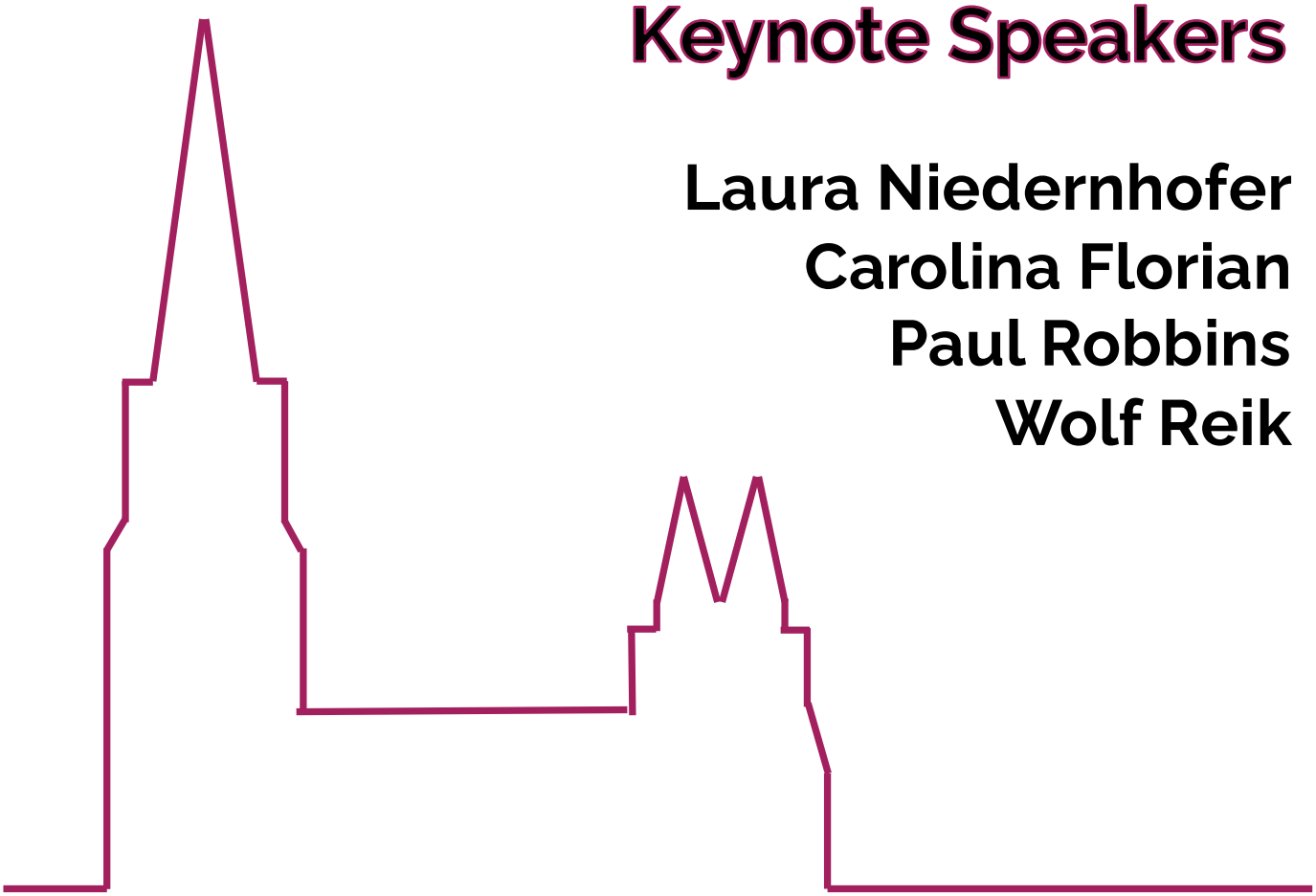
DGfA | DEUTSCHE GESELLSCHAFT FÜR ALTERNSFORSCHUNG / GERMAN ASSOCIATION FOR AGING RESEARCH

Annual Meeting of the German Association for Aging Research

26 - 27 June 2025
Ulm, Germany

Keynote Speakers

Laura Niedernhofer
Carolina Florian
Paul Robbins
Wolf Reik



UNIVERSITÄTS KLINIKUM ulm

arc aging research center uulm

AGING AT INTERFACES

universität ulm

Thursday, June 26

12.00 - 01.00 pm **Arrival + Registration + Snacks**

01.00 - 01.05 pm **Opening Remarks**

01.05 - 01.50 pm **Keynote Lecture 1**

Carolina Florian

The Bellvitge Institute for Biomedical Research (IDIBELL), Barcelona, Spain

Reducing nuclear stretching rejuvenates aged hematopoietic stem cells

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01.55 - 03.30 pm **Session 1**

Chair: Michael Milsom + Hartmut Geiger

01.55 - 02.10 pm

Albert Kallon Koroma, Department of Dermatology and Allergic Diseases, Ulm University Medical Center

Unrestrained CdC42 Activation Impairs the Cytotoxic Function of NK Cells From Old Adults Promoting the Accumulation of Senescent Fibroblasts in Aging Skin – A Successful Rescue by the CdC42 Inhibitor CASIN

02.10 - 02.25 pm

Mona Vogel, Institute of Molecular Medicine, Ulm University

The extent of myeloid skewing might serve as a novel biological marker for the extent of aging in both mice and humans

