mohamed Aghyad Al Kabbani^{1,2}; Andrew Naylor^{1,2}; David Benitez Alvarez^{1,2}; Hans Zempel^{1,2}

¹*Institute of Human Genetics, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany*

²*Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany*

Adeno-associated viruses (AAVs) are powerful and safe gene delivery tools widely used in biomedical research and therapeutic development. To meet the growing demand for standardized, high-quality AAV vectors, we propose the establishment of a dedicated AAV Core Facility at the Center for Molecular Medicine Cologne (CMMC). The facility will centralize vector design, production, purification, and quality control, offering researchers cost-effective and reproducible solutions. Under the scientific leadership of Dr. Hans Zempel, and managed by Dr. Aghyad Al Kabbani and Dr. Andrew Naylor, the core will provide technical support, training, and collaborative opportunities across disciplines such as neuroscience, oncology, and gene therapy. By streamlining access to AAV resources and fostering interdisciplinary collaboration, the facility will strengthen the University of Cologne's research competitiveness and serve as a cornerstone for future translational initiatives, including the envisioned Gene Therapy Center.

Laser-targeted Optogenic Activation of Channelrhodopsin in iPSC Derived Cells Using High-Resolution Microscopy.

Alabed, L.¹; Xia, X.¹; Zempel, H.^{1,2,3}

¹Institute of Human Genetics, University Hospital Cologne, Cologne, Germany

²Center for Molecular Medicine Cologne, University of Cologne, Germany

³Center for Genomic Medicine, RWTH University Hospital Aachen

Introduction: Mutations in the POLG gene impair mitochondrial DNA replication and oxidative phosphorylation, leading to progressive neuronal dysfunction. As neurons depend on efficient mitochondrial function to meet high energy demands, investigating activity-dependent mitochondrial responses may provide insight into the mechanisms underlying POLG-associated neurodegeneration.

Hypothesis/Aims: We hypothesize that POLG iPSC-derived neurons show impaired mitochondrial responses to neuronal stimulation compared with wild-type controls. This project aims to assess changes in mitochondrial membrane potential following localized optogenetic stimulation and to compare these responses between neuronal compartments and genotypes.

Methods: Human iPSC-derived neurons from POLG patients and healthy controls were transfected with a Channelrhodopsin-ABCB-eYFP construct and labeled with TMRM to monitor mitochondrial membrane potential. Using a Leica fluorescence microscope, targeted laser stimulation was applied while TMRM fluorescence changes were recorded in real time in neuronal somata and axons.

Expected Results: We expect POLG neurons to exhibit altered TMRM fluorescence dynamics following optogenetic stimulation, indicating impaired mitochondrial adaptability and bioenergetic function. These findings may reveal activity-dependent deficits in mitochondrial regulation that contribute to POLG-related neurodegeneration.

Active Genomic Reprogramming Drives Neuroresilience in MAPT-Knockout Human Neurons: An Integrative Multi-Omics Approach

Elnaz Amanzadeh **Jajin**¹, Zempel, Hans¹

¹Center of Molecular Medicine Cologne (CMMC),, Institute human genetics

Background: The microtubule-associated protein Tau (MAPT) is central to Alzheimer's disease (AD) pathogenesis. While Tau loss confers resistance to amyloid-beta (A β) toxicity, the active molecular reprogramming driving this neuroresilience remains poorly defined.

Methods: We employed an integrative multiomics approach, combining temporal transcriptomics (3h and 24h post-A β exposure) with newly processed proteomic layers from human iPSC-derived neurons (WT vs. MAPT-KO). Analysis included DESeq2, WGCNA, and Limma, followed by cross-layer integration to identify convergent molecular signatures and master regulators.

Results: Our analysis revealed a striking transcriptomic stagnation in WT neurons, which failed to mount an adaptive response to A β stress. In contrast, MAPT-KO neurons exhibited a pre-established resilience signature characterized by profound baseline reprogramming. WGCNA identified a turquoise module significantly linked to KO neurons ($r=0.74$), enriched in mitochondrial transport, autophagy, and synaptic homeostasis. Key hub genes including SIM1, INPP5F, and AKRIC2 established a mitochondrial metabolic defense mechanism. Multiomics integration identified 27 core consensus hits (e.g., COL17A1, PER3, ST3GAL6) consistently preserved across both layers. Furthermore, transcription factor enrichment identified KLF4 (Kruppel-like Factor 4) as the master regulator orchestrating this protective network.

Conclusion: These findings demonstrate that Tau deletion induces an active, multilevel gain-of-resilience rather than passive resistance. By identifying a KLF4-driven molecular signature, this study highlights novel therapeutic targets that could be leveraged to induce synthetic resilience in AD patients, protecting neurons from amyloid-induced bioenergetic and structural collapse.

Keywords: Alzheimer's Disease, MAPT knockout, Multiomics Integration, Neuroresilience, Proteomics, Transcriptomics.

Changes in the 3D chromatin structure during aging using the turquoise killifish

Lorena Ardila-García¹ (lardgar@upo.es); Sofía Manzo¹ (sman2@upo.es); Lucía Muñoz-Gil¹ (lmungil@upo.es); Valle Hernández¹ (mdherper@upo.es); Silvia Naranjo (snardur@upo.es)¹; Juan J. Tena¹ (jjtenagu@upo.es)

¹Centro Andaluz de Biología del Desarrollo (CABD), CSIC/UPO/JA, Seville, Spain

Aging has emerged as a major biomedical challenge associated with the progressive increase in global lifespan. Our research investigates how senescence alters gene transcription through changes in three-dimensional chromatin architecture, a key regulator of genome organization and promoter–enhancer interactions. Using *Nothobranchius furzeri*, the shortest-lived vertebrate in captivity, we study how aging remodels chromatin structure across different organs and sexes. By integrating Hi-C chromatin conformation assays with transcriptomic and epigenomic analyses, we will characterize the dynamic relationship between genome architecture and gene regulation during aging. Our objectives include generating multi-organ chromatin maps at different aging stages and performing comparative analyses with other vertebrates to identify chromatin features associated with lifespan and age-related disease. Preliminary results reveal tissue- and sex-specific chromatin alterations, highlighting the importance of integrative and comparative approaches in aging biology.

Proteomic characterization of Selinexor-mediated rescue of the healthy phenotype in progeroid fibroblasts

Ayala, Y.¹; Encarnación, S.²; Navarro-García, F.¹ & Cisneros, B.³

¹ Department of Cell Biology, Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico

² Center for Genomic Sciences, National Autonomous University of Mexico (UNAM), Cuernavaca, Morelos, Mexico

³ Department of Genetics and Molecular Biology, Center for Research and Advanced Studies (Cinvestav), Mexico City, Mexico

Hutchinson–Gilford Progeria Syndrome (HGPS) is a genetic disorder that mimics physiological aging. HGPS is caused by a mutation in the LMNA gene that produces progerin, a toxic lamin A variant that triggers nuclear disorganization, DNA damage, and premature senescence. Progerin upregulates exportin XPO1, disrupting nucleocytoplasmic proteostasis. Although the XPO1 inhibitor Selinexor reverses the progeroid phenotype, its molecular mechanism remains unclear. To elucidate it, we assessed the effect of Selinexor on nucleocytoplasmic proteome distribution in healthy and HGPS cells. Mass spectrometry of these subcellular fractions showed that Selinexor globally attenuates protein synthesis while selectively increasing nuclear localization of proteasome subunits. In silico analysis linked these nuclear-enriched subunits to p62, an XPO1 cargo. We experimentally validated Selinexor-mediated nuclear relocalization of p62, which accumulates as biomolecular condensates in the nucleoplasm. We are currently investigating how nuclear p62 and proteasome activation restore cellular homeostasis in HGPS.

The combined treatment with rapamycin and trametinib partially recapitulates the in vitro molecular effects of genetic variants identified in exceptionally long-lived individuals

Mara Neuerburg¹; Joris Deelen^{1,2}; **Maarouf Baghdadi**¹

¹Max Planck Institute for the Biology of Ageing, Cologne, Germany

²Leiden University Medical Center, Leiden, the Netherlands

Rare genetic variants in the IIS signalling network from long-lived individuals cluster into two groups that show shared and diverging (i.e. on stress resistance) in vitro effects associated with lifespan across species. However, whether these effects can be recapitulated pharmacologically is unknown. We compared transcriptomic and proteomic profiles of CRISPR-engineered mouse stem cells carrying these mutations with wild-type cells treated with rapamycin (mTOR inhibitor), trametinib (MEK inhibitor), or their combination (RT). RT produced a transcriptional state distinct from either drug alone, partially mimicking the stress-resistant group of mutant cells. This omics-based signature is characterised by an activation of the ETS-FOXO longevity circuit driving stress defence and autophagy targets at the RNA and protein level. Convergence was partial, mutants uniquely upregulated proteasome subunits, a programme absent under RT. The shared omics-based signature supports RT as potentially relevant to human longevity, although mutant-specific effects highlight that this drug combination only partly captures the mutant's in vitro effects.

Aging of the blood–brain barrier: An in vitro metabolomic and lipidomic resource of mouse brain endothelial cell senescence

Hari Baskar Balasubramanian^{1,2,†}; Ammar Tahir^{3,4,†,*}; Dominik Kahr¹; Florian Haage¹; Marietta Zille^{1,*}

† Authors contributed equally

* Corresponding authors

¹Department of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, University of Vienna, 1090 Vienna, Austria

²Vienna Doctoral School of Pharmaceutical, Nutritional and Sport Sciences, University of Vienna, 1090 Vienna, Austria

³Department of Pharmaceutical Sciences, Division of Pharmacognosy, University of Vienna, 1090 Vienna, Austria

⁴Section of Biomedical Sciences, Department of Health Sciences, Hochschule Campus Wien (HCW) University of Applied Sciences Campus Wien, Vienna, Austria

Contact: Hari Baskar Balasubramanian: hari.baskar.balasubramanian@univie.ac.at

The blood–brain barrier (BBB) is a critical interface between the peripheral circulation and the brain, maintained by brain endothelial cells (BECs) through tight junction and efflux transporters. Aging of the BBB is associated with endothelial senescence and metabolic remodeling, yet systematic metabolomic and lipidomic profiles of BECs in this context are lacking. Here, we provide an in vitro, discovery stage resource characterizing metabolite and lipid signatures of BEC senescence to inform BBB aging research. Senescence was induced in mouse BECs using the chemotherapeutic doxorubicin and oxidative stress mediator hydrogen peroxide. Both treatments led to increased senescence-associated β -galactosidase activity and upregulated p16 and p21, along with increased IL-6 mRNA as senescence-associated secretory phenotype marker. At the BBB structural level, senescence was accompanied by reduced protein abundance of the tight junction proteins zonula occludens 1 and occludin. Untargeted mass spectrometry of cell lysates and supernatants revealed both shared and inducer specific changes. Pathway mapping highlighted prominent remodeling of lipid metabolism (including glycerophospholipids, sphingolipids, acylcarnitines) and amino acid pathways. This dataset recapitulates several metabolites previously linked to senescence in the brain or other organs and nominates additional candidates for follow-up. To support translation, we outline a staged roadmap from monoculture discovery to validation in advanced co/tri culture and flow-based BBB models, followed by in vivo confirmation. Together, these data provide a curated molecular fingerprint of BEC senescence to guide mechanism driven studies of BBB aging and the development of potential therapeutic targets.

Physiological role and mediation of TAU-based neuronal dysfunction via specific TAU isoforms in vivo

David Benitez Alvarez^{1,2}; Qiang Guo^{1,2,3}; Meng Hou^{1,2}; Andrew S. Naylor^{1,2}; Hans Zempel^{1,2,4}

¹Institute of Human Genetics, University Hospital Cologne

²Center for Molecular Medicine Cologne (CMMC).

³Department of Clinical Laboratory, Anhui Provincial Children's Hospital, Hefei, Anhui, China

⁴Center for Human Genetics and Genomic Medicine, RWTH University Hospital, Aachen

Contact: david.benitez-alvarez@uk-koeln.de; hans.zempel@uk-koeln.de

Alzheimer's disease (AD) and related tauopathies are progressive, age-associated neurodegenerative disorders characterized by cognitive decline, motor impairment, and neuronal loss. A pathological hallmark is hyperphosphorylated TAU accumulation and neurofibrillary tangle formation, leading to neuronal dysfunction. Although six TAU isoforms are expressed in the adult human brain, their individual contributions to neuronal function and disease remain unclear. We hypothesize that TAU isoforms confer distinct functional properties to neurons, shaping cytoskeletal dynamics, synaptic activity, and differential vulnerability to tauopathy. Therefore, this project aims to investigate isoform-specific roles using complementary in vitro and in vivo approaches. In vitro, human iPSC-derived MAPT (TAU) knockout neurons were lentivirally transduced with individual TAU isoforms. Sholl analysis showed that expression of 0N3R isoform, but not other isoforms tested, enhances neurite outgrowth, increasing dendritic complexity and maximal distance from the soma. Additional experiments assessing microtubule dynamics and neuronal activity, including EB3-life imaging and multi-electrode array (MEA) recordings, have been performed, pending analysis. For in vivo studies, we first characterized the developmental expression patterns in the central nervous system of a humanized TAU (hTAU) mouse model (full gene swap). Low molecular weight isoforms (0N3R and 0N4R) predominated during early postnatal stages (one week), whereas all isoforms were detected in adulthood (after eight weeks). In parallel, hippocampal proteomics across wild-type, heterozygous, and hTAU mice will establish a baseline molecular framework and assesses TAU-dependent changes in the hippocampal proteome, alongside structural analyses of neuronal integrity, including dendritic complexity and spine density. AAV vectors encoding TAU isoforms shRNAs were generated and have already been injected (ICV in P1-2 and I.V. in 1 year old mice), I.V. for selective in vivo modulation in hTAU mice; subsequent analysis is pending. In combination with APP-Swe/Ind overexpression, this approach models AD-like pathology and enables analysis of region-specific changes in TAU pathology, protein expression, and neuronal structure. Hippocampal proteomics from AAV-treated animals will be used to identify pathways sensitive to TAU modulation. Effects on cognition and motor function will be assessed using behavioral paradigms, including open field, Barnes maze, elevated plus maze, and novel object recognition, linking molecular and cellular alterations to disease-relevant phenotypes. Altogether, this study aims to define the specific contributions of TAU to neuronal vulnerability and tauopathy progression, providing insight into the role of the TAU isoforms and potential therapeutic targets for AD and related disorders.

Keywords: AAV-gene therapies, Alzheimer's disease, Neurodegeneration, Neuronal vulnerability, Tau isoforms, Tauopathies

Epigenetic Aging Indicators are Associated with Bone Mineral Density in a Population-Based Setting

Mengyuan Cai^{1,2}; Evert F S van Velsen¹; Lieke Kuiper¹; Jinluan Chen^{1,2}; Katerina Trajanoska^{1,2}; Joyce B J van Meurs¹; Fernando Rivadeneira¹; M Carola Zillikens¹

¹ Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands.

² Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands.

Background. Epigenetic ageing markers capture biological ageing, but their links to bone mineral density (BMD) and trabecular bone score (TBS) remain inconsistent across measures.