02.25 - 02.40 pm

Yiwen Zhang, German Cancer Research Center (DKFZ), Heidelberg

Extreme heterogeneity in hematologic aging is predominantly driven by stochastic variation, not by environmental nor genetic variation

02.40 - 02.55 pm

Dominik, Schlotter, Department of Obstetrics and Gynecology, Ulm University

Replication stress responses in human lymphocytes change sex-specifically during aging

02.55 - 03.10 pm

Irfana Jan, Institute of Immunology, Ulm University Medical Center

Role of B cells in chronic inflammation and their implication in Inflammaging

03.10 - 03.25 pm

Karin Prummel, European Molecular Biology Laboratory (EMBL), Heidelberg

Disrupted Harmony: Inflammatory Remodeling Of The Bone Marrow Niche In Clonal Hematopoiesis And Myelodysplasia

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03.30 - 04.00 pm **Coffee Break**

04.00 - 04.45 pm **Keynote Lecture 2**

Paul Robbins

Biochemistry, Molecular Biology & Biophysics University of Minnesota, USA

Development of Novel Senotherapeutics for Extending Healthspan

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04.00 - 06.20 pm **Session 2**

Chair: Karin Scharffetter-Kochanek + Maja Funk

04.50 - 5.05 pm

Maja Funk, Institute of Lung Health and Immunity, Helmholtz Munich

The role of aging and environmental stress along the gut-lung axis in chronic inflammation

05.05 - 05.20 pm

Ahmed Sayed, Institute of Molecular Endocrinology and Physiology, Ulm University

Glucocorticoid receptor in adipocytes mediates metabolic pathologies during aging

05.20 - 05.35 pm

Thea Stephan, University Medizin Halle

SASP-Panel as a biomarker for ageing: GDF-15 identified as key driver of age-related disease

05.35 - 05.50 pm

Larissa Smulders, Max Planck Institute for Biology of Ageing, Köln

Characterization of two transgenic mouse lines harbouring rare protein-altering variants in a gene involved in insulin/insulin-like growth factor-1 signalling identified in long-lived individuals

05.50 - 06.05 pm

Prerana Chaudhari, Leibniz Institute on Aging- Fritz Lipmann Institute, Jena

Barrier to Aging: Enhancing Intestinal Barrier Function to Prevent Biological Decline

06.05 - 06.20 pm

Yidong Wang, Research Group on Stem Cell and Metabolism Aging, Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena

Foxa transcription and aging-associated limits to dietary restriction responses

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06.20 - 07.30 pm	**POSTERSESSION + wine + beer + ice cream**

07.30 - 09.30 pm	**FOODTRUCKS + drinks**

Friday, June 27

08.30 - 09.00 am **Arrivals + Coffee + Snacks**

09.00 - 09.45 am **Keynote Lecture 3**

Wolf Reik

Altos Labs - Cambridge Institute of Science

Single cell multiomics landscape of development and ageing

09.55 - 11.00 am **Session 3**

Chair: Wolfgang Wagner + Andreas Simm

09.55 - 10.10 am

Juan-Felipe Perez-Correa, Helmholtz Institute, Institute of Stem Cell Biology, Aachen

Beyond Linear: A Robust Non-Parametric Approach to Epigenetic Age Estimation

10.10 - 10.25 am

Felix Böhm, Institute for Geriatric Research, Ulm University Medical Center

Biological Age Estimation based on blood biomarkers – the ActiFE study

10.25 - 10.40 am

Denise Posadas Pena, Institute of Biochemistry and Molecular Biology, Ulm University

Can zebrafish regeneration reveal anti-aging strategies?

10.40 - 10.55 am

Jana Riegger-Koch, Klinik für Orthopädie, Ulm University Medical Center

Growth differentiation factor 15 (GDF-15) as a new mediator between stress-induced premature senescence and regenerative processes in post-traumatic osteoarthritis

11.00 - 11.30 am **Coffee Break**

11.30 - 12.20 pm **Keynote Lecture 4**

Laura Niedernhofer

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota Medical School, USA

Endogenous DNA damage as a primary driver of aging

11.30 - 01.40 pm **Session 4**

Chair: Kristina Endres + Dario Valenzano

12.25 - 12.40 pm

Julia Popow, Center for Molecular Medicine Cologne, University Hospital Cologne

Suppression of G-quadruplex DNA and interconnected genome instability to counteract aging in a cellular model of the Hutchinson-Gilford progeria syndrome

12.40 - 12.55 pm

Daniel Sauter, Institute for Medical Virology and Epidemiology of Viral Diseases, University Hospital Tübingen

HIV-1 activates an endogenous retrovirus regulating p53 signaling and senescence

12.55 - 01.10 pm

Qingwen Yang, Department of Internal Medicine I, Ulm University Medical Center

Notch/NF-kB crosstalk signaling critically affects SASP gene expression in brain aging

01.10 - 01.25 pm

Thi Tinh Nguyen, Faculty of Computer Sciences and Microsystems Technology, Kaiserslautern University of Applied Sciences, Campus Zweibrücken

Enteric neuron transcriptomics in accelerated and diseased aging

01.25 - 01.40 pm

Dennis M. de Bakker, Leibniz Institute on Aging - Fritz Lipmann Institute (FLI), Jena

Mapping The Genetic Architecture Of Brain Aging In Killifish

01.45 - 2.00 pm **DGfA PRIZES FOR BEST POSTER + BEST TALK**

2.00 - 2.45 pm **DGfA Member Assembly**

From 01.30 pm **Packed Lunches**

Internet:

Access to the WLAN “welcome” is free.

TAXI:

Call: +49 (0)731 66066

Public Transportation:

DING: Donau-Iller-Nahverkehrsverbund-GmbH

https://www.ding.eu/

unser-DING-App: Tickets and Timetables

Meeting Venue:

Multimedia Room 2.069 in Building N27

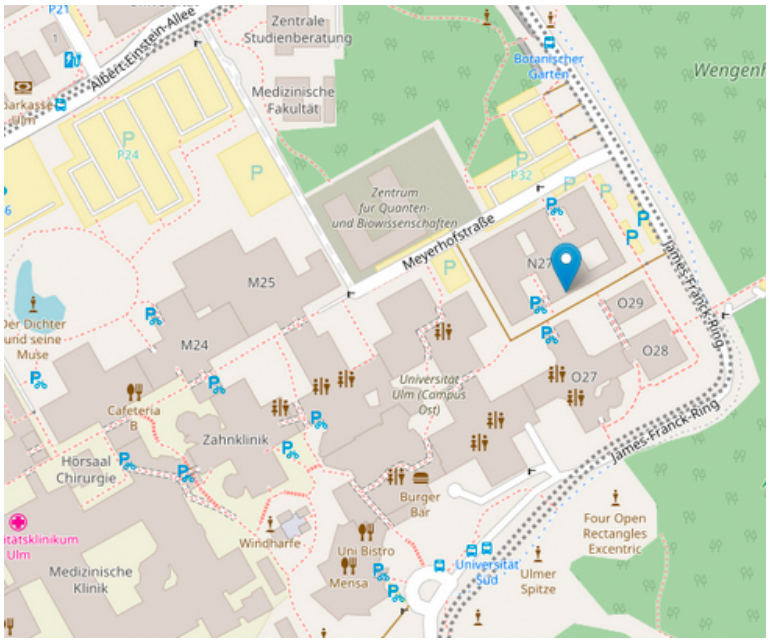
Meyerhofstraße 1

Ulm University

Tram stop: Botanical Garden

From the city center take Tram Line 2

Direction SCIENCE PARK II



Myelin load dictates NG2-glia vulnerability to aging

A. Skaf, L. Dimou
ulm university, molecular and translational neuroscience, ulm, baden-württemberg, germany

Aging is a process characterized by a progressive functional decline in all organs of an organism. In the central nervous system (CNS), aging leads to biological malfunctions causing cognitive and motor decline. The link between aging and neurodegenerative diseases is robust, with aging and its traits considered major risk factors for developing such disorders. The functional decline seen in aging is thought to be partially caused by alterations in the oligodendrocyte lineage cells and myelination. This lineage includes Oligodendrocytes (OLs) and NG2-glia, a type of glial cells also known as oligodendrocyte progenitor cells. Oligodendrogenesis and myelination persists throughout life. This phenomenon is crucial for proper brain function allowing the fine-tuning of the existing myelin network and the maintenance of myelin integrity. It is known that the rate of NG2-glia proliferation and differentiation declines with age leading to a lower baseline generation of OLs. Yet, the vulnerability of the CNS to aging is not uniform but varies in a spatial and temporal manner. Here, we show that the age-dependent changes in NG2-glia proliferation and differentiation are spatially restricted to areas of high myelin content. Moreover, we show that the aging corpus callosum, a major white matter tract, undergoes a strong inflammatory shift that coincides with the loss of OLs. Notably, we find that allowing aged mice to engage in physical activity mitigates some of the age-related alterations. Our results strongly suggest a link between myelin content, inflammation and the age-related changes in NG2-glia highlighting a potential entry point for attenuating CNS aging.

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Sodium Butyrate Accelerates Diabetic Wound Healing through Epigenetic Modifications of Dysfunctional Macrophages

Karmveer Singh[1,2], Albert Kallon Koroma[1,2], Yongfang Wang[1], Jinnan Cheng[1], Rajeev Kumar Pandey[1], Mahyar Agarpour[1], Philipp Haas[1], Linda Krug[1,2], Adelheid Hainzl[1], Meinhard Wlaschek[1,2], Pallab Maity[1,2,3] and Karin Scharffetter-Kochanek[1,2,3]

[1]Department of Dermatology and Allergic Diseases, Ulm University, Ulm, Germany
[2]Aging Research Center (ARC), Ulm, Germany
[3]Corresponding author (*Contributed equally*)