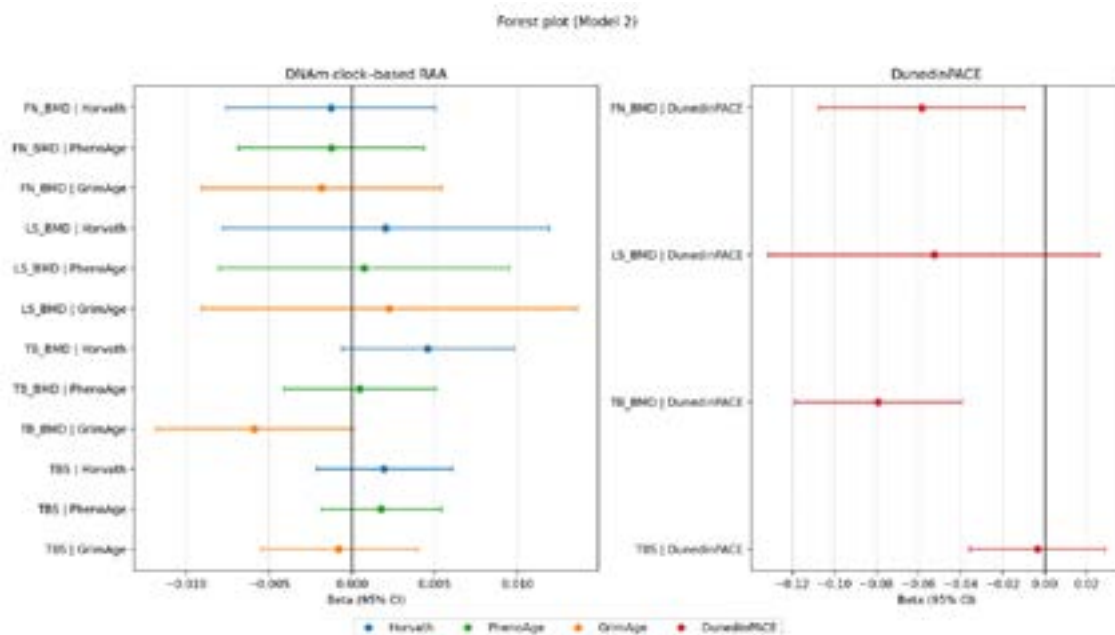
Objectives. To examine associations of epigenetic ageing indicators with site-specific BMD and TBS.

Methods. We examined four DNA methylation-based aging indicators: three epigenetic clocks (Horvath, GrimAge, and PhenoAge) that focus on relative age acceleration (RAA), and one that measures the pace of aging (DunedinPACE). RAA was defined as residuals from regressing DNAm age on chronological age (z-scored). Outcomes were femoral neck (FN), lumbar spine (LS), total body (TB) BMD, and TBS. Linear regression models were fitted with technical and blood cell composition covariates, with additional adjustment for lifestyle and clinical covariates. False discovery rate (FDR) was controlled across 16 primary tests (Benjamini-Hochberg).

Results. In the full sample (n=2,246), higher DunedinPACE was associated with lower TB BMD in the fully adjusted model ($\beta = -0.08$, 95% CI -0.12 to -0.04; FDR $q < 0.05$). Before multiple-testing correction, DunedinPACE also showed an inverse association with FN BMD ($\beta = -0.06$, 95% CI -0.11 to -0.01). In the basic model, DNAm clock-based RAAs were associated with TB BMD (Horvath: $\beta = 0.006$; GrimAge: $\beta = -0.006$), but these associations did not remain significant after FDR correction. No associations were observed for LS BMD or TBS.

Conclusion. A faster pace of ageing was associated with lower BMD, most consistently for TB BMD, whereas DNAm clock-based RAA measures showed no robust associations with BMD or TBS after multiple-testing correction.

Keywords: DNA methylation; DunedinPACE; bone mineral density; trabecular bone score; biological aging



A Combination Approach to Modulating NAD⁺ Precursor–Induced Lifespan Extension in *C. elegans*

Alexander D. Carlsson¹; He-Ling Wang^{1,*}; Evandro F. Fang^{1,2,*}

¹ Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital, Lørenskog, Norway

² The Norwegian Centre on Healthy Ageing (NO-Age) and The Norwegian National Anti-Alzheimer's Disease (NO-AD) Networks, Oslo, Norway

* Correspondence

Lead correspondence: E.F.F. e.f.fang@medisin.uio.no

NAD⁺ metabolism has become a central theme in aging research over the past decade^{1,2}. Yet recent evidence shows that animals can tolerate a large reduction in NAD⁺ without overt loss of fitness³, suggesting that its role in aging is not explained by bulk levels alone. Supplementation with the precursor nicotinamide riboside (NR) extends lifespan in some aging models⁴, albeit modestly. Because NAD⁺ is consumed by enzymes with opposing effects on aging, we hypothesize that raising NAD⁺ while simultaneously inhibiting a detrimental NAD⁺-consuming enzyme, PARP1⁵, will extend lifespan more than either intervention alone. Our preliminary data in *C. elegans* show a trend toward lifespan extension following PARP1 inhibition. We next aim to directly compare the lifespan effects of NR, the PARP1 inhibitor olaparib, and their combination.

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The Role of MARK Isoforms in Neuronal Structure and Function: Implications for Neurodegenerative Diseases

Hanin Chmes¹; Nadine Allroggen¹; Jana Chudobová¹; Hans Zempel²

¹ *Institute of Human Genetics, Köln, Germany,*

² *Institute of Human Genetics and Center for Molecular Medicine, Cologne, Germany*

Background: PAR-1/MARK kinases (Microtubule Affinity-Regulating Kinases) are serine/threonine kinases known for regulating microtubule dynamics, cell migration, neuronal polarity, and synaptic plasticity. Mammals express four MARK isoforms: MARK1 (Par1c), MARK2 (Par1b/EMK), MARK3 (Par1a/C-TAK1), and MARK4 (MARKLI/Par1d).

MARK kinases have significant roles in neuronal function, influencing microtubule stability, axon/dendrite polarity, and synaptic plasticity. Dysregulation of these pathways is implicated in neurodegenerative diseases such as Alzheimer's disease (AD). However, the distinct functions of individual MARK isoforms remain poorly understood.

Objectives: This study aims to dissect the distinct roles of MARK isoforms in neuronal structure and function, with a focus on neurodegenerative disease mechanisms. Preliminary data in primary rat neurons show that MARK isoforms differentially regulate Alzheimer-like TAU missorting. In iPSC-derived neurons, MARK isoforms differentially regulate microtubule dynamics, with MARK1 leading to fewer comets per μM , indicating isoform-specific functions.

Materials and Methods: We will use iPSC-derived human neurons to perform overexpression (OE) and knockdown (KD) of individual MARK isoforms. Dendritic complexity and spine formation will be quantified via Sholl analysis; microtubule dynamics will be assessed using EB3-GFP live imaging. Calcium imaging will evaluate activity changes following MARK modulation. Fluorescence microscopy with phosphorylation-specific antibodies will assess isoform-specific effects on TAU and PSD-95 phosphorylation, localization, and transport. MARK activity will be monitored with a FRET-based biosensor. We will also use TAU pseudophosphorylation mutants (at MARK-targeted KxGS motifs) and APP overexpression as a tauopathy model to study MARK response under disease-relevant conditions.

Results and Conclusions: Initial results show isoform specific activity, with MARK2 and MARK4 overexpression leading to significantly shorter neurites in neurons as young as day 7. This effect is not observed in TAU KO iPSC-derived neurons, indicating a dependency on TAU. MARK2 and MARK4 overexpression also induced a higher level of Tau phosphorylation, leading to more prominent mislocalisation of TAU into the somatodendritic compartment.

By elucidating the mechanisms through which MARK isoforms regulate neuronal structure and function, this study can possibly provide valuable insights into their roles in neurodegenerative diseases, with the potential to identify new therapeutic targets for intervention in tauopathies.

Challenges in Detecting Biotoxins and Biomolecules by using Surface-enhanced Raman Scattering Spectroscopy on Nanopatterned Substrates

N. E. Dina^{1*}; A. Colniță¹; D. Marconi¹; I. A. Brezeștean¹; A. S. Tătar¹

¹ Department of Isotopic and Molecular Technologies, National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania

The incorporation of nanopatterned substrates with plasmonic properties into **surface-enhanced Raman spectroscopic (SERS)** sensing platforms is a crucial part of the development of new miniaturized detection devices with on-site applications. We employed **nanoimprint lithography (NIL)** and magnetron sputtering to fabricate plasmonic nanostructures able to detect environmental contaminants and toxins. Thus, networks of tunable nanopillars were imprinted in thermoplastic polymers and metallized with Ag/Au thin films using magnetron sputtering technique. The structural and topographical properties were assessed by SEM and AFM techniques (**Figure 1**). The nanostructured sensing platforms were very suitable for SERS detection of crystal violet, for example, reaching a limit of detection of 10 pM [1] and of 1 μ M in case of nodularin [2]. After assessing these figures of merit for the fabricated SERS-active substrates, relevant biotoxins from natural acquifers or biomolecules present as biomarkers in **chronic non-communicative diseases** [3] and **neurodegeneration diseases** [4] were considered as analytes for testing their performance in practice.

Keywords: nanopatterning, biomedical applications, biotoxins, SERS, nanoimprint lithography, sensing.

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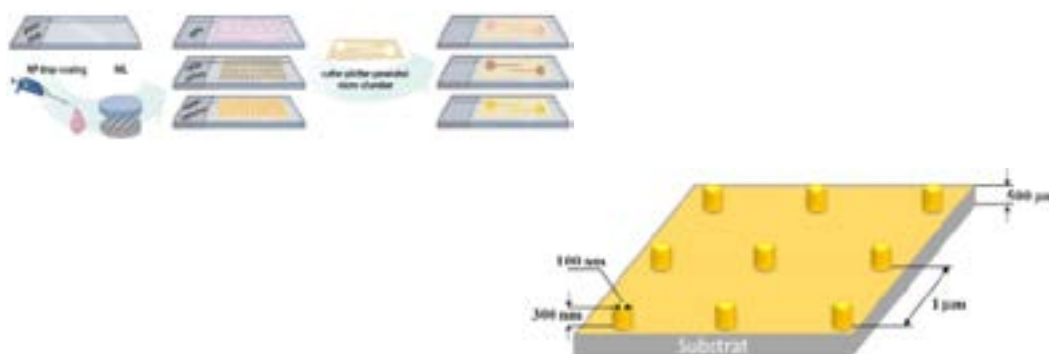


Figure 1: *Flexible substrates*: a network of periodical pillars (width: 100 nm, pitch: 1 μ m, height: 300 nm) fabricated by thermal nanoimprint lithography (NIL) and their envisioned integration in a miniaturized device.

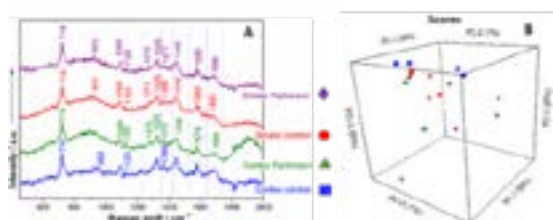


Figure 2: *SERS spectra of neural tissue* and their integrated chemometric analysis showing clear discrimination between in vivo healthy models and neurodegenerated models [4].

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Aggregate Lifespan Encyclopaedia (ALE) – A new way to record animal lifespans

Ariel Eraso¹

¹ Senckenberg, Translational Biodiversity Genomics, Frankfurt

Lifespan differs by orders of magnitude across mammals. Differences in lifespan are often attributed to the positive correlation between mass and lifespan. Though humans live five times longer than expected for their mass, there are mammals that live proportionately longer still. To better understand the genetic factors that underpin human longevity, we can leverage a wealth of newly available genomics data. Unfortunately, longevity records are currently limited to maximum recorded lifespans. These data can bias analysis as maximum lifespan records correlate with population size.

Here we present the Aggregate Lifespan Encyclopaedia (ALE), a resource expanding current lifespan records in three key ways: 1) Updated records, 2) New metrics, and 3) Transparency. ALE provides maximum lifespan data for 1,383 mammalian species, 300 more than AnAge. New individual-level lifespan data from zoos and recent publications enabled us to update existing maximum lifespan records. We also leveraged these individual-level lifespan data to calculate new metrics: Q90, E90, and bootstrapped maximum. Q90 and E90 represent the 90th percentile and average of the top 10% lifespans, respectively. Bootstrapped maximum lifespan was calculated averaging the maximum lifespan of 1000 subsets of the individual data. These metrics substantially reduce correlations between lifespan and sample size, thus enabling comparative genomics analysis to identify molecular underpinnings of natural lifespan differences. For each metric we also curated a final value, and provided informative curation rationales. Altogether we hope our updated records, new metrics, and transparency will make ALE the new gold standard for lifespan estimation and unbiased genomics.

Division-dependent regulation of cell fate & positioning in epidermis by aPKC

Sena Ezgin^{1,2}; Skyler Perot^{1,2}; Gladiola Goranci-Buzhala²; Jabiz Nafisi^{1,2}; Frederik Tellkamp^{2,4}; Marcus Krüger^{2,4}; Carien M. Niessen^{1,2,3}

¹ *Department Cell Biology of the Skin*

² *Cologne Excellence Cluster on Aging and Aging-associated Diseases (CECAD)*

³ *Center for Molecular Medicine Cologne (CMMC), University Hospital Cologne, University of Cologne*

⁴ *Institute for Genetics, University of Cologne*

Stem cell loss and changes in cell fate are a hallmark of aging. Oriented divisions regulate daughter cell fate and the capacity of tissues to self-renew. How division controls chromatin re-organization to regulate daughter cell fate is not known. The cell polarity protein aPKC is a cell fate regulator. We found that aPKC phosphorylates NuMA to control spindle orientation and now ask whether aPKC directs cell fate during division. Proteomics identified an interaction of NuMA with nuclear lamina proteins that regulate chromatin organization. We thus hypothesize that aPKC directs daughter cell fate by coordinating division orientation to nuclear and chromatin re-organization. Loss of aPKC promotes chromosome lagging and alters the localization of lamin proteins and epigenetic markers during mitosis. As a potential consequence nuclear shape, nuclear lamina and chromatin organization are changed in interphase. Combining high-resolution imaging and single-cell omics we now investigate how aPKC coordinates chromatin dynamics in division. Overall, our data identify aPKC as a key regulator of mitotic bookmarking likely to control daughter cell fate.

Age-related selection and liver-deposition of an autoantibody in mice

Arthur Fischbach¹; Niklas Müller¹; Aki Minoda²; Sarah Kreuz¹; Adam Antebi¹

¹Max Planck Institute for Biology of Ageing, Cologne, Germany.

²Radboud University, Nijmegen, Netherlands

Aging is associated with clonal selection and somatic evolution within the immune system. We identified a specific B-cell clone that expands 19-fold during aging, accounting for 13% of B-cell clonality. Plasma cells from this clone produce an antibody that accumulates specifically within liver tissue. We isolated this clone to generate hybridomas and purified the antibody for characterization; mass spectrometry confirmed this clonotype represented 17% of the hybridoma population. In aged tissues, antibody deposition was observed primarily at the endothelium, spatially coinciding with perivascular fibrosis in the liver. Immunoprecipitation-mass spectrometry (IP-MS) experiments suggest the antibody binds inflammatory factors, indicating it functions as an autoantibody. These results suggest that age-related clonal B-cell expansion contributes to tissue fibrosis via endothelial antibody deposition and autoimmune mechanisms.