Diabetes mellitus, an aging-related disease which is due to the senescence of pancreatic β-cells with reduced insulin release and disrupted lipid metabolism, is profoundly increasing. Diabetic non-healing ulcers remain a major unmet medical need and pose a difficult challenge to healthcare systems worldwide. The persistent accumulation of dysfunctional pro-inflammatory macrophages has previously been identified as a fundamental cause of chronic wounds, however, the role of saturated fatty acids on their dysfunctional state remains elusive. Here we report that histone H3K27 acetylation, an epigenetic mark that regulates macrophage gene transcription, is significantly suppressed under metabolic conditions closely resembling that of diabetes. We identify palmitate, a saturated fatty acid that occurs at high concentrations in diabetic patients and *db/db* mice, to be the responsible culprit. High palmitate concentrations are correlated with the occurrence of a new pro-inflammatory macrophage subpopulation in diabetic skin as shown in single cell RNA seq analysis. In a series of *in vitro* and *in vivo* experiments, we found that palmitate supplementation – via activation of HDAC-dependent histone deacetylation pathways - significantly suppressed histone acetylation and results in a the occurrence of a new . Furthermore, a shift in transcriptional control from STAT1 to JUN was observed in LPS/IFNγ stimulated macrophages exposed to palmitate. The histone deacetylation inhibitor sodium butyrate profoundly promoted cutaneous wound healing in diabetic mice. Butyrate induced restoration of the histone H3K27 acetylation-dependent transcriptional signature and corresponding pathways in wound-associated macrophages and in consequence, reestablished pro-regenerative STAT1 signaling. Furthermore, we found that butyrate-mediated inhibition of HDAC preserves the morphological features, rewired the pro-inflammatory subpopulation and, more importantly, improves the phagocytic activity and migration of macrophages even under the palmitate-imprinted inflammatory conditions mimicking that in diabetes. These findings are clinically relevant as butyrate concentrations are severely reduced in diabetic patients and mice. Since butyrate is a metabolic product of gut bacteria, its decrease also indicates a dysfunctional gut microbiome in diabetic mice and humans. Our study highlights a novel pathological mechanism controlling dysfunctional macrophages, which could be exploited therapeutically to improve or even diabetic wounds dominated by macrophage dysregulation in diabetes and possibly other chronic wound healing disorders.

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A Novel Human 3D Skin Model for Aging Research

Sebastian Huth[1,], Yvonne Marquardt[1], Laura Huth[1], Karmveer Singh[2,3], Karin Scharffetter-Kochanek[2,3,], Jens Malte Baron[1], Pallab Maity[2,3]

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Abstract

Current models used to study skin aging, including in vivo murine models, ex vivo human skin, and in vitro 2D cell cultures, present significant limitations in replicating the complexity of chronological human skin aging. To address this gap, we developed a novel 3D human full-thickness skin aging model using primary dermal fibroblasts and epidermal keratinocytes harvested from the same aged donors (average age 80 years). Comprehensive histological, immunostaining, and transcriptomic analyses of this aging model, compared to a young 3D skin model (average age 20 years), revealed distinct hallmarks of chronological skin aging, including reduced epidermal and dermal thickness, decreased extracellular matrix content, diminished cell proliferation, and increased cellular senescence. Furthermore, 3D aging skin model also showed reduced IGF-1 expression and induction of AP1/JunB, which were consistent with observations in aged human skin. Transcriptomic profiling further identified upregulated pathways associated with extracellular matrix degradation, cellular senescence, and immune responses, aligning closely with published data from human aged skin. This novel in vitro model faithfully recapitulates several key features of chronological skin aging, offering a robust platform for studying aging mechanisms and testing anti-aging therapeutic interventions.

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Unrestrained CdC42 Activation Impairs the Cytotoxic Function of NK Cells From Old Adults Promoting the Accumulation of Senescent Fibroblasts in Aging Skin – A Successful Rescue by the CdC42 Inhibitor CASIN

Albert Kallon Koroma[1,3], Karmveer Singh Shekhawat[1,3], Yongfang Wang[1], Mahyar Aghapour-Ask[1], Meinhard Wlaschek[1,3], Hartmut Geiger[2,3], Pallab Maity[1,3]*, Karin Scharffetter-Kochanek[1,3]*

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*Contributed equally

Cellular senescence is known as a state of permanent cell cycle arrest, and a prime hallmark of tissue aging. Senescent cells – by the release of bioactive molecules such as inflammatory chemokines, cytokines, extracellular vesicles and matrix degrading metalloproteases, collectively referred to as **S**enescence **A**ssociated **S**ecretory **P**henotype (SASP) – even enforce age-related disorders such as non-healing states of wounds, osteoporosis, neurodegenerative disease among other aging-related conditions. Fibroblasts, which physiologically and anatomically constitute the principal component of the connective tissue, play an important role in organ homeostasis, however – senescent cells – drive organ and skin aging. We previously showed that senescent fibroblasts gradually accumulate in human skin with age. Under transient conditions of senescence during embryogenesis and acute wound healing, senescent fibroblasts are successfully removed by Natural Killer cells (NK cells) which belong to the innate immune system. We here wished to understand whether (1) NK cells fail to remove senescent fibroblasts and - if so, (2) what are the underlying mechanisms and and (3) whether there is any therapeutic approach to rescue the impaired cytotoxic function of old NK cells. Using a NK cell mediated killing assay with magnetic negative selection of NK cells, we were able to show that primary NK cells from old human donors (~70 years) and old mice (~700 days) were less efficient in killing senescent fibroblasts as opposed to those from young human donors (~23 years) and young mice (~100 days), respectively. While there was change d in the number of resident NK cells in the skin between young and old human adults and mice, employing western blot and cytometry analysis we observed a significant reduction in the content of NK cell cytolytic granules – which comprises the pore forming protein perforin and the apoptosis inducing serine protease granzyme B, in NK cells isolated from old individuals. The impaired content of these cytotoxic substances unequivocally leads to a profoundly impaired cytotoxic ability of old NK cells towards senescent fibroblasts. A global approach of transcriptome profiling of NK cells from old and young adults was employed to understand the over- and underrepresentation of pathways, the expression of distinct genes and their role in mediating NK cell cytotoxicity. Transcriptome enrichment analysis of old versus young NK cells, identified among others, the upregulation of gene signature sets for Rho GTPases in old NK cells. Pull down experiments found an unrestrained overactivation of Cdc42, a family member of Rho GTPases. Using a small molecule pharmaceutical approach, we found CASIN to inhibit the unrestrained Cdc42 activity in old human NK cells as wellas NK cells isolated from CASIN treated old mice and, in consequence, profoundly improved the impaired cytotoxic function of NK from old human and murine adults/mice towards senescent fibroblasts. As perforin and granzyme B granules are moved and focused to the synaptic cleft between NK cells and target senescent fibroblasts by the action of microtubules which are regulated by Cc42 activity, unrestrained CdC42 activity - as occurring in old NK cells - disrupts this fine tuning which is remarkably rebalanced to the Cdc42 activity of young NK cells by CASIN.