A Spatial Multi-Omic Atlas of Cellular Senescence in Mouse and Human Skin

Ana Catarina Franco^{1,2}; Yaeun Han^{1,2}; Anthony Agudelo^{4,5}; Dominik Saul^{1,3}; Anthony Lagnado^{1,2}; Yukang Li^{1,2}; Ines Sturmlechner^{1,6}; Saranya Wyles⁷; James Kirkland⁸; Luigi Ferrucci⁹; Nathan LeBrasseur^{1,10}; Nicola Neretti^{4,5}; João F Passos^{1,2}

¹ Robert and Arlene Kogod Center on Aging Mayo Clinic, Rochester, MN, USA;

² Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA;

³ Department of Trauma and Reconstructive Surgery, Eberhard Karls University Tübingen, Germany;

⁴ Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI, USA.

⁵ Center on the Biology of Aging, Brown University, Providence, RI, USA.

⁶ Department of Immunology, Mayo Clinic, Rochester, MN, USA

⁷ Department of Dermatology, Mayo Clinic Rochester, MN, USA;

⁸ Center for Advanced Gerotherapeutics, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

⁹ Translational Gerontology Branch, Intramural Research Program, National Institute on Aging, Baltimore, Maryland, USA.

¹⁰ Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, Minnesota 55905, United States

The skin, the body's outermost barrier, is continuously exposed to environmental stressors, making it particularly susceptible to aging and senescence accumulation—a key driver of tissue decline. Here, we present the first spatially-resolved atlas of senescence in mouse and human skin, revealing its cell type specificity, and functional impact during aging. We first analyzed skin from young and aged mice. Preliminary spatial proteomics revealed age-related increase in senescence markers, and cell type-specific expression of cyclin-dependent kinase inhibitors. Using the p16-Ink-ATTAC mouse model, we observed a significant reduction of p16 protein levels in the dermis, where it is predominantly expressed, accompanied by increased epidermal thickness, indicating that clearance of p16-positive cells rescues epidermal thinning, a key hallmark of skin aging.

We then profiled sun-protected human skin from young and aged individuals using spatial multi-omics, including iterative indirect immunofluorescence imaging (4i), Akoya Phenocycler (CODEX), and Xenium spatial transcriptomics, with custom protein and RNA panels targeting senescence-associated features.

To define senescence, we implemented a combinatorial framework in which cells were classified as senescent if they were positive for three core hallmarks: cell cycle arrest markers, negative for proliferation markers, and positive for senescence-associated secretory phenotype (SASP). This approach revealed a significant age-related increase in senescent cells across the different platforms, with keratinocytes and fibroblasts showing the highest proportions.

Spatial analyses demonstrated that senescent cells form clusters both at protein and transcriptomic levels. These clusters were enriched for Notch and TGF- β signaling, consistent with secondary senescence. Aged skin showed increased immune infiltration, with immune cells—including cytotoxic CD8 T cells and FOXP3-positive CD4 T cells—also scoring positively for senescence based on the criteria. Senescent clusters were located proximally to immune cells, suggesting immune surveillance and recruitment.

Together, these findings provide a spatially resolved framework for understanding senescence in skin aging across species.

Investigating the link between physical activity, depressive symptoms and epigenetic aging: findings from the Moli-sani study

Gialluisi, A.^{1,2}; Bracone, F.¹; Pepe, A.¹; Limone, A.G.¹; Di Castelnuovo, A.¹; Costanzo, S.^{1,3}; Tirozzi, A.⁴; Bonaccio, M.¹; Morelli, M.¹; Licastro, D.⁵; Cerletti, C.¹; Donati, M.B.¹; de Gaetano, G.¹; Iacoviello, L.^{1,2}

¹ Epidemiology and Prevention Research Unit, IRCCS Neuromed, Pozzilli, Italy

² Department of Medicine and Surgery, LUM University, Casamassima, Italy

³ Research Center in Epidemiology and Preventive Medicine (EPIMED), Department of Medicine and Surgery, University of Insubria, Varese, Italy

⁴ Medical Genetics Unit, Department of General and Emergency Pediatrics, AORN Santobono-Pausilipon, Naples, Italy

⁵ Area Science Park, Trieste, Italy

Objective: To investigate the link between physical activity (PA), depressive symptoms and DNA methylation (DNAm) aging clocks.

Methods: In an Italian cohort (N=1,098 subjects recruited at 2005–10 and 2017–20; mean (SD) age 58.8 (5.6) y, 51.3% women; median (IQR) follow-up 12.6 (1.1) y), we tested associations of PA levels (MET-h/day in sport activities) with depressive symptoms (standardized PHQ-9 scale) and DNAm aging (Hannum, Horvath, GrimAge, DNAmPhenoAge, DunedinPACE) clocks, through multivariable linear mixed models.

Results: PA was associated with lower depressive symptoms (Beta (SE): -0.16 (0.05)), including both somatic (-0.12 (0.05)) and cognitive domains (-0.15 (0.05)). DNAm aging clocks revealed significant inverse associations of PA with the Horvath (-0.31 (0.13)) and the DunedinPACE clock (-0.01 (0.004)).

Conclusions: These findings suggest that ~3.3 additional MET-h/day of sport PA (equivalent to ~50 min/day of brisk walking), would correspond to a 2-point lower PHQ-9 score, a change that may be relevant at the population level. A potential mediation of DNAm clocks in this link is currently under investigation.

Adeno Associated Virus (AAV) Based Gene Therapy Approach for the Treatment of L-2-Hydroxyglutaric Aciduria

Qiang Guo^{1,2,3}; David Benitez Alvarez^{1,2}; Xinyu Xia^{1,2}; Meng Hou^{1,2}; Hans Zempel^{1,2,4}

¹ Institute of Human Genetics, University Hospital Cologne

² Center for Molecular Medicine Cologne, University of Cologne

³ Department of Clinical Laboratory, Anhui Provincial Children's Hospital, Hefei, Anhui, China

⁴ Center for Human Genetics and Genomic Medicine, RWTH University Hospital, Aachen

Contact to: qiang.guo@uk-koeln.de; hans.zempel@uk-koeln.de

Background

L-2-hydroxyglutaric aciduria (L-2-HGA) is a rare autosomal recessive neurometabolic disorder characterized by developmental delay, epilepsy, cerebellar dysfunction, and progressive cognitive and motor impairment. Clinical manifestations are typically slowly progressive, leading to increasing neurological disability with age. Brain magnetic resonance imaging reveals characteristic abnormalities involving the subcortical white matter reflecting widespread neurodegeneration.

The disease is caused by mutations in the L2HGDH gene, which encodes the mitochondrial enzyme L-2-hydroxyglutarate dehydrogenase. Loss of enzyme activity results in pathological accumulation of L-2-hydroxyglutarate (L-2HG), an off-pathway metabolite that is markedly elevated in blood, cerebrospinal fluid, and urine and represents a characteristic biochemical hallmark of the disease.

Despite its severe and age-dependent course, treatment options for L-2-HGA remain limited to supportive care, and no disease-modifying therapies are currently available.

Objective

This study aims to establish an adeno-associated virus (AAV)-mediated L2HGDH gene replacement strategy and to evaluate its therapeutic potential in L-2-hydroxyglutaric aciduria (L-2-HGA). We hypothesize that restoration of L2HGDH expression can compensate for the enzymatic deficiency, normalize mitochondrial metabolism, and attenuate progressive neurodegeneration. Furthermore, we aim to investigate the molecular mechanisms underlying disease progression and therapeutic response using histological, biochemical, transcriptomic, and bioinformatic approaches.

Methods and Results

Plasmids encoding human L2HGDH were constructed and used for in-house production of recombinant AAV9 and AAV-PHP.eB vectors, which were subsequently validated in HEK293 cells and human induced pluripotent stem cells (iPSCs) neurons. Western blot analysis confirmed expression of L2HGDH, while immunofluorescence demonstrated correct mitochondrial localization in both cellular systems. Functional assessment using a dichlorophenolindophenol (DCIP)-based enzymatic assay further showed increased L2HGDH activity, indicating preservation of catalytic function following AAV-mediated gene transfer.

To evaluate in vivo transduction, AAV9 and AAV-PHP.eB vectors were administered to C57BL/6N mice via intravenous (IV) and intracerebroventricular (ICV) injection. immunofluorescence analyses revealed successful transgene expression and appropriate mitochondrial localization in the brain following both delivery routes and for both serotypes. Reporter expression analyses by qPCR and western blot suggested enhanced brain transduction following ICV administration.

Therapeutic studies in L2hgdh knockout mice are currently ongoing. Future investigations will combine histological, biochemical, transcriptomic, and bioinformatic approaches to evaluate therapeutic efficacy and elucidate the molecular mechanisms underlying disease progression and treatment response.

Conclusion

We successfully established and validated an AAV mediated L2HGDH gene delivery system. In vitro studies and in vivo experiments in wild type mice demonstrated stable expression and correct mitochondrial localization of the transgene, supporting further evaluation of this strategy in in-vivo disease models of L-2-hydroxyglutaric aciduria and swift therapeutic translation

Keywords: AAV; gene therapy; mitochondrial metabolism; neurometabolic disorders; L2HGDH

Metabolic signaling controls intergenerational adaptive responses via peroxisomal catabolism

Gupta P.¹; Gevelhoff R. A.^{1,5}; Zuo S.¹; Paul D.¹; Mani S.¹; Fahrlander J.¹; Armand B.¹; Spiewag S.¹; Yan Y.^{1,4}; Chen X.^{1,5}; Schulze E.¹; Qi W.¹; Wu G.¹; Baumeister R.^{1,2,3,5}; **Heimbucher T.**^{1,*}

¹ *Bioinformatics and Molecular Genetics, Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany*

² *Center for Biochemistry and Molecular Cell Research, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany*

³ *Signalling Research Centres BIOSS and CIBSS, University of Freiburg, 79104 Freiburg, Germany*

⁴ *Faculty of Pharmaceutical Sciences, Shenzhen University of Advanced Technology, China*

⁵ *Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, 79104 Freiburg, Germany*

Transmission of stress-associated metabolic signals and resources across generations can induce strong adaptive responses in offspring, promoting their survival and supporting species continuity. However, the mechanisms driving the intergenerational transmission of signals, as well as the signalling molecules and their downstream effectors in progeny, remain largely unknown. Here, we identify signalling inputs converging on the serum- and glucocorticoid-inducible kinase SGK-1 as regulators of adaptive, intergenerational lipid metabolic processes in cold stress (CS). We find that adaptive responses benefiting offspring involve the reallocation of lipid resources from the soma to developing oocytes, at the expense of maternal survival after CS. By controlling peroxisomal catabolism and lipid mobilization to oocytes, SGK-1 and its downstream transcriptional effector PQM-1 regulate the availability of peroxisomal dicarboxylic acids (DCAs), which are essential for intergenerational signalling to the offspring. DCA availability for oocytes is determined by the peroxisomal catabolic enzyme ECH-9, which is tightly regulated by the SGK-1/PQM-1 signalling axis. Remarkably, a single DCA metabolite, the C16-DCA hexadecanedioic acid, can function as an intergenerational signalling molecule. Hexadecanedioic acid is transferred by lipoproteins to oocytes and induces the nuclear receptor NHR-49 in embryos to promote offspring survival in CS. Our data support a model in which a peroxisomal lipid metabolite can induce an intergenerational adaptive response in progeny.

Multiplexed Quantitative Epigenomic Profiling across Tissues and Liquid Biopsies

Herholz, M.¹; Alekseenko, A.¹; Elsässer, S.^{1,2}

¹ *Epigenica AB, Stockholm, Sweden*

² *Karolinska Institutet, Stockholm, Sweden*

Epigenetic remodeling is a hallmark of aging and a central mechanism through which environmental exposure, disease, and emerging interventions influence cellular function. While DNA methylation-based clocks have transformed biological age assessment, they capture only one layer of epigenetic regulation. Emerging evidence suggests that integrating histone post translational modifications with DNA methylation may improve the predictive power of aging biomarkers, yet current technologies lack the throughput and quantitative rigor required to generate integrated multi-layer epigenetic data at scale.

To address this challenge, we developed EpiFinder™ GenomePro and EpiFinder™ cNUC, complementary platforms enabling quantitative multiplex profiling of histone modifications and DNA methylation across tissue and liquid biopsies. Together, they support epigenetic discovery and longitudinal monitoring of aging-associated changes, providing a scalable framework for developing next-generation multiomic aging clocks, identifying biomarkers of intervention response, and investigating the molecular mechanisms underlying biological aging.

A neonatal-like cardiac program underlies adult heart regeneration in the naked mole-rat

Jacome Burbano, M.S.¹; Wagner, E.^{2,3}; Wang, N.¹; Grimm, S.²; Zimmermann, F.³; Geissen, S.³; Lackmann, J-W.⁴; Antczack, P.^{2,4}; Winkels, H.^{2,3}; Mollenhauer, M.³; Reznick, J.¹

¹University of Cologne, Faculty of Medicine and University Hospital Cologne, Cluster of Excellence Cellular Stress Responses in Aging-associated Diseases (CECAD), Cologne Germany

²Centre for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

³Heart Center, Department of Cardiology, Faculty of Medicine and University Hospital Cologne, University of Cologne, 50937 Cologne, Germany;

⁴Center for Molecular Medicine Cologne (CMMC), University of Cologne, 50937 Cologne, Germany
Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany

Cardiac regeneration is severely restricted in most adult mammals, leading to irreversible damage, fibrosis, and loss of function after myocardial infarction (MI). In contrast, neonatal mammals transiently regenerate the heart through cardiomyocyte (CM) proliferation and minimal scarring, but this capacity is rapidly lost after birth.

Here, we identify the naked mole-rat (NMR, *Heterocephalus glaber*) as a striking exception. Adult NMR hearts retain neonatal-like metabolic and structural traits, including reduced fatty-acid oxidation, a subset of mono-nucleated diploid CMs, and fetal-like contractile proteins. Following MI, NMR mount a regenerative response. Despite comparable initial injury to mice, NMR hearts show sustained CM cell-cycle re-entry, progressive scar resolution and up to 30% recovery. ECM proteomics identified a factor enriched in injured NMR hearts. Injection of this factor into adult mice after MI stimulated CM proliferation and reduced fibrosis, suggesting NMR ECM components can reactivate regenerative programs. Overall, these results establish the naked mole-rat as a unique adult mammalian model of heart regeneration.

Microtubule surface regulation by polyglutamylating TTLL enzymes in human tauopathy models – Implications for neurodegeneration and healthy brain aging

Laura Köhler¹; Hans Zempel¹

¹Institute of Human Genetics & Center for Molecular Medicine Cologne
[laura.koehler@uk-koeln.de]

Background:

Age-associated neurodegenerative diseases such as Alzheimer's disease are characterized by progressive cytoskeletal dysfunction, impaired axonal transport, and synaptic loss. Central to these processes is the destabilization of neuronal microtubules and the pathological redistribution of TAU. Microtubule function is critically regulated by post-translational tubulin modifications, including polyglutamylation mediated by tubulin tyrosine ligase-like (TTLL) enzymes. However, the contribution of individual TTLL enzymes to neuronal integrity and TAU-dependent pathology in human neurons remains poorly understood.

Methods:

Human iPSC-derived cortical neurons are used to investigate how distinct TTLL enzymes regulate neuronal microtubule organization and dynamics during neuronal maturation. Expression of selected TTLL enzymes is modulated using lentiviral overexpression and shRNA-mediated knockdown approaches. Cytoskeletal organization, tubulin post-translational modifications, and TAU localization are analyzed by immunocytochemistry. Neuronal morphology and dendritic architecture are assessed by quantitative image analysis, while live-cell EB3 imaging is applied to characterize microtubule growth dynamics. In addition, neuronal network activity and functional connectivity are examined using multielectrode array (MEA) recordings. To evaluate the translational relevance of TTLL-dependent mechanisms in vivo, stereotactic delivery of TTLL1 knockdown constructs is performed in hTAU transgenic mice.

Preliminary Results / Ongoing Work:

Initial findings indicate that modulation of individual TTLL enzymes differentially affects neuronal morphology, microtubule dynamics, and TAU localization in human iPSC-derived neurons. TTLL overexpression induced stage-dependent alterations in tubulin post-translational modifications, including increased acetylation and tyrosination in mature neurons. Furthermore, TTLL1 and TTLL6 differentially affected axonal and dendritic architecture, with several structural phenotypes showing reduced effects in TAU knockout neurons, suggesting TAU dependency. Live-cell EB3 imaging revealed distinct enzyme-specific effects on microtubule growth behavior, including increased growth velocity and run length following TTLL6 overexpression. In parallel, in vivo studies using AAV-mediated TTLL1 targeting in hTAU mice are currently ongoing to investigate the translational relevance of TTLL-dependent microtubule regulation in tauopathy-associated neurodegeneration.

Conclusion:

Our findings suggest that polyglutamylating TTLL enzymes differentially regulate microtubule surface properties, neuronal morphology, and TAU compartmentalization in human neurons. The observed effects on cytoskeletal organization, microtubule dynamics, and neuronal network activity support a role for TTLL-dependent tubulin modifications in mechanisms associated with neuronal vulnerability and tauopathy progression. Ongoing in vivo studies will further address the translational relevance of TTLL1 modulation in hTAU mice. Together, this work provides mechanistic insight into microtubule dysregulation during neurodegeneration and may contribute to the development of therapeutic strategies aimed at preserving neuronal integrity during brain aging.

Unravelling the role of H3K4me2 in response to DNA damage and transcription stress during development and ageing

Angelina Job Kolady^{1,3}; Walter Sandt³; David H. Meyer^{1,3}; Anna Eva Koch^{1,4}; Robert Hänsel – Hertsch^{1,3,4}; Björn Schumacher^{1,3,4}; Siyao Wang^{1,2,3}

¹ Institute for Genome Stability in Ageing and Disease, Medical Faculty, University of Cologne, Germany

² Institute of Molecular Biology, Mainz, Germany

³ Cologne Excellence Cluster for Aging and Aging-Associated Diseases (CECAD), Cologne, Germany

⁴ Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

Cells are continuously exposed to DNA damage that can stall RNA Pol II, threatening genome stability^{1,2} and contributing to diseases such as cancer, neurological disorders, and cardiovascular disease. We investigated the role of histone H3 lysine 4 dimethylation (H3K4me2) in transcription recovery after transcription-blocking stress using *C.elegans* and mouse dermal fibroblasts. H3K4me2 levels increased during recovery and correlated with productive transcription elongation. Reduced H3K4me2 delayed development and impaired recovery, while elevated levels accelerated recovery, indicating a conserved role in restoring transcription. Temporal analyses showed that RNA Pol II Ser2P reappears before H3K4me2 accumulation, suggesting H3K4me2 sustains rather than initiates elongation. CUT&Tag profiling revealed coordinated H3K4me2 and RNA Pol II Ser2P regulatory patterns during transcription recovery. Ongoing IP-MS studies are examining how the H3K4me2 is recruited during transcription recovery. These findings identify H3K4me2 as a conserved chromatin feature that promotes transcription recovery and genome stability during ageing.

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Hesperidin and Melatonin Synergistically Enhance Longevity and Mitigate Amyloid- β Toxicity Through ACR-16-Dependent Activation of DAF-16/NRF2 Driven Autophagy

Kumar, S.^{1,2} & Suchiang, K.^{1,3}

¹ Department of Biochemistry and molecular Biology, Pondicherry university, Puducherry, India

² The College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

³ Department of Biochemistry, North-Eastern Hill University, Shillong, India

Aging is closely associated with dysregulation of autophagy, and the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) has emerged as a key regulator of this process. Given its central role in neurodegenerative and metabolic diseases, the $\alpha 7$ nAChR signaling network represents a promising target for gero-therapeutic drug repurposing. Hesperidin, a well-established gero-protective compound, and melatonin, a known modulator of $\alpha 7$ nAChR signaling, were evaluated individually and in combination for their effects on lifespan and healthspan in *Caenorhabditis elegans*.

Hesperidin significantly extended lifespan, while its combination with melatonin produced an additive effect. The combination treatment enhanced healthspan parameters and improved resistance to oxidative stress. Mechanistically, treatment upregulated ACR-16/ $\alpha 7$ nAChR, DAF-16/FOXO, and SKN-1/NRF2 signaling, as demonstrated in transgenic strains. RT-PCR analysis revealed that *daf-16* and *skn-1/nrf2* expression showed no significant changes in *acr-16* mutant worms, whereas significant upregulation was observed in wild-type N2 animals, indicating ACR-16 dependency. Furthermore, autophagy induction was confirmed by increased GFP::LGG-1 puncta in treated worms, along with elevated expression of autophagy-related genes (*lgg-1* and *bec-1*), with maximal effects observed under combination treatment.

In the Alzheimer's disease model strain CL2331 expressing $A\beta_{3-42}$, both hesperidin and melatonin reduced amyloid- β aggregation, with the most pronounced reduction observed in the combination group. This was accompanied by significant downregulation of reactive oxygen species levels in CL2331 worms and a marked delay in paralysis progression, again with maximal efficacy in the combined treatment. Gene expression analysis further confirmed significant upregulation of *acr-16*, *daf-16*, *skn-1*, *lgg-1*, and *bec-1*, particularly in the combination group.

Collectively, these findings demonstrate that hesperidin and melatonin synergistically promote longevity, enhance autophagy, and mitigate amyloid- β toxicity via an ACR-16-dependent DAF-16/NRF2 driven autophagy. This study highlights the therapeutic potential of this combination as a candidate strategy for gero-protection and neurodegenerative disease intervention.

Keywords: Hesperidin; Melatonin; Autophagy; Oxidative stress; $\alpha 7$ nAChR; *C. elegans*; Amyloid- β

Molecular signatures of sex-specific liver aging

Merve Kutay¹, Andreas Beyer¹

¹ *Cologne Excellence Cluster on Aging and Aging-associated Diseases (CECAD)*

Chronic liver diseases and liver ageing are global health issues that trigger complex transcriptomic pathways at the cellular level. Recent studies have demonstrated that transcriptomic responses to liver damage and ageing processes exhibit significant biological variations between female and male organisms. However, existing research using murine models has been strongly biased towards using male animals only, which poses a major barrier towards elucidating sex-dependent effects of age on liver health. To investigate sex-specific liver aging at the molecular level, this study identified transcriptional marker genes that facilitate an unbiased quantification of cellular damage to hepatocytes. The resulting hepatocyte damage score was subsequently used to analyze how male versus female transcriptional aging trajectories distinctly impact on liver health. To avoid sex bias, diverse liver damage datasets were split into two training groups: 13 female and 21 male. In each group, genes that were statistically significant in at least 50% of the datasets and up- or down-regulated in the same direction in at least 75% of them were filtered. The 165 common genes identified from the intersection of the female and male groups were tested using 13 independent datasets and selected as the “top 101 global marker genes” with the most robust transcriptomic signal. In the next step, age-related genes will be identified using the female and male age datasets. The accuracy of these genes in predicting hepatocyte damage scores (HDS) will be modelled using Elastic Net regression analysis, and the sex-specific changes in damage profiles with aging will be analysed. Ultimately, this sex-unbiased bioinformatics approach establishes a robust framework for identifying universal hepatocyte diagnostic markers while opening new ways to untangle how aging dynamics drive liver pathology with sex.

Keywords: Liver Aging, Hepatocyte Damage Score (HDS), Bioinformatics, Sex Bias, Elastic Net Regression

Old muscle, new rules: muscle derived FGF21 drives systemic resistance to protect failing muscle

Lienau, J.^{1,3}; Gillum, M.P.¹; Gerhart-Hines, Z.² & Finger, F.¹

¹ Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

² Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

³ joelina.lienu@sund.ku.dk

Aging creates a paradox for FGF21: while analogous are therapeutics showing efficacy in metabolic disease, endogenous FGF21 is chronically elevated in aging. As homeostatic control declines with age, stress pathways become activated, leading to increased circulating FGF21. Skeletal muscle – a major source of FGF21 under mitochondrial stress and aging – remains poorly understood as a driver of systemic signaling stress. We used a muscle-specific mitochondrial myopathy model that accelerates skeletal muscle aging and drives circulating muscle-derived FGF21 to near-pharmacological levels. We found no canonical response of FGF21, consistent with an actively induced resistance state safeguarding failing muscle in aging. Together, these findings sharpen the paradox of FGF21 biology in aging: sustained endogenous elevation does not yield productive signaling. Instead, FGF21 responsiveness is shaped by tissue context and chronic stress. Defining when FGF21 is adaptive and when resistance emerges will be critical for identifying biomarkers, stratifying patients, and guiding personalized FGF21-based therapies for aging and age-related disease.

Ex vivo expansion of hematopoietic stem and progenitor cells as a platform for partial reprogramming

Loyva, K.^{1,2}; Arif, N.^{1,2,3}; Robertson, N.^{1,4}; Chandra, T.^{1,4} & Kirschner, K.^{1,2}

¹ Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, USA

² Department of Hematology, Mayo Clinic, Rochester, MN USA

³ Graduate School of Biomedical Sciences, Mayo Clinic, Rochester, USA

⁴ Department of Quantitative Health Sciences, Mayo Clinic, Rochester, USA

Partial reprogramming has emerged as a promising rejuvenation method after researchers observed a reduction in the epigenetic age of cells during early reprogramming. Because this reduction in epigenetic age occurs early in the reprogramming time course, cells are still able to maintain their somatic identity without transitioning to a pluripotent state. The application of partial reprogramming to the blood system offers the potential for rejuvenation of multiple organ systems due to the systemic nature of blood with hematopoietic stem and progenitor cells (HSPCs) serving as the ideal target. HSPCs reside at the top of the hematopoietic hierarchy where a rejuvenation-based intervention could have the greatest impact, and these cells possess a range of established in vitro assays to assess function, making them an ideal system to test partial reprogramming-based rejuvenation. However, maintenance of primary human HSPCs in vitro presents challenges due to the rarity of this cell population in the peripheral blood and the specific requirements needed for long-term culture. In this study, we compared four different HSPC media conditions in addition to the traditional media conditions used in early reprogramming to assess how culture condition affects progenitor cell subpopulations. We then compared the effects of these media conditions on early reprogramming by comparing the proportion of TRA-1-60+ cells (an early pluripotency marker) to CD34+ cells (an HSPC marker), allowing us to assess the change in somatic identity and conversion to pluripotency within the reprogrammed HSPC population. These data provide guidance on in vitro conditions for human HSPCs and how best to maintain somatic identity during partial reprogramming-based rejuvenation techniques.

Harnessing Stem Cell Glycoengineering for Healthy Aging: Current Progress and Future Directions

Makinde, I.¹; Adetunmibi, F.O.²

¹*Independent Researcher, Lagos, Nigeria*

²*Independent Researcher (PharmD), Lagos, Nigeria*

Stem cell exhaustion and declining tissue regeneration are hallmarks of aging. Cell therapies are hampered by poor homing, accelerated senescence, and compromised survival in aged microenvironments. An underexplored contributor is age-associated remodeling of cell-surface glycosylation. The glycocalyx governs niche adhesion and immune recognition, yet targeted glycan manipulation for longevity medicine remains uninvestigated. This review examines glycoengineering as a precision tool to optimize stem cell performance in aging. We synthesize evidence that glycan modification specifically upregulating sialyl Lewis X to enhance selectin-mediated homing (Sackstein, 2022) and hypersialylation to engage inhibitory host Siglec receptors to cloak cells from clearance (Läubli & Borsig, 2019) reduces immune destruction and attenuates SASP signaling (Chen et al., 2021). Chemical platforms include metabolic labeling, exoglycosidase treatment, and glycosyltransferase remodeling (Alghazali et al., 2024). We propose a roadmap integrating glycoengineering into autologous therapies to promote healthy ageing.

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Increasing membrane fluidity preserves endolysosomal integrity and limits tau propagation and toxicity

Martin, N.¹; Sandhof, C.A.^{2,3}; Tittelmeier, J.¹; El-Kabarity, D.¹; Ngonza-Nito, S.-B.⁴; Melki, R.⁴; Nussbaum-Krammer, C.^{1,2}

¹ Chair of Neuroanatomy, Institute of Anatomy, Faculty of Medicine, LMU Munich, Munich, Germany

² Center for Molecular Biology of Heidelberg University (ZMBH) and German Cancer Research Center (DKFZ), DKFZ-ZMBH Alliance, Heidelberg, Germany

³ Chemical Neurobiology Laboratory, Center for Genomic Medicine, Departments of Neurology and Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

⁴ Institute Francois Jacob (MIRCen), CEA, and Laboratory of Neurodegenerative Diseases, CNRS, Fontenay-Aux-Roses, France

Aging is the major risk factor for neurodegenerative disorders such as Alzheimer's disease that are characterized by the accumulation and spreading of tau aggregates. During cell-to-cell propagation, tau seeds enter recipient cells via endolysosomal uptake. Subsequent vesicle rupture allows tau seeds to escape into the cytosol and template further aggregation, suggesting that endolysosomal integrity limits tau propagation. To identify protective mechanisms, we established a *C. elegans* tau spreading model and performed a genome-wide RNAi screen for regulators of endolysosomal integrity, identifying sphingolipid metabolism as a key pathway. Silencing sphingolipid metabolism genes decreased membrane fluidity and increased vesicle rupture, while tau aggregation further exacerbated membrane rigidification. Conversely, supplementation with unsaturated fatty acids restored membrane fluidity, reduced rupture, and ameliorated tau-associated defects in human cell models and *C. elegans*. Together, these findings identify endolysosomal membrane fluidity as a potential therapeutic target to preserve vesicle integrity and limit tau pathology.

Metabolic reprogramming as a quality control strategy in long-lived bats

Asya Martirosyan¹; Björn Schumacher¹

¹ Cologne Excellence Cluster on Aging and Aging-associated Diseases (CECAD)

Bats (*Chiroptera*) exhibit extraordinary longevity relative to their body size, yet the cellular mechanisms underlying this phenomenon remain poorly understood. Using primary fibroblasts from *Myotis myotis* (long-lived, ~37 years), *Rousettus aegyptiacus* (shorter-lived, ~20 years), and *Homo sapiens* as a reference, we investigated species-specific responses to UVC-induced genotoxic stress through an integrated multi-omics approach encompassing quantitative proteomics, phosphoproteomics, Seahorse respirometry, live-cell apoptosis profiling, and mitochondrial functional assays.