Collectively, we here unveiled a previously unreported mechanism of the functional impairment of NK cells from old adults and defined a therapeutic strategy to counteract the accumulation of senescent fibroblasts in old skin and likely other organs. In perspective, our data hold also promise to develop novel strategies against age-related disorders.

The extent of myeloid skewing might serve as a novel biological marker for the extent of aging in both mice and humans.

Julian Niemann[1], Selina Stahl[1], Vadim Sakk[1], Juan Felipe Perez Correa[2], Wolfgang Wagner[2], Andrea Jaensch[3], Dietrich Rothenbacher[3] und Hartmut Geiger[1], Mona Vogel[1,4]

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Myeloid skewing is a central and often cited hallmark of hematopoietic aging. Myeloid skewing refers to the aging-associated shift in hematopoietic stem cell (HSC) differentiation, resulting in an elevated myeloid cell over lymphoid cell ratio in aged compared to young mice, which further correlates with immune dysregulation and reduced hematopoietic regenerative capacity. Interestingly, whether the extent of myeloid skewing might be in itself a quantitative biological marker of aging has not been addressed yet, nor whether this parameter has also relevance for the extent of aging in humans.

We analyzed aging parameters in aged mice with low (<30% of myeloid cells) or a high (>50% myeloid cells) myeloid skewing in peripheral blood (PB). Mice with high myeloid skewing exhibited clear signs of enhanced hematopoietic aging compared to mice with a low myeloid skewing, including increased aging-associated splenic B cells, decreased naïve $CD8^+$ T cells, altered erythrocyte parameters as well as an increased level of inflammatory cytokines, together with an impaired HSC repopulation capacity. Epigenetic clock analyses indeed confirmed that high myeloid skewed mice present with a biological age that is older than their chronological age, while low skewed mice match their chronological age. We then investigated whether the extent of myeloid skewing, as observed in the mouse, might be also linked to the extent of aging in humans. The study included 1,425 individuals aged 65 or older from the population-based ActiFE study cohort. Participants were classified into quartiles based on their degree of myeloid skewing in PB, and age-associated parameters were compared between quartiles. A high extent of myeloid skewing was, also in humans, associated with elevated levels of inflammatory markers, reduced mobility, an increased burden of comorbidities and increased mortality, even evident after adjustment for age and sex. Taken together, the data support that, besides myeloid skewing being a central hallmark of aging in the hematopoietic system, the **extent** of myeloid skewing might also serve as a biological marker of the extent of aging in both mice and humans.

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Growth differentiation factor 15 (GDF-15) as a new mediator between stress-induced premature senescence and regenerative processes in post-traumatic osteoarthritis

Sara Sofi Marques[a], Alexandra Liebaug[a], Svenja Maurer[a], Dietrich Rothenbacher[b], Rolf E Brenner[a], **Jana Riegger**[a]

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Aim: Osteoarthritis is the most common age-related joint disease worldwide. Preceding joint injuries are considered as major risk factor, leading to a so-called post-traumatic osteoarthritis (PTOA). Although there are some minor differences between the clinical characteristics of patients diagnosed with idiopathic OA (IOA) and PTOA, no molecular marker has yet been identified to distinguish between IOA and PTOA. In the present study, we investigate the role of the all-cause mortality marker and stress-responsive cytokine, growth differentiation factor 15 (GDF-15), in PTOA.

Methods: GDF-15 was assessed in synovial fluid of end-stage OA patients from the Ulm OA study cohort (PTOA: n =12; IOA: n=54). Human fibroblast-like synoviocytes (hFLS) and chondrocytes (hCH) were isolated from OA patients undergoing arthroplasty. Highly degenerated human cartilage tissue was harvested without culturing. Macroscopically intact human cartilage explants were cultured and traumatized using a drop tower device (0.59 J). Expression of GDF-15 and GFRAL were analyzed by RT-PCR, immunohistochemistry (IHC), and ELISA, respectively. P53 was determined by immunofluorescence (IF) or IHC. To induce oxidative stress or senescence, hCH were exposed to 200 µM H2O2 for 48 h or 0.1 µM Doxorubicin for 5 consecutive days. N-acetylcysteine (NAC, 2mM) was refreshed every other day.