Under genotoxic stress, *Myotis* fibroblasts mounted a pronounced upregulation of TCA cycle and fatty acid oxidation enzymes, accompanied by increased mitochondrial OXPHOS activity and remodelling of mitochondrial dynamics. This metabolic shift was coupled with apoptotic priming — evidenced by pro-apoptotic protein phosphorylation and live-cell caspase activation — suggesting that *Myotis* preferentially eliminates damaged cells through a metabolically-fuelled quality control program. *Rousettus*, despite its comparatively shorter lifespan, showed a markedly different metabolic and bioenergetic stress response, pointing to divergent cellular strategies even within the same order. Human fibroblasts displayed robust ATM-mediated DNA damage signalling with comparatively muted metabolic engagement.

Together, these findings support a model in which the longevity of *Myotis* is underpinned by a metabolic-first quality control strategy: rapid energetic commitment to mitochondrial function primes efficient apoptotic clearance of genotoxically damaged cells, potentially limiting the accumulation of senescent or mutagenised cells over a long lifespan. This work positions bat comparative biology as a productive framework for identifying actionable metabolic nodes in healthy ageing.

The Dynamic Duo: How p16 and p21 Drive Senescence in Cardiovascular Disease.

Mathison L.¹; Booth L.¹; Redgrave R.¹; Booth P.¹; Korolchuk V.¹; White S.¹; Gavin Richardson¹

¹ *Vascular Biology and Medicine Theme, Biosciences institute, Newcastle University, UK*

Cellular senescence is recognised as a contributor to age-related cardiovascular disease and may represent a fundamental driver of myocardial remodelling. Using single-nuclei RNA sequencing of human donor hearts, we identified distinct senescence-associated signatures, with p21 correlating with cardiovascular disease markers, including proBNP and fibrosis, whereas p16 increased with age. Importantly, cardiomyocytes (CMs) expressed p21 yet demonstrated little to no detectable p16, supporting the concept that senescence in post-mitotic CMs is predominantly p21-associated and stress-induced, rather than p16-driven or replicative. We therefore generated a doxycycline-inducible p21 AC16 cardiomyocyte model to activate senescence independently of broad chemical or physical stressors, such as doxorubicin or X-ray irradiation, which may induce senescence independent effects. This approach is particularly important in CMs, where ageing cannot be modelled via and replicative senescence. p21 induction, did not activate p16, but induced several senescence-associated features, including a hypertrophic morphology, increased expression of the CM SASP protein GDF-15, and induction of PURPL, an epigenetic driver and biomarker of cellular senescence. This model will be used to determine how p21-driven senescence alters cardiomyocyte signalling, electrophysiological conduction and contraction, to understand why senescent CMs are detrimental to the ageing and diseased heart. We will also establish engineered 3D cardiac models for functional analysis and drug screening. Together, this work will improve our understanding of cardiomyocyte and post-mitotic senescence and guide the development of novel therapeutic strategies for cardiac ageing and disease.

Methylation-associated Gene Expression (MaGE) proxies of cellular senescence: scalable systemic senescence biomarkers in relation to disease severity and 174 incident disease outcomes.

Daniel Simpson^{1,2,*}; Samuel Crofts^{1,2,3,*}; **Christos Mavrommatis**^{1,4,*}; Kirstyn Loyva^{1,5}; Yossawat Suwanlikit^{1,2,6}; Thomas White¹; Riccardo Marioni⁷; Kristina Kirschner^{1,5}; Nathan Lebrasseur^{1,8,†}; Tamir Chandra^{1,9,†}

* These authors contributed equally to this work

† These authors jointly supervised this work

1 Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester MN, USA

2 Mayo Clinic Graduate School of Biomedical Sciences, Rochester MN, USA

3 School of Biological Sciences, University of Edinburgh, Edinburgh, UK

4 Graduate School for Biological Sciences, University of Cologne, Cologne, Germany

5 Department of Hematology, Mayo Clinic, Rochester MN, USA

6 Department of Pharmacology, Faculty of Science, Mahidol University, Thailand

7 Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK

8 Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, Minnesota, USA

9 Department of Quantitative Health Sciences, Mayo Clinic, Rochester MN, USA

Cellular senescence is a key hallmark of aging, yet its measurement remains limited by invasive, tissue-specific techniques — unsuitable for large-scale cohort research. We present MaGE (Methylation-associated Gene Expression predictors) — the first DNA methylation-based proxies of senescence gene expression, providing a whole-blood-derived, systemic measure of senescence burden, establishing a new class of tool via DNAm-based RNA prediction. MaGE was developed via Elastic Net on paired DNAm and qPCR for p16^{INK4A}, p14^{ARF} and p21^{CIP1} (n=514). MaGE scores associated with COVID-19 severity and Crohn's disease. Across 174 incident disease outcomes and all-cause mortality (10-year follow-up; n=18,859), MaGE generated 49 Bonferroni-significant associations across 18 unique diseases and mortality ($P < 0.05/174$), with strongest associations for MaGE-p16/Pulmonary Fibrosis (HR=2.10, $P=1.99 \times 10^{-5}$) and MaGE-p21/Alcoholic Liver Disease (HR=2.64, $P=1.94 \times 10^{-5}$) — outperforming 14 epigenetic clocks and standard clinical risk factors, including FEV₁ and smoking. MaGE transforms a specialized assay into a scalable senescence biomarker.

Regulatory Bias in Epigenetic Aging Dynamics

Morozova, T.¹; Polster, A.¹ & Axelson-Fisk, M.²

¹Department of Life Sciences, Chalmers University of Technology, Gothenburg, Sweden

²Department of Mathematical Statistics, Chalmers University of Technology, Gothenburg, Sweden

Aging reflects both stochastic fluctuation and biological regulation [1]. We present a Markov chain framework for epigenetic aging that extends noise-driven models [2] by adding a state-dependent bias term representing regulatory constraint. Using DNA methylation data from mice, rats, and bats, we show that the biased model restricts state-space exploration, rendering certain methylation states progressively inaccessible, consistent with empirical data across species. The results indicate that regression-based estimates of drift and covariance achieve lower mean, covariance, and correlation errors than the biased model. The pattern of state loss is consistent with discrete bifurcation events, suggesting resilience declines stepwise rather than continuously. This implies that the solution space for intervention narrows irreversibly at each transition, making intervention timing structurally critical.

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Redox-Regulated Chaperones at the Interface of Protein and Redox Homeostasis During Early Aging in *Caenorhabditis elegans*

Shayan Motiei¹; Kathrin Ulrich¹

¹ *Molecular and Cell biology, Saarland University, Saarbrücken Germany*

Disruption of redox and protein homeostasis represents an early consequence of aging. Importantly, these processes are tightly interconnected, such that impairment of one accelerates dysfunction of the other. Redox-regulated chaperones represent a first line of defense against oxidative stress-induced protein aggregation. To elucidate their role, we investigate the dual-functional protein ASNA-1 in *C. elegans*. Under non-stress conditions, ASNA-1 functions as a targeting factor in the GET pathway. Upon oxidative stress, however, ASNA-1 becomes an ATP-independent chaperone holdase that binds unfolding protein intermediates, prevents aggregation, and facilitates aggregate clearance. Our data demonstrate that ASNA-1 depletion results in protein aggregation and a shift toward a more oxidized milieu. We aim to determine how ASNA-1 regulates protein and redox homeostasis early in life and whether its dysregulation affects aging phenotypes later in life.

Restoring Mitochondrial Resilience: From Patient-Derived Preclinical Models to Therapeutic Intervention and Setup of Clinical Trials for POLG-associated Mitochondriopathies

Naylor, A. S.^{1,2}; Xia X.^{1,2}; Wojke, M.^{1,2}; Borgart, E.^{1,2}; Al Abed, L.^{1,2}; Cakmak, C.^{1,2}; Langerscheidt, F.^{1,2}; Mantey, I.^{1,2}; Zempel, H.^{1,2,3}

¹ Institute of Human Genetics, University Hospital Cologne, University of Cologne, Kerpener Str. 34, 50931 Cologne, Germany

² Center for Molecular Medicine Cologne (CMMC), University of Cologne, Robert-Koch-Str. 21, 50931 Cologne, Germany

³ Center for Human Genetics and Genomic Medicine, RWTH University Hospital Aachen, Germany

Background: Mitochondrial dysfunction is a hallmark of ageing and contributes to neurodegeneration. POLG-spectrum disorders (POLG-SDs) provide a genetically defined human model of impaired mitochondrial DNA maintenance, chronic mitochondrial dysfunction and reduced lifespan. **Methods & Results:** Using patient-derived fibroblasts, keratinocytes, hiPSCs and differentiated neurons, we identify deficits in mtDNA content, mitochondrial function and bioenergetics. Pharmacological stimulation of mitochondrial biogenesis partially restores disease-relevant phenotypes, supporting mitochondrial targeted therapeutic approaches. **Ongoing Work & Future Perspectives:** Building on these findings, we are evaluating gene-based strategies targeting key regulators of mitochondrial maintenance and function. Together, these studies are guiding the development of clinical trials, for which patient recruitment has begun, establishing a translational pathway from patient-derived models to therapeutic intervention in POLG-SDs and related mitochondrial disorders.

Age, SASP and Cellular Senescence Impact Influenza Vaccine Response

Ovsyannikova, I.G.; Quach, H.Q.; Ratishvili, T.; Haralambieva, I.H.; Kennedy, R.B.

Vaccine Research Group, Mayo Clinic, Rochester, Minnesota, United States

Background. While immunologic aging impacts immune responses to influenza vaccination, reliable biomarkers associated with aging of the immune system and suboptimal serologic response to influenza vaccination remain to be elucidated.

Objectives. The purpose of our study was to investigate whether age, SASP and senescent cells contribute to impaired immune response following influenza vaccination.

We quantified multiple serum and cell-based parameters related to immune aging (SASP, cytokines/chemokines, senescent T-cell frequencies) in a cohort of influenza vaccine recipients and evaluated their associations with vaccine-induced HAI response.

Results. We found that age had an impact on the SASP profile, with positive correlations observed for GDF-15/TNFR1/MMP-3/RAGE/MMP-2 levels ($p < 0.05$), while IL-1a/TRAIL levels were inversely associated with age. Notably, IL-18/MMP-3/GDF-15 were elevated in males compared to females ($p < 0.05$). At 28 days post-vaccination, MCP-1/TNFR2 levels were higher in those that seroconverted compared to those that did not. We observed higher p16ink4a expression ($n=149$, ≥ 65 yo) in CD4+T-cells by RT-qPCR in females compared to males ($p=0.0004$). We found that p16ink4a expression was significantly higher in individuals who did not seroconvert (median 6.2 [3.5;11.1] vs 3.8 [2.3;6.5], $p=0.011$), an effect that was larger in males ($p=0.003$). We assessed senescent T cell frequencies in older (≥ 65 yo) and younger adults (18–40yo). SA- β -gal+CD28-CD57+ CD4+ cells were detectable at low frequencies and correlated with p16ink4a PCR ($r=0.46$, $p=0.01$). The CD8+T-cell compartment was enriched in senescent T-cells in older adults. We detected a marginally significant negative association between senescent CD8+ T-cells and HAI titer ($r=-0.28$, $p=0.07$). Senescent CD4+ and CD8+T cells were highly correlated ($r=0.72$, $p < 0.0001$). SASP components (MMP-2/GDF-15/MMP-9; $p < 0.05$) were associated with senescent T-cell frequencies.

Conclusions. These data demonstrate a complex interplay between senescent cells in the CD4+ and CD8+ compartments, confirm a relationship with decreased vaccine response, and highlight connections with markers of immunosenescence.

Exercise does not slow aging: comparison with drugs, supplements and gerotherapeutics

Pabis, K.^{1,2}; Yeo, C.³; Liu, Y.⁴; Gruber, J.^{1,2}; Tolwinski, N.S.^{5,6} & Kennedy, B.K.^{1,2,7}

¹ Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

² Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

³ Biomedical Engineering, College of Design and Engineering, National University of Singapore, Singapore

⁴ Yong Loo Lin School of Medicine, National University of Singapore, Singapore

⁵ Science Division, Yale-NUS College, Singapore

⁶ Cancer and Stem Cell Biology Program, Duke-NUS Medical School, Singapore

⁷ Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Exercise improves health, but whether it slows aging biology has not been rigorously tested and there exist few comparisons against geroprotective candidates. We tested this using fly lifespan experiments and performed a comparative meta-analysis of exercise, drugs and supplements. We show here convergent evidence from five different species, including humans, that the benefits of exercise on all-cause mortality – a gold standard for impacts on aging – are inconsistent. Several interventions showed clinical evidence equal to or stronger than exercise, whereas others had stronger preclinical support. Except for exercise, few interventions have been tested across both human and preclinical study designs, limiting direct comparison. Where exercise was tested experimentally, evidence did not support large effects on aging biology. Cohort-study benefits were partly explained by weight loss, residual lifestyle confounding and reverse causation. Based on these results, we propose that trials of putative geroprotectors should receive more funding and that combinatorial therapies, rather than exercise as a monotherapy, are the most promising therapy.

Disentangling cellular damage and aging

Tsimafei Padvitski; Andreas Beyer

¹ *Cologne Excellence Cluster on Aging and Aging-associated Diseases (CECAD)*

The main message: Aging and damage are largely inseparable at the transcriptomic level, with identity erosion mediating substantial part of their shared variance. In other words, a dominant characteristic of aging and damage is loss of cellular identity.

Previously, we have developed Podocyte and Hepatocyte damage scores, a transcriptome-based metric that quantifies cellular damage with high accuracy. Podocytes are critical for kidney filtration and become progressively compromised with age, contributing to age-related decline in kidney function. Hepatocytes are the principal parenchymal cells of the liver, responsible for metabolic homeostasis, detoxification, and protein synthesis, and likewise undergo functional deterioration during aging and chronic injury.

It remains unclear whether aging and damage represent distinct axes or collapse onto a shared transcriptional trajectory. In this study, we quantified the extent to which cellular aging, damage, and cell-type identity represent overlapping versus distinct transcriptional programs in podocytes and hepatocytes. We employed transcriptome-based scores for damage (DGS), aging (AGS), and cell-type identity (CTS) as quantitative proxies of these biological processes. We found that the three scores are collinear within each cell type, sharing a substantial portion of transcriptional variance. Notably, DGS most accurately tracked morphological and physiological damage in podocytes. In an independent aging cohort, all three scores predicted chronological age, indicating that aging is tightly coupled to both progressive cellular damage and erosion of cell-type identity.