Results: Synovial GDF-15 levels were significantly higher in samples from end-stage PTOA patients as compared to IOA patients (mean ± SD: PTOA 625.9 ng/mL ± 210.2; IOA 452.8 ng/mL ± 150.3). Additionally, we confirmed that hFLS excessively release GDF-15 and other cytokines (IL-6, IL-1b, TNF) in response to medium of *ex vivo*-traumatized cartilage. The expression of GDF-15 and its receptor, GFRAL, was significantly elevated in chondrocytes of highly degenerated OA cartilage (both markers: > 60 %) as compared to cells of macroscopically intact tissue (both markers: < 20%) in patient-matched samples. 24h, 7d, and 14d after *ex vivo* cartilage trauma, chondrocytes secreted high amounts of GDF-15 in a time-dependent manner, while antioxidative treatment using NAC attenuated GDF-15 release. We confirmed that oxidative stress-induced GDF-15 was driven by the activation of the transcription factor p53. Accordingly, GDF-15 was strongly expressed by senescent chondrocytes. Addition of exogenous GDF-15 after cartilage trauma induced proliferation and had cell protective effects on chondrocytes. Similarly, GDF-15 induced proliferation and migratory activity in isolated hCH, but not enhanced expression of SA markers.

Conclusions: Overall, our findings demonstrate that chondrocytes release GDF-15 after cartilage injury. This stress response is mediated by oxidative stress and subsequent activation of p53, which is a key regulator of senescence. However, GDF-15 promotes primarily pro-regenerative processes but did not cause paracrine senescence in our study. Additionally, the stress-responsive cytokine might serve as a future biomarker of PTOA.

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Beyond Linear: A Robust Non-Parametric Approach to Epigenetic Age Estimation

Juan-Felipe Perez-Correa[1,2], Thomas Stiehl[3], Riccardo Marioni[4], Janie Corley[5], Simon Cox[5], Ivan Costa[6], Wolfgang Wagner[1,2,7,*]

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Abstract

Epigenetic aging signatures provide valuable insights into the complex process of human aging. However, traditional epigenetic clocks primarily use linear regression models based on DNA methylation levels, which inherently assume that age-related changes follow linear trajectories over time. In this study, we present a novel non-parametric approach that employs 2D-kernel density estimation to determine accurately epigenetic age. Our weighted methodology demonstrates predictive accuracy that is comparable to conventional clocks when applied in an independent validation dataset (R^2 = 0.81, mean absolute error = 4.00 years). A significant advantage of our approach is the introduction of a variation score that quantifies the inherent variability in age-related epigenetic changes across different samples. This variation score is notably elevated in various diseases, including acute myeloid leukemia (AML), HIV, and Down syndrome, indicating its potential clinical relevance. Furthermore, we found that an increase of one unit in the variation score associates with a 9.2% decrease in mortality risk (95% CI (0.8387, 0.9872), P = 0.0160) within the Lothian Birth Cohort 1921. Utilizing pyrosequencing data, we demonstrate that our methodology can effectively construct targeted assays with as few as 9 methylation sites while maintaining high accuracy across various platforms such as Illumina EPICv2 Beadchip. We also applied our method to the elderly ActiFe cohort from Ulm University and found it exhibited greater robustness compared to traditional linear regression-based clocks when assessing chronological age. In conclusion, our weighted 2D-kernel density estimation not only facilitates precise predictions of epigenetic age but also introduces an additional variable for biological age estimation, significantly enhancing our understanding of the intricate processes underlying human aging.

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With aging, you cannot afford errors anymore- how ER-stress signaling shapes protein synthesis in aging human fibroblasts

Max Hartmann, Zhouli Cao, Maximilan Wagner, Amy Lee Schug, Karin Scharffetter-Kochanek, Sebastian Iben

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In previous studies, our lab identified disturbed ribosomal biogenesis in premature aging diseases like Cockayne syndrome and trichothiodystrophy. This pathomechanism might contribute to the neurodegeneration observed in these diseases. Disturbed ribosomal biogenesis results in higher translational infidelity, which causes a loss of protein homeostasis (Alupei et al. 2018, Phan et al. 2021, Khalid et al. 2023). A loss of protein homeostasis characterizes most neurodegenerative diseases of the aging body (Wagner et al. 2024). Comparing skin fibroblasts from healthy young and old donors, we find an improved protein synthesis (decreased error rate of the ribosomes) with aging. To gain mechanistic insights, we could identify endoplasmic reticulum (ER) stress as the main regulator of translational fidelity. In cells from old donors, protein kinase R-like endoplasmic reticulum kinase (PERK) expression is increased. In contrast, the protein level of the PERK repressor GRP78 is reduced, resulting in a higher phosphorylation of eIF2α, the main translation regulator. Phosphorylation of eIF2α increases the accuracy of protein translation at the cost of inhibition of protein synthesis. Counteracting ER stress, especially blocking PERK, leads to a higher error rate in translation. By Nanopore sequencing, we compared the transcriptome of young and old donors and after the induction of ER stress in young donors. Differentially expressed gene analysis revealed that 75% of genes responding to ER stress induction are differently expressed in old compared to young fibroblasts. This indicates that translation regulation in aging is closely connected to ER stress. We now hypothesize that healthy aging might depend on the sustained accuracy of protein synthesis by the ribosome. Cellular compensation mechanisms that balance the proteome are decreasing with aging, a misregulated translational error rate might disturb the homeostasis mechanisms of cells and organs. Interestingly, this aging mechanism was not observed in fibroblasts isolated from old and young C57BL/6 mouse ears.

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Generation and first analysis of two *C. elegans* strains carrying mutations in the *semo-1* gene with putative effects on aging and methanethiol oxidase activity

Josephine Priebs[a], Lars-Oliver Klotz[a] and Holger Steinbrenner[a]

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Objective: The SELENBP1 orthologue, SEMO-1, affects aging and stress resistance in the model organism *C. elegans*. Like human SELENBP1, SEMO-1 is a copper-dependent methanethiol oxidase (MTO) converting methanethiol to hydrogen sulphide (H_2S), hydrogen peroxide (H_2O_2) and formaldehyde. SEMO-1-deficient worms lack MTO activity, showing life span extension and better resistance to oxidative stress in comparison to wild-type worms. This project shall investigate whether the beneficial effects of a *semo-1* knock-out on aging are related to suppressed MTO activity.

Methods: Two SEMO-1 mutant *C. elegans* strains were generated through CRISPR-Cas technology by introducing specific amino acid exchanges, previously demonstrated by us to result in suppressed MTO activity of bacterially-produced recombinant SEMO-1: (i) a *C. elegans* SEMO-1 mutant, G223W, homologous to a natural variant of human SELENBP1 identified in MTO-deficient patients with extraoral halitosis, and (ii) a *C. elegans* SEMO-1 mutant, E252Q, with deficient copper binding. Both mutants were analysed with respect to SEMO-1 protein levels using immunoblotting, MTO enzyme activity through detection of methanethiol-derived H_2S generation and life span, in comparison to wild-type and *semo-1* knockout worms.

Results: SEMO-1 was detectable in protein lysates of both mutant strains. Interestingly, SEMO-1-G223W levels were very low as compared to wildtype worms, suggesting that G to W change renders the protein unstable. E252Q mutants and wild-type worms showed similar SEMO-1 levels. In contrast to the MTO-defective bacterially produced recombinant SEMO-1 mutant proteins as well as to *semo-1* knock-out worms, the *C. elegans* E252Q and G223W mutant strains still exhibited some MTO activity, with ~44 % and ~17 %, respectively, of the MTO activity of wild-type worms. Strikingly, the two mutants behaved differently in the life span assay: G223W mutant worms had increased life expectancy, whereas the life span of E252Q mutant worms was similar to that of wild-type worms.

Conclusions: The G223W mutant strain closely resembles the *semo-1* knock-out strain with respect to the three hitherto analyzed parameters, showing strongly suppressed SEMO-1 protein levels and MTO activity combined with increased life expectancy. In contrast, no anti-aging effect was observed in the E252Q mutant strain, despite a decrease of more than 50 % in its MTO activity.

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Effects of CNS-related amyloidogenic peptides on enteric glia cells

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Background

Neurotoxic amyloidogenic peptides such as amyloid-β (Aβ) and alpha-synuclein (α-syn) can accumulate in the gut during aging and might lead to amyloid-mediated intestinal inflammation. There is growing evidence, that intestinal amyloids might spread to the brain via neuronal processes or the blood stream, suggesting the gut as starting point for neurodegenerative diseases of the central nervous system, such as Alzheimer's and Parkinson's disease, common conditions in aged individuals.

Enteric glia cells (EGC), as part of the enteric nervous system, play a crucial role in the immunosurveillance of the gut and are key regulators of the maintenance of gastrointestinal homeostasis. While a first study points to the adoption of a proinflammatory phenotype upon contact with the bacterial amyloid curli, to date nothing is known about the contribution of EGC in amyloid-mediated disturbance of gut homeostasis (or amyloid proceeding towards the brain) during aging.

Objective

Our aim is to understand the contribution of aged EGC during amyloid-mediated gastrointestinal dysfunction which might be the starting point of neurodegenerative diseases.

Methods

We address this question by incubating primary murine EGC cultures with the amyloidogenic peptides Aβ and α-syn. Amyloid-mediated glia cell activation is assessed and compared between EGC cultures derived from SAMP8 mice (an accelerated aging model) and the respective control strain (SAMR1) to investigate age-dependent functional changes. Read-out parameters for EGC activation include changes of intracellular calcium levels measured by Calcein AM assay and nitric oxide release measured by Griess reagent assay. Future experiments will include proteomic analysis with amyloid-challenged EGC cultures to identify cellular pathways involved in amyloid-mediated (patho)physiology.

Results

Primary murine EGC cultures were successfully prepared from adult and aged SAMR1 and SAMP8 mice and the preparation protocol was optimized to achieve the highest yield and purity of EGC. Pretests for Calcein AM loading of the cells were performed to quantify amyloid-mediated changes in EGC activation in SAMR1- and SAMP8-derived EGC cultures.

Conclusion

Our results will help to understand the role of EGC in age-dependent amyloid-mediated gastrointestinal dysfunction. The identification of involved cellular glial pathways might pave the road for future pharmacological intervention strategies.

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Can zebrafish regeneration reveal anti-aging strategies?

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Abstract

An important question in regeneration research is whether species and organs that regenerate well are immune against challenges that inhibit regeneration during aging in mammals. Zebrafish achieve complete heart regeneration via dedifferentiation and proliferation of cardiomyocytes and efficient bone regeneration in the fin via dedifferentiation of osteoblasts. Surprisingly, we found that regenerating cardiomyocytes experience DNA replication stress, which represents one reason for declining tissue regeneration during aging in mammals. Likewise, dedifferentiating osteoblasts in regenerating fins upregulate gene signatures indicative of DNA damage responses as well. Pharmacological inhibition of ATM and ATR kinases revealed that DNA damage response signaling is essential for zebrafish heart and fin regeneration. This suggests that regenerating fins and hearts are not immune against DNA stress that limits regeneration in aged mammals. Rather, the ability to overcome DNA stress appears to represent a key factor for the elevated regenerative capacity of these organs. Manipulation of Bone Morphogenetic Protein (BMP)-Smad signaling using transgenics and mutants showed that BMP signaling alleviates cardiomyocyte replication stress. BMP signaling also rescues neonatal mouse cardiomyocytes, human fibroblasts and human hematopoietic stem and progenitor cells (HSPCs) from replication stress. DNA fiber spreading assays indicate that BMP signaling facilitates re-start of replication forks after replication stress-induced stalling. Our results reveal a conserved role for BMP signaling in promotion of stress-free DNA replication and more broadly suggest that a thorough understanding of zebrafish regeneration could inspire anti-aging interventions.