Proteomics of Germ Line Reset Across Species

Leon Peshkin (peshkin@gmail.com)

Beth Israel Deaconess Medical Center, Boston, MA 02115, USA

Germ line reset is an ancient rejuvenation program implemented in oocytes and embryos, first described by Weismann and revealed by Gurdon nuclear transfer. Cloned animals reset age, indicating broad cytoplasmic reprogramming beyond epigenetics. We highlight cytoplasmic quality control, including proteolytic cleanup, sorting defective mitochondria and extra-chromosomal DNA out, and handling aggregated proteins across *Xenopus*, mice, and yeast. Recent work shows oocytes sequester and reprocess damaged proteins in meiosis, linking cell cycle dynamics to rejuvenation. We present vertebrate oocyte proteomics using quantitative mass spectrometry to follow nontranscriptional stages of oogenesis, maturation, and early development. This system enables detailed analysis of proteome and phosphoproteome changes. Our goal is to map when and which proteins aggregate and are cleared, and how this differs with age. Identifying timing and mechanisms of Germ Line Reset may inspire strategies to improve proteostasis and support rejuvenation in somatic cells, tissues and bodies.

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A nutraceutical strategy against senescence by using oleic acid

Pilar Picallos-Rabina*; Bertien Dethmers-Ausema; Marco Demaria

European Research Institute for the Biology of Ageing (ERIBA), University of Groningen and University Medical Center, Groningen, Groningen, the Netherlands

Dietary composition exerts a profound influence on aging trajectories and susceptibility to age-related diseases. While specific diets such as the Mediterranean diet are associated with longevity and reduced incidence of chronic conditions, the molecular mechanisms linking nutrient intake to cellular aging remain incompletely defined. Cellular senescence—a state of stable proliferative arrest coupled with a pro-inflammatory senescence-associated secretory phenotype (SASP)—has emerged as a key driver of tissue dysfunction and systemic aging. Modulating the SASP without eliminating senescent cells represents a promising therapeutic avenue. In this study, we investigate the role of oleic acid (OA), the predominant monounsaturated fatty acid in the Mediterranean diet, as a dietary senomorphic compound. Our data demonstrate that OA robustly suppresses the expression and secretion of canonical SASP factors across multiple cell types and senescence-inducing stimuli, while also enhancing the sensitivity of senescent cells to the senolytic agent ABT-263. Furthermore, OA treatment limits the paracrine propagation of senescence and reduces SASP amplification in response to secondary stress, thereby stabilizing the senescent cell phenotype and its microenvironmental impact. Additionally, OA treatment extends the replicative lifespan of fibroblasts in vitro and is associated with a reduced burden of DNA damage.

In an in vivo model of chemotherapy-induced accelerated aging, preliminary findings suggest that OA treatment improves grip strength and preserves intestinal barrier integrity. Ongoing studies in naturally aged mice further suggest that OA administration, even when initiated late in life, may confer beneficial effects on median lifespan, as well as physical and cognitive performance.

Collectively, these findings identify OA as a promising nutraceutical senomorphic capable of modulating senescence-associated dysfunction and enhancing resilience to age-related decline.

Spectrin coordinates cell shape and signaling essential for epidermal differentiation

Soffer, A.^{1*}; Bhosale, A.^{2,3*}; Ghodrat, R.^{2,3}; Peskoller, M.^{2,3,4*}; Matsui, T.⁵; Niessen, C.M.^{2,3,4#}; Luxenburg, C.^{1#}; Rübsam, M.^{2,3,4#}

¹ *Department of Cell and Developmental Biology, Faculty of Medical & Health Sciences, Tel-Aviv University, Tel-Aviv, Israel*

² *Department Cell Biology of the Skin*

³ *Cologne Excellence Cluster for Stress Responses in Ageing-associated diseases (CECAD)*

⁴ *Center for Molecular Medicine Cologne (CMMC), University Hospital Cologne, University of Cologne, Cologne, Germany*

⁵ *School of Bioscience and Biotechnology, Tokyo University of Technology, Tokyo, Japan,*

* *These authors contributed equally to this work*

shared corresponding authorship

Abstract: The epidermis is a barrier towards external challenges and dehydration. It's constant renewal and barrier function declines with age. To renew, basal stem cells delaminate and replace suprabasal differentiated cells by integration of mechanical cues of neighbors and extracellular matrix that stiffens with age. Mechanics dependent changes in cell shape and fate are linked, yet how the cytoskeleton integrates both is unclear. Using the multi-layered epidermis as a paradigm for cell shape-guided changes in differentiation we identify spectrin as an organizer of cortical actomyosin to integrate transitions in shape with spatial organization of signaling. E-cadherin organizes actin and spectrin into layer-specific sub-membranous networks with discrete structural properties to dissipate tension, and regulate the growth factor receptor EGFR and cation channel TRPV3 to induce differentiation. These findings reveal how organization of the cortical cytoskeleton directs transitions in cell shape and fate at tissue scale to establish epithelial barriers. We are now investigating how age dependent stiffening alters cortical networks and impaired renewal.

Accelerated Aging is a Determinant of Arterial Thrombosis Risk in Individuals with Low Genetic and Cardiovascular Risk Factor Burden

Ayesha Sanober; Alexander Gieswinkel; Yufan Yang; Harsini Raaja Sulochana; the GHS Research Consortium; Philipp Lurz; Philipp Wild; Vincent ten Cate

Background

The relative contribution of genetics and exposomal cardiovascular risk factors (CVRFs) to arterial thrombosis incidence varies across the lifespan. Individuals with low genetic and CVRF burden also experience arterial thrombosis.

Aims

To evaluate how genetic and exposomal risk contribute to arterial thrombosis risk over the lifespan, and to assess whether accelerated biological aging explains residual events in the subgroup of low genetic and exposomal risk.

Methods

Data from the longitudinal, population-based Gutenberg Health Study (GHS; 1,001 events) and the UK Biobank (UKB; 44,108 events) were used for attained-age time-varying Cox regression with restricted cubic splines. A polygenic risk score (PRS) for arterial thrombotic events was constructed from SNPs previously identified in genome-wide association studies, via LASSO-regularized Cox regression in UKB. In GHS, the CVRF burden was defined repeatedly over a 10-year follow-up. The low-PRS-low-CVRF subgroup was defined as individuals in the lower 50% of the PRS distribution with ≤ 1 CVRF. Two measures of accelerated aging (PhenoAgeAccel and C-reactive protein-based inflammaging) were calculated as baseline chronological age-adjusted residuals.

Results

In UKB, the PRS hazard ratio per SD (HR) declined from 1.85 at 53 years of age to 1.10 by age 80. In GHS, the HR was 1.22 [95% CI 1.07–1.39] at age 55 and reached 1.10 [95% CI 1.01–1.20] by age 75. Concurrently, the median [interquartile range] **CVRF burden** increased from 1 [0–2] at age 55 to 2 [1–2] at age 75. The CVRF burden HR declined from 2.36 [95% CI 2.16–2.58] at age 55 to 1.09 [95% CI 1.02–1.17] by age 75 (**$p < 0.001$**). The **low-PRS-low-CVRF** subgroup represented 27.3% of participants ($n = 3,312$) and accounted for 17.3% of all arterial events ($n = 173$). In this subgroup, PhenoAgeAccel (HR = 1.21; 95% CI 1.08–1.36; $p = 0.0012$) and inflammaging (HR = 1.18; 95% CI 1.01–1.37; $p = 0.033$) were robust predictors of residual risk. **By age 75, low-PRS-low-CVRF individuals ≥ 90 th percentile of PhenoAgeAccel had a predicted cumulative incidence of 21.7% (95% CI 16.8–26.3) versus 14.7% (95% CI 12.9–18.3) in the ≤ 10 th percentile.**

Conclusions

Genetics and the exposome have attenuating effect sizes in old age. Accelerated biological aging and inflammaging are predictors of arterial thrombosis in those at low genetic and exposomal risk. These results support incorporating biological age into risk assessment and prevention strategies.

Evolution of primary cells through replicative crisis to immortality

Schmidt, T.T.^{1,*}; Cosenza, M.R.^{2,*}; Popey, T.³; Przetocka, S.¹; Haggblom, C.¹; Allsing, N.⁴; Lande, K.⁴; Grimstead, J.⁵; Williams, A.⁴; Baird, D.⁵; Dixon, J.³; Korb, J.O.^{2,#}; Karlseder, J.^{1,#}

¹ *Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA*

² *Genome Biology, European Molecular Biology Laboratory, Heidelberg, Germany*

³ *Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA*

⁴ *Integrative Genomics and Bioinformatics Core, Salk Institute for Biological Studies, La Jolla, CA, USA*

⁵ *Division of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, UK*

^{*,#} *These authors contributed equally*

Shortening of telomeres, the repetitive, protective ends of eukaryotic chromosomes, restrict the proliferation capacity of human somatic cells by inducing replicative senescence and crisis. During malignant transformation, premalignant cells must overcome these barriers and acquire replicative immortality. Here, we investigate this fundamental step during tumorigenesis using a spontaneous crisis escape model. Single-cell DNA strand-sequencing of crisis cells revealed high genome instability characterized by complex structural variants (SVs). Unexpectedly, spontaneous emerging postcrisis clones showed relative few and simple SVs, suggesting that crisis evolved to stringently counterselect against cells with high genomic instability. However, with accumulating divisions post crisis escape, the immortal postcrisis clones' genomes evolve and amass genomic alterations, including tetraploidization events with subsequent losses and gains of individual chromosomes. Thus, our data indicate that the highly rearranged genomes found in some cancers are not originating from crisis but are acquired after crisis escape and immortalization.

Lower blood cell mitochondrial DNA abundance associates with higher all-cause mortality and aging: a 30-year prospective epidemiological study

Sebe, A.¹; Lautaoja-Kivipelto, J.¹; Jokelainen, J.²; Skarp, S.¹; Kerkelä, R.¹; Pirinen, E.^{1,3,4,5} & Ukkola, O.^{1,3}

¹ Research Unit of Biomedicine and Internal Medicine, Faculty of Medicine, University of Oulu, Oulu, Finland

² Northern Finland Birth Cohorts, Arctic Biobank, Infrastructure for Population Studies, Faculty of Medicine, University of Oulu, Oulu, Finland

³ Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland

⁴ Biocenter Oulu, University of Oulu, Oulu, Finland;

⁵ Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Recent epidemiological studies suggest blood cell mitochondrial DNA (mtDNA) abundance as a promising biomarker for aging and disease, though findings remain inconsistent and pathomechanisms unclear. We investigated blood mtDNA abundance in our OPERA cohort (n=1045), assessed in the 1990s and followed for over 30 years, with a second visit in the 2010s. We quantified mtDNA abundance in blood samples from both time points with RT-qPCR. RNA sequencing was performed in 452 follow-up samples. At both time points, lower mtDNA levels were associated with higher overall morbidity and mortality. MtDNA abundance showed a small, but significant decline over two decades. Transcriptomic analyses linked lower mtDNA levels to enhanced inflammatory pathways in immune cells. Our findings highlight blood mtDNA as a potential biomarker for aging and morbidity, with lower levels possibly linked to inflammatory processes. With three decades of follow-up, these results support its use in preventing morbidity and mortality of diverse etiology. Additionally, blood mtDNA emerges as a promising inflammatory biomarker, warranting further exploration in future studies.

HEAL-DO: A Healthy Aging and Longevity Domain Ontology for Semantic Knowledge Extraction in Aging Research

Greta Willing¹; Astghik Sargsyan¹; Astha Anand¹; Jasmeer Singh Kalra²; **Pegah Shakibnia**²; Rojin Sadat Yasini¹; Atefeh Habibi¹; Rudi Schmidt²; Marc Jacobs¹; Martin Hofmann-Apitius^{1,2}; Negin Sadat Babaiha^{1*}

¹ Department of Bioinformatics, Fraunhofer Institute for Algorithms and Scientific Computing (SCAI), Schloss Birlinghoven 53757 Sankt Augustin, Germany

² Bonn-Aachen International Center for Information Technology (b-it), University of Bonn, Bonn 53115, Germany

*Corresponding author

Aging research has generated extensive evidence on biological mechanisms, molecular biomarkers, aging clocks, interventions, exposures, disease trajectories, and functional outcomes. However, these findings remain distributed across publications and databases, described with heterogeneous terminology, and difficult to connect across biological scales. While established biomedical ontologies provide rich representations for biological processes, phenotypes, diseases, chemicals, investigations, and relations, there is still a need for an aging-focused semantic layer that consistently links mechanisms, biomarkers, interventions, exposures, outcomes, and longitudinal aging-related trajectories.

We present **HEAL-DO, a Healthy Aging and Longevity Domain Ontology** designed to organize aging-related knowledge and support automated biomedical information extraction and discovery. HEAL-DO is being developed as an OWL-based ontology in Protégé through an iterative workflow combining computational assistance with expert review. Candidate concepts and relations are generated with Large Language Model support, curated by domain experts, and aligned with established biomedical ontology resources. Current modules focus on biological aging processes, biomarkers, aging clocks, phenotypes, interventions, exposures, outcomes, and longitudinal health trajectories, while treating aging, healthy aging, and longevity as related but distinguishable conceptual layers.

As an early result, the current ontology prototype provides an OWL/RDF scaffold anchored in upper-level biomedical ontology principles, with initial alignment notes to established resources and a first set of aging-relevant classes covering concepts such as aging processes, aging biomarkers, longevity interventions, geroprotective agents, age-related disorders, healthspan trajectories, and temporal dimensions. This scaffold provides the basis for testing how semantic guidance can improve automated extraction of aging-related evidence from biomedical literature.

HEAL-DO is intended to support more reliable automated discovery by helping computational systems identify aging-relevant concepts, harmonize equivalent terms, and represent biologically meaningful relationships in context. In this work, we demonstrate the current HEAL-DO ontology structure, representative concept and relation types, alignment strategy with existing biomedical ontologies, and selected proof-of-concept showcases. These include ontology-guided extraction of aging-related evidence from biomedical literature, comparison of extraction results with and without semantic guidance, and organization of extracted findings into a healthy aging knowledge graph. Together, these showcases illustrate how HEAL-DO can provide a reusable semantic foundation for connecting evidence across mechanisms, biomarkers, aging clocks, interventions, exposures, and health outcomes, supporting more transparent and biologically grounded computational discovery in aging research.

Keywords: Healthy aging; longevity; aging biomarkers; domain ontology; ontology-guided extraction; biomedical knowledge graph; semantic representation; AI-assisted discovery.

Clinical Testing of Fat-Soluble Vitamins (A, D, E, K) and Glutathione: Methodological Diversity, Biomarker Standardization, and Clinical Realities in Longevity Medicine

Ravinder Singh

Mayo clinic, Rochester

Background and Analytical Realities

The paradigm of modern healthcare is undergoing a structural shift from the management of acute disease states to the proactive extension of human health span. Within longevity and anti-aging medicine, micronutrient optimization and cellular redox management have emerged as central pillars of preventative therapeutic strategies. Specifically, the fat-soluble vitamin network (vitamins A, D, E, and K) and the endogenous anti-oxidant glutathione function as a highly integrated biological defense system. They work synergistically to maintain genomic stability, regulate cellular differentiation, preserve skeletal integrity, and mitigate chronic, age-related systemic inflammation.

However, the integrity of clinical research and subsequent therapeutic interventions regarding aging depends entirely on the analytical validity of the laboratory methodologies used to measure these diverse compounds. Historically, the clinical laboratory landscape has relied heavily on automated, high-throughput immunoassays and basic colorimetric tests. While operationally efficient for large workloads, these automated platforms frequently suffer from significant matrix interferences, cross-reactivity with structurally similar metabolites, and a pronounced lack of standardization across manufacturing platforms.