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Abstract:

Extreme heterogeneity in hematologic aging is predominantly driven by stochastic variation, not by environmental nor genetic variation

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The combinatorial interaction of genetic and environmental variables acting cumulatively over long periods of time is broadly acknowledged to drive different rates and biological outcomes of organismal aging. In regenerating tissues, age-associated defects in the stem cell compartment or the stem cell niche are thought to ultimately result in the evolution of aged phenotypes, such as the myeloid biased linage output; anemia; and expansion of functionally compromised hematopoietic stem cells (HSCs) that are observed within the aged hematopoietic system. However, the degree of heterogeneity in these aged phenotypes has been largely unexplored. Here, we generated a multi-layered hematopoietic dataset using a large cohort (>100 individuals) of young (2 months), middle aged (18 months) and old (>24 months) inbred female C57BL/6 mice house under specified pathogen free conditions, to gain a comprehensive characterization of the aging process. Despite the lack of genetic and environmental variability across these cohorts, we observed highly variable hematologic phenotypes across the aged mice, with some 24-month-old mice displaying parameters comparable to young controls, while others showed extreme aging outcomes. Surprisingly, an cohort-wide correlation analysis of the aged individuals discounted the previously accepted cause-effect relationships between HSC functionality (measured by transplantation output), HSC expansion, myeloid bias and anemia, with these outcomes being spread across different individual mice. Nonetheless, this data set revealed two novel correlations relating to molecular programs existing in specific niche cell types and the presence of aged mature blood cell phenotypes. Thus, anemia correlated with elevated TGFβ signaling in a unique subset of bone marrow fibroblasts, while increased myelopoiesis correlated with altered TGFβ signaling in osteo-cxcl12-abundant-reticular cells (CARs) and, to a lesser extent, in adipo-CARs. These phenomena occur in different mice, suggesting that systemic TGFβ signaling is not the sole factor involved. Our results provide a comprehensive characterization of variability in hematological aging, challenging the notion of a uniform aging process driven by compromise HSCs, but rather indicating that age-associated programming of the bone marrow niche may directly act on more mature blood cells to drive aged hematopoiesis.

Chemerin Expression in Perivascular Adipose Tissue Differs Between Atherosclerosis-Prone and -Resistant Arteries in Patients Undergoing Coronary Artery Bypass Surgery

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Background:

It is estimated that 70% of individuals over 70 years develop coronary artery disease (CAD). Most patients undergoing elective coronary artery bypass grafting (CABG) are overweight or obese. Our previous work in adipocyte-specific eNOS knockout mice demonstrated that chemerin derived from perivascular adipose tissue (PVAT) promotes vascular inflammation and remodeling in high-fat diet (HFD)-induced obesity. While the aorta and coronary arteries are prone to atherosclerosis, the internal mammary artery (IMA) is relatively resistant to atherogenesis and is widely regarded as the gold-standard graft for coronary artery bypass grafting (CABG).

Methods and Results:

Vascular tissues (aortic and IMA segments) and the corresponding PVAT (aortic PVAT, or C-PVAT, and IMA-PVAT) were obtained from 60 patients undergoing elective CABG. The mean body mass index of the cohort was 29 kg/m², indicating overweight or obesity. Expression levels of fibrosis markers, inflammatory genes, oxidative stress-related genes, adipokines (adiponectin, leptin, and chemerin), and eNOS were assessed in PVAT using quantitative PCR. Fibrosis in vessels and PVAT was analyzed by Masson's trichrome staining.

Aortic tissue exhibited significantly higher fibrosis staining and expression of fibrosis-associated genes compared to IMA. Aortic PVAT also showed markedly higher fibrosis and expression of fibrotic markers than IMA-PVAT. While adiponectin and leptin expression were comparable between the two PVAT sites, chemerin expression was significantly higher in aortic PVAT relative to IMA-PVAT. This pattern paralleled increased expression of genes related to inflammation and oxidative stress in aortic PVAT.

Conclusions:

Chemerin expression is significantly higher in PVAT surrounding atherosclerosis-prone aortic segments compared to atherosclerosis-resistant IMA segments. This suggests that PVAT-derived chemerin may contribute to local vascular inflammation and atherogenesis, particularly in the context of obesity-related cardiovascular disease.

How somatic mutations cause aging without causing aging

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Evolutionary theory identifies reduced extrinsic mortality as driving the evolution of slower aging, but there is no consensus about what, if anything, impedes the evolution of increased longevity. Classic mechanisms such as antagonistic pleiotropy and resource allocation efficiency fail to identify the specific molecular mechanisms determining life span.

The tumor suppression theory of ageing is a reductionist evolutionary theory of aging proposing cancer risk as the dominant force preventing indefinite self-renewal and constraining the evolution of longevity in mammals. In this work, most hallmarks of aging are shown to be tumor suppressors and rearranged into a causal hierarchy that puts forward cell proliferation