For instance, automated immunoassays evaluating vitamin D have demonstrated a high degree of variability, clouding large-scale epidemiological results. Similarly, measuring highly labile antioxidants like glutathione or lipophilic molecules like vitamins A, E, and K requires sophisticated separation techniques to accurately distinguish between active, bioavailable forms and inactive or degraded metabolites.

To build a scientifically sound foundation for longevity medicine, modern clinical laboratories leverage an integrated suite of advanced analytical techniques. By balancing High-Performance Liquid Chromatography (HPLC) with ultraviolet or fluorescence detection, Capillary Electrophoresis (CE), and targeted Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), laboratories can achieve the ideal combination of throughput, cost-efficiency, and analytical specificity. This abstract outlines how rigorous, multi-platform quantification of vitamins A, D, E, and K, alongside precise measurements of the glutathione redox state, is vital for safely evaluating and modifying the biological mechanisms of human aging.

Methods and Technological Integration

The clinical assessment of fat-soluble vitamins and cellular antioxidants requires matching each biomarker to its optimal analytical methodology based on molecular abundance and chemical properties.

High-Performance Liquid Chromatography (HPLC) coupled with ultraviolet (UV) or fluorescence detection serves as the primary analytical workhorse for high-abundance lipophilic vitamins. For vitamin A (retinol) and vitamin E (α -tocopherol), HPLC-UV offers excellent chromatographic resolution and reproducibility. This approach efficiently separates active forms from dietary carotenoids and alternative tocopherols without the cost and operational complexity of mass spectrometry.

In contrast, vitamin K presents an extreme analytical challenge due to its exceptionally low circulating picogram-per-milliliter concentrations. To accurately differentiate vitamin K₁ (phylloquinone) from the long-chain forms of vitamin K-2 (menaquinones, such as MK-4 and MK-7), laboratories deploy highly sensitive, targeted LC-MS/MS setups optimized for trace-level extraction.

Evaluating the intracellular antioxidant defense system requires a different technological approach. Glutathione exists in a dynamic equilibrium between its reduced active form (GSH) and its oxidized dimeric form (GSSG). Because GSH is highly unstable and prone to rapid autoxidation after blood collection, strict pre-analytical stabilization using acid deproteinization and chemical alkylation is mandatory.

Once stabilized, the highly charged GSH and GSSG molecules are ideally suited for analysis via Capillary Electrophoresis (CE) or microfluidic chip-based capillary systems. CE utilizes high-voltage electrical fields to separate molecules within a fused-silica capillary based on their size-to-charge ratio. This technique provides rapid, high-resolution separation of glutathione variants using minimal sample volumes, making it an excellent tool for real-time cellular redox monitoring.

Validating this multi-method testing suite requires strict alignment with reference materials from the National

Institute of Standards and Technology (NIST) to guarantee long-term analytical stability across decades of patient follow-up.

Clinical Findings, Longitudinal Data, and Physiological Mechanisms

Applying these standardized methodologies over extended chronological timelines yields deep insights into population-level trends and age-related biological variations. Analyzing multi-year clinical datasets reveals distinct patterns in micronutrient retention, seasonal fluctuations, and demographic variations across aging cohorts. As individuals age, endogenous biosynthetic pathways decline sharply—including cutaneous vitamin D synthesis, intestinal absorption of lipophilic nutrients, and hepatic glutathione production—reinforcing the absolute necessity of accurate multi-marker monitoring.

Simultaneously, vitamins A and E work in tandem with glutathione to combat the chronic, age-related accumulation of reactive oxygen species (ROS) and lipid peroxidation. Vitamin E protects the polyunsaturated fatty acids in cellular and mitochondrial membranes from oxidative destruction. Once oxidized, vitamin E is regenerated back to its active state by a cascade driven by vitamin C and reduced glutathione (GSH).

Glutathione is the cell's primary endogenous defense mechanism. It acts as a substrate for glutathione peroxidase to neutralize harmful peroxides and functions as a direct conjugating agent in hepatic detoxification. As mitochondrial efficiency wanes with advanced age, the GSH/GSSG ratio drops precipitously. This loss of cellular reducing power triggers a state of chronic, low-grade systemic inflammation (often termed „inflammaging“) and accelerates endothelial dysfunction.

Excessive vitamin A intake can induce hepatic stellate cell activation and bone toxicity, while acute vitamin D toxicity causes severe hypercalcemia and soft tissue calcification. Extreme over-supplementation of vitamin E can disrupt the blood-clotting cascade by interfering with vitamin K metabolism, increasing hemorrhagic risk. This double-edged sword underscores the critical need for continuous, multi-technique laboratory monitoring.

Conclusion and Future Directions

Ultimately, true longevity medicine cannot safely exist without multi-method analytical precision. The relationship between clinical testing and healthy aging demonstrates how robust laboratory medicine directly informs preventative clinical practice. Moving forward, the field must advance beyond basic, static nutrient cutoffs toward comprehensive, dynamic, biomarker-driven optimization profiles.

By leveraging the unique advantages of HPLC-UV for high-abundance vitamins, capillary electrophoresis for highly labile redox markers like glutathione, and targeted mass spectrometry for trace-level nutrients like vitamin K, the clinical laboratory provides a comprehensive, cost-effective roadmap for healthy aging. This meticulous approach allows clinicians to safely maximize skeletal integrity, preserve endothelial function, and enhance overall metabolic health span while entirely avoiding the systemic dangers of toxic over-supplementation and cellular oxidative stress. Precision diagnostic oversight remains the ultimate bridge between anti-aging theory and safe, evidence-based clinical reality.

Spatiotemporal Mapping of Neurodegenerative Stress in the Locus Coeruleus in a Cell-type Specific *Erccl* Deficiency Model

Steppek C.¹; Chennavajula, K.²; Carey, J.¹ & Niedernhofer, L.J.¹

¹*Masonic Institute on the Biology of Aging and Metabolism and the Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, United States*

²*Institute of Translational Neuroscience, University of Minnesota, Minneapolis, Minnesota, United States*

The locus coeruleus (LC) is a noradrenergic brain nucleus that is particularly vulnerable in age-related neurodegenerative diseases such as Alzheimer's and Parkinson's, showing early neuronal tau and α -synuclein pathology leading to neuronal loss. In this study, we used mice in which the DNA repair gene *Erccl* was deleted specifically in tyrosine hydroxylase-expressing cells, leading to accelerated degeneration of LC noradrenergic neurons due to failure to repair endogenous DNA damage. To identify the mechanism behind the unique vulnerability of LC neurons to stress, the LC and surrounding microenvironments were profiled by GeoMx® Digital Spatial Profiler for whole-transcriptome analysis. Expression profiles were compared to controls at timepoints representing pre- and post- neurodegeneration. A tissue microarray was utilized to reduce batch effects and to accommodate scan-area and reagent constraints, providing a scalable workflow across genotypes and ages. Transcriptomic signatures were identified that may predispose noradrenergic neurons to protein aggregation and loss in LC circuits, which underlie neurodegenerative disease.

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Mechanosensitive Calcium Signalling Regulates Mitochondrial Quality Control in Ageing Tendon Cells

Tarasova, Karyna¹; Mahadik, Shrunkhala¹; Gültekin, Sinan¹; Gerner, Iris^{1,2} & Jenner, Florian^{1,2}

¹Department for Small Animals and Horses, Centre for Equine Health and Research, Equine Surgery Unit, VETERM, University of Veterinary Medicine Vienna, Austria

²Austrian Cluster for Tissue Regeneration, Austria

Tendinopathy is an age-related degenerative disease characterized by chronic inflammation and extracellular matrix disorganization¹. Tenocyte adaptation to mechanical load depends on PIEZO1-mediated mechanosensitive Ca²⁺ signalling², however how cellular senescence alters this pathway remains unclear³. We hypothesized that senescence uncouples PIEZO1-mediated Ca²⁺ influx from adaptive mitochondrial buffering, shifting mechanotransduction from anabolic to maladaptive signalling.

Replicative senescence was induced in primary equine tenocytes (n=6 donors) by serial passaging, comparing low-passage (LP) and high-passage (HP) cells. Cells were stimulated with the PIEZO1 agonist Yoda1 (10, 50 µM) under basal and inflammatory conditions for 24–72 h.

HP cells showed elevated basal cytosolic and mitochondrial Ca²⁺ levels, reduced mitochondrial polarization, increased senescence-associated β-galactosidase, elevated oxidative stress, upregulated p21, and decreased collagen type I. Yoda1 reduced senescence markers at 24 h in LP cells, whereas in HP cells reduction occurred only at 72 h exclusively under inflammatory conditions.

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Transcriptomic Characterization of Cellular Senescence in Human Donor Livers Before Transplantation

Adam M. Thorne^{1,2*}; Sabrina Stimmeder^{1,2,3*}; Kirill Ustyantsev⁴; Niels Mulder⁵; Laura Niedernhofer⁶; Marco De-maria⁴; Ton Lisman¹; Eugene Berezikov⁴; Folkert Kuipers^{4,5}; Vincent E. de Meijer^{1,2#}

¹ Department of Liver Transplantation and HPB Surgery, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands

² UMCG Comprehensive Transplant Center, Groningen, the Netherlands

³ Department of Surgery, Division of General, Visceral and Transplant Surgery, Medical University of Graz, Graz, Austria

⁴ European Research Institute for the Biology of Ageing (ERIBA), University of Groningen and University Medical Center Groningen, Groningen, the Netherlands

⁵ Department of Pediatrics, University of Groningen, University of Groningen and University Medical Center Groningen, Groningen, the Netherlands

⁶ Masonic Institute on the Biology of Aging and Metabolism, University of Minnesota, Minneapolis, MN, USA;

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA

Background and Aims: With an aging donor population, the response to ischemic and inflammatory injury caused during donor organ procurement and preservation could promote senescence and inflammation in organ recipients. While injury patterns between donor types are known, their role in driving cellular senescence and impact on downstream inflammatory responses remains unclear.

Methods: We performed smart-3SEQ bulk RNA-sequencing on tissue obtained from human donor livers after brain death (DBD) or circulatory death (DCD). Senescence signatures were assessed using the SenMayo gene panel and a liver-specific senescence panel. Immunohistochemistry was performed to assess senescence-associated protein expression.

Results: A total of 66 DBD (n=24) and DCD (n=42) livers were included. On a global transcriptional level, more than 1,700 differentially expressed genes separated DBD and DCD grafts. Functional enrichment analysis showed distinct biological programs, with DBD grafts enriched for metabolic pathways, epithelial proliferation, and angiogenesis regulation, whereas DCD grafts showed enrichment of immune activation, chemokine signaling, and injury-associated pathways. Senescence-associated analyses using both the SenMayo (125 genes) and the liver-specific senescence panels (61 genes) recapitulated this separation of DBD and DCD grafts. These differences were reflected by broader coordinated senescence-associated transcriptional programs rather than a unidirectional shift. DCD livers showed increased immunohistochemical expression of p16- and p21-positive stained nuclei than DBD livers (median 9.8 vs. 6.0%, p<0.001; and 7.3% vs. 4.7%, p<0.001; respectively).

Conclusion: Donor type reflects distinct transcriptional states in human donor livers, with senescence-associated profiles reflecting donor type-specific injury. Beyond its potential role as a marker of graft quality, injury-induced senescence-associated signaling may influence both graft function and recipient (patho)physiology, thereby positioning it as a promising modifiable gerotherapeutic target in liver transplantation.

Skin insulin resistance as an initiator of diabetes-associated barrier dysfunction and systemic insulin resistance

Lea Trojahn^{1,2}; Zirui Yu¹; Julia Löhr¹; Emmi Wachsmuth^{1,2}; Philipp Hammerschmidt³; Eva von Gablenz^{4,5}; Jens C. Brüning³; Ruth E.-M. Hanßen^{4,5}; Carien M. Niessen^{1,2}

¹Department Cell Biology of the skin and CECAD,

²Center of Molecular Medicine Cologne (CMMC),

³Max Planck Institute for Metabolic Research,

⁴Polyclinic for Endocrinology, Diabetology and Prevention Medicine,

⁵University Hospital Cologne

Obesity and type 2 diabetes mellitus (T2DM) are increasingly global health burdens. T2DM is associated with a wide range of comorbidities including the skin. However, whether these complications are driven by long-term hyperglycemia or inherent insulin resistance remains unknown. We have shown that short-term high fat diet disturbs epidermal stratification and barrier function, mimicking loss of skin insulin/IGF-I signaling and drives skin insulin resistance before systemic resistance occurs. Here we explored the hypothesis that skin insulin resistance accelerates the development of T2DM. Female epidermal IGF-IR-/- on short term high fat diet were more insulin resistance than control. Interestingly, brown adipose tissue showed a changed insulin response and, irrespective of diet, whitening. Our data reveal a novel communication axis between the skin and brown adipose tissue and identify the skin as an endocrine organ important for organismal metabolic homeostasis. These findings highlight the potential of skin as an early diagnostic marker and therapeutic target for T2DM.

Optimized glycogen metabolism supporting cardiac resilience in naked mole-rats

Vyshkvorkina, I.¹; Bundgaard, A.^{1,2}; Wang, N.¹; & Reznick, J.¹

¹University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Cologne, Germany

²Section for Zoophysiology, Department of Biology, Aarhus University, Aarhus, Denmark

Naked mole-rats (NMRs) are exceptionally long-lived rodents that maintain cardiac function with age. To protect their hearts from ischemia, NMRs rely on carbohydrate metabolism and store large, liver-like glycogen granules in their hearts. We investigated NMR genes contributing to glycogen handling by overexpressing them in heterologous systems. Among those candidates, Ppp1r3b, a liver glycogen-targeting subunit, showed increased cardiac expression in NMRs. Overexpressing Ppp1r3b raised glycogen content and promoted the formation of large glycogen granules in non-liver HEK293 cells, resembling those found in the NMR heart. We discovered that during ischemic stress, oligosaccharides accumulate in the NMR heart, and we investigated whether amylase drives their release from glycogen. We detected amylase mRNA and protein in the NMR heart and in the livers of NMRs and mice, pointing to an underappreciated liver-type route for amylase-mediated glycogen breakdown that is co-opted into the NMR heart. Overexpressing NMR amylase in Hepal-6 cells increased oligosaccharides, supporting this mechanism, and also revealed unique features of NMR amylase.

Engineering an Inducible HEK293 Suspension Cell Platform for Controlled AAV Vector Production

Torabi, M.^{1,2}; **Wojke, M.**^{1,2}; Al Kabbani, Mhd. A.^{1,2}; Naylor, A.S.^{1,2}; Zempel, H.^{1,2,3}

¹*Institute of Human Genetics, University Hospital Cologne, University of Cologne, Kerpener Str. 34, 50931 Cologne, Germany*

²*Center for Molecular Medicine Cologne (CMMC), University of Cologne, Robert-Koch-Str. 21, 50931 Cologne, Germany*

³*Center for Human Genetics and Genomic Medicine, RWTH University Hospital Aachen, Germany*

Adeno-associated viral (AAV) vectors are the current backbone of gene replacement for neurogenetic disease and promising for age-associated disease. However, AAV production still relies on transient multi-plasmid transfection approaches, complicating standardization and batch-to-batch variability. Currently we are establishing a viral core facility for (pre)clinical translation.

We aim to establish an inducible stable HEK293 suspension producer cell platform for controlled AAV vector manufacturing. Our strategy is based on transposon-mediated genomic integration of AAV production cassettes, enabling stable maintenance of the genetic elements required for vector production. In combination with chemogenetic or optogenetic inducible ON/OFF switches, the system will allow on-demand activation of AAV production while cells are expanded under non-induced conditions. By establishing a versatile platform for controlled AAV manufacturing using alternative strategies, we can approach to simplify the manufacturing workflow and provide greater control over vector production. This engineered cell line is expected to facilitate scalable bioprocessing and support the development of more reproducible production processes promising for future gene therapy applications to be realized in an academic setting.

Mitochondrial control of TAU-based neurodegeneration and gene-therapy approaches

Xinyu Xia^{1,2}; Andrew Naylor^{1,2}; Qiang Guo^{1,2,3}; Leila Al Abed^{1,2}; Hans Zempel^{1,2,4}

¹ Institute of Human Genetics, University Hospital Cologne

² Center for Molecular Medicine Cologne, University of Cologne

³ Department of Clinical Laboratory, Anhui Provincial Children's Hospital, Hefei, Anhui, China

⁴ Center for Human Genetics and Genomic Medicine, RWTH University Hospital, Aachen

Correspondance to: xinyu.xia@uk-koeln.de; hans.zempel@uk-koeln

Introduction

Aging is the major risk factor for Alzheimer's disease (AD) and related tauopathies. Increasing evidence suggests that age-associated mitochondrial dysfunction contributes to the onset and progression of neurodegeneration. TAU is a microtubule-associated protein predominantly localized to axons, which becomes aberrantly mislocalized and subsequently aggregates in tauopathies, including sporadic AD and various forms of inherited dementia. As central regulators of neuronal energy metabolism and calcium homeostasis, mitochondria are essential for neuronal function, and their dysfunction has been closely linked to synaptic impairment and neurodegeneration. Recently, we found that global and axon-initial-segment (a neuronal compartment crucial for axonal targeting of TAU) specific induction of mitochondrial dysfunction is sufficient to cause AD/tauopathy typical TAU missorting. Thus, we hypothesize that mitochondria dysfunction may be upstream of TAU pathology, and may thus be the most important therapeutic target for AD/tauopathy.

Aim

This ongoing study aims to determine whether mitochondrial dysfunction is sufficient to induce TAU pathology and to investigate the effects of mitochondria-targeted therapeutic strategies on TAU and TAU-related pathological alterations.

Methods and Results

Human induced pluripotent stem cell (iPSC)-derived neurons are currently being used as the primary disease-relevant experimental system. Future studies will extend these findings to K320E-Twinkle mouse models and murine POLG shRNA knockdown models to investigate the in vivo consequences of mitochondrial dysfunction on TAU pathology. Mitochondrial dysfunction was induced using pharmacological inhibitors (IMTIB, Actinonin, Antimycin A, Rotenone, ddc) as well as genetic perturbation of mitochondrial DNA maintenance pathways, including POLG knockdown using shRNA.

POLG knockdown efficiency measured by qPCR resulted in a reduction of mitochondrial DNA copy number. In parallel, pharmacological inhibition of mitochondrial function (IMTIB, ddc) similarly reduced mtDNA content, indicating convergent impairment of mitochondrial genome maintenance.

Importantly, TAU missorting was consistently observed following individual pharmacological treatments (Actinonin, Antimycin A, Rotenone, and ddC) as well as genetic POLG knockdown, whereas IMTIB-induced mtDNA depletion alone did not result in detectable TAU alterations.

In parallel, preparatory work in the laboratory has established viral gene delivery platforms, including lenti-viral POLG shRNA systems and adeno-associated virus (AAV) production pipelines, enabling bidirectional modulation of mitochondrial function in vitro and in vivo. In addition, the laboratory has generated a panel of expression constructs for mitochondrial function enhancement, including POLG, TFAM, TWINKLE, PGC-1 α , POLG2 (Pol- β), and SSBP1. Ongoing work aims to further characterize neuronal and mitochondrial structural alterations and to perform transcriptomic profiling of mitochondrial dysfunction-induced TAU pathology.

Conclusion

These findings suggest that mitochondrial dysfunction may contribute to the early stages of TAU pathology and is closely associated with TAU missorting and early tauopathy-related phenotypes. This study may provide a theoretical foundation for the development of therapeutic strategies targeting mitochondrial dysfunction in aging-associated neurodegenerative and mitochondrial diseases and associated neuronal dysfunction.

A ttntv cardiomyopathy model in African Turquoise Killifish enables identification of aging hallmark-associated gerotherapeutic strategies

Feixiang Yan^{1,2}; Yonghe Ding^{1,2}; Paul Yoon^{1,2}; Xiaolei Xu^{1,2}

¹Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, USA

²Department of Cardiovascular Medicine, Mayo Clinic, Rochester, USA

Background: Truncating variants in TTN (TTNtv) are the most common genetic cause of dilated cardiomyopathy (DCM). Given the age-dependent penetrance of TTNtv-associated DCM, an aging-compatible vertebrate model would open doors to systematic studies of contribution of aging to ttntv DCM. African Turquoise Killifish (ATK) is a vertebrate model with an extreme short lifespan. However, no cardiomyopathy model has been generated in this animal.

Methods: We annotated ttn homologues in ATK and then generated a ATK ttntv mutant using CRISP technology. Progression of DCM-like phenotypes were defined at 8 weeks, 16 weeks, and 24 weeks of age, corresponding to 30, 60 and 90 years of age in humans. In the meantime, we analyzed hallmarks of aging, including de-regulated nutrient sensing and cellular senescence, and then assessed hallmark-based gerotherapeutics such as metformin, rapamycin, and Erki for deregulated nutrient sensing, and dasatinib plus quercetin (DQ) for cellular senescence. Finally, transcriptome changes of ttntv along the aging process were defined by single-cell RNA sequencing.

Results: Homozygous ttn mutants were embryonic lethal, whereas heterozygous mutants developed progressive DCM-like phenotypes. While the lifespan is not affected by ttntv, healthspan is affected – ejection fraction was significantly reduced by 8 weeks of age, followed by progressive cardiac wall thinning, reduced swimming capacity by 24 weeks, and myofibrillar disarray by 30 weeks. In contrast to cellular senescence that is activated in ttntv ATK at 16 weeks, deregulated nutrient response, another aging hallmark that normally occurs in wild-type killifish at 16 weeks, was observed in ttntv model as early as 8 weeks. We tested hallmark-associated gerotherapeutic compounds and found that the deregulated nutrient response in WT fish at 16 weeks could be rescued by metformin and DQ, but not rapamycin nor Erki. However, the nutrient-response abnormalities in young ttn mutants appeared distinct from those in normally aged wild-type fish, suggesting mechanistic divergence.

Conclusion: Some aging hallmarks such as deregulated nutrient response is activated earlier than other hallmarks such as cellular senescence in an ATK ttntv DCM model. It is anticipated that age-dependent gerotherapeutic strategies will be established for ttntv-associated DCM.

The Effects of Cycloastragenol and Its Derivatives on Age-Related Pathways

Yilmaz, S.^{1,2,3}; Mingardo, E.^{1,2}; Müller, J.^{1,2}; Ballar-Kirmizibayrak, P.⁴; Bedir, E.⁵

¹Faculty of Life Science, Biochemistry of Nutrition, Kulmbach, Germany

²Faculty of Mathematics, Physics and Computer Science, Applied Computer Science, Bayreuth, Germany

³Department of Biotechnology, Graduate School of Natural and Applied Sciences, Ege University, Bornova, Izmir, Turkey

⁴Department of Biochemistry, Faculty of Pharmacy, Ege University, 35100 Bornova, Izmir, Turkey, email: petek.ballar@ege.edu.tr (Correspond author)

⁵Department of Bioengineering, Izmir Institute of Technology, 35430 Urla, Izmir, Turkey, email: erdalbedir@iyte.edu.tr (Correspond author)

Aging is characterized by progressive decline in cellular functionality and is associated with hallmarks including telomere erosion, loss of proteostasis, oxidative stress, and cellular senescence (López-Otín et al., 2013, Salmon et al., 2010). These features contribute to age-related disorders, including neurodegenerative and metabolic diseases. Cycloastragenol (CA), a triterpenoid saponin derived from *Astragalus* species, has been investigated as a telomerase-activating compound with relevance to age-associated dysfunction (Harley et al., 2011).

In this study, we evaluated novel CA derivatives generated through fungal biotransformation on aging-related pathways, including NRF2/proteasome/telomerase regulation and replicative senescence in human primary epidermal keratinocytes (HEKn). CA derivatives enhanced NRF2 transcriptional activity, increasing cytoprotective enzyme expression, proteasome activity, and proteasome-associated protein levels.

Continuous treatment with CA and its derivatives delayed replicative senescence, accompanied by modulation of senescence-associated markers and increased NRF2, proteasome, and hTERT levels, even in senescent cells. We further evaluated the lead compound CA in induced pluripotent stem cells (iPSCs) during prolonged culture. CA treatment was associated with preservation of OCT4 expression and modulation of cell-cycle dynamics.

Overall, our findings suggest that CA and its derivatives modulate aging-related pathways through the NRF2/proteasome/telomerase axis and support their role as modulators of cellular senescence.

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Epicardial Remodeling in Cardiomyopathies and Cardiac Aging: A New Gerotherapeutic Strategy

Baul Yoon^{1,2}; Yonghe Ding³; Maryam Moossavi^{1,2}; Song Zhang^{2,4}; Yifeng Xu⁵; Ping Zhu^{1,2}; Joerg Herrmann²; Yuji Zhang^{5,6}; Xiaolei Xu^{1,2}

¹Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN, United States

²Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, United States

³The Affiliated Hospital of Qingdao University & Biomedical Sciences Institute, Qingdao Medical College of Qingdao University, Qingdao, Shandong 266021, China

⁴Center for Regenerative Medicine, Mayo Clinic, Rochester, MN, United States

⁵Division of Biostatistics and Bioinformatics, Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD, United States

⁶University of Maryland Marlene and Stewart Greenbaum Comprehensive Cancer Center, Baltimore, MD, United States

Epicardium is the outmost layer of cells that enclose a heart. In contrast to widely recognized functions of the epicardium in cardiac development and heart regeneration, functions of these cells during cardiomyopathies and cardiac aging have been poorly characterized. Here, through mechanistic studies of fatty acid binding protein 7a (fabp7a) as a salutary modifier of cardiomyopathies, we uncovered a previously unrecognized function of epicardium in cardiomyopathies and normative cardiac aging. We identified epicardial remodeling, which is characterized by ultrastructural changes in the epicardium and Fabp7 and Cav1 induction in the epicardium, as a pathological event in both anthracycline-induced cardiotoxicity and bag3-associated cardiomyopathy, as well as a geriatric event during normative cardiac aging. Genetic or pharmacological inhibition of Fabp7 significantly rescued epicardial remodeling, restored cardiac function, and reduced cellular senescence across cardiomyopathies and aging models, whereas epicardial-specific fabp7a overexpression is sufficient to induce epicardial remodeling and activate cellular senescence. Single-cell RNA sequencing of the aging African Turquoise Killifish heart dataset suggested additional features of epicardial remodeling, including reduced epicardial signaling hub activity, increased immune response and extracellular matrix remodeling activity, and altered epicardial cell states shifting towards epicardium-derived cells. Altogether, our data suggest epicardial remodeling as a common molecular event in cardiomyopathies and normative cardiac aging, which can be harnessed for cardioprotective or gerotherapeutic benefits.

The Therapeutic Effect of Selinexor Partially Rescues Colon Physiology in a Murine Model of Progeria Syndrome

Zavala–Torres Norma Gabriela^{1,2}; De Ita Marlon²; Nava Porfirio³; García–Mena Jaime²; Rodríguez–Dorantes Mauricio⁴; Silva–Tinoco Rubén⁵; Arrieta–Cruz Isabel⁶; Baay–Guzman Guillermina⁷ and Bulmaro Cisneros^{2*}

¹ *Cologne Excellence Cluster for Cellular Stress Responses in Aging–Associated Diseases (CECAD), University of Cologne, Joseph Stelzmann Strasse 26, 50931 Cologne, Germany.*

² *Department of Genetics and Molecular Biology, Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV–IPN), Mexico City, Mexico.*

³ *Department of Physiology, Biophysics, and Neurosciences, Center for Research and Advanced Studies of the National Polytechnic Institute, Mexico City, Mexico.*

⁴ *Oncogenomics Laboratory, National Institute of Genomic Medicine, Ministry of Health, Mexico City, Mexico.*

⁵ *Public Health Services of Mexican Institute of Social Security, Mexico City, Mexico.*

⁶ *Department of Basic Research, Division of Research, National Institute of Geriatrics, Ministry of Health, Mexico City, Mexico.*

⁷ *Unit of Investigative Research on Oncological Diseases, Children’s Hospital of Mexico “Federico Gomez”, Mexico City, Mexico*

**Correspondence: Bulmaro Cisneros*

Hutchinson–Gilford progeria syndrome (HGPS) is a premature aging disorder caused by a point mutation in the LMNA gene, resulting in the production of the aberrant protein progerin. Progerin disrupts nuclear architecture and essential cellular functions, leading to genomic instability, stem cell exhaustion, impaired intercellular communication, and defective proteostasis. Previously, we demonstrated that HGPS fibroblasts exhibited altered nuclear protein export due to elevated exportin CRM1. Remarkably, pharmacological inhibition of CRM1 with selinexor alleviates senescent hallmarks in HGPS fibroblasts. The LmnaG609G/G609G knock-in mouse model accurately mimics progerin expression and the primary clinical features of HGPS, including reduced growth, cardiovascular problems, a shortened lifespan, and low weight gain. Recent findings show that these mice develop intestinal dysbiosis and gastric atrophy.

This study aim to determine whether Selinexor has a beneficial effect on colon homeostasis and dysbiosis. LmnaG609G mice, maintained at the UPEAL–CINVESTAV–IPN facility (ID #031420), were treated with selinexor (2.5 mg/kg) or vehicle twice weekly by intragastric gavage from weeks 6 to 14 after weaning. Proliferation was tracked via EdU. Histological analysis included H&E staining and AB/PAS staining. Immunohistochemistry and immunofluorescence was performed using antibodies against PCNA, Muc2 and progerin. Western blot of isolated crypts assessed PTEN, AKT1, pAKT473, S6, pS6, progerin, and PI3K. Our findings reveal significant histopathological changes of the colonic mucosa of LmnaG609G mice, including shortened colon length, reduced crypt size, diminished intestinal cell proliferation, increased epithelial cell death, and impaired mucus secretion. Mechanistically, progerin increases PTEN stability, partially inhibiting the PI3K/Akt/mTOR pathway. Significantly, pharmacological treatment with selinexor activates the PI3K/Akt/mTOR signaling pathway, while also decreasing PTEN levels and reduced progerin levels in the colon. These effects reverse colon shortening, enhance cell proliferation, reduce epithelial cell death, and restore mucus secretion.

In summary, progerin impairs intestinal epithelial homeostasis via PI3K/Akt/mTOR inhibition, while selinexor reverses these pathologies, including reducing colonic progerin.

Keywords: Progeria, Selinexor, Intestinal Aging, PTEN, PI3K/Akt/mTOR

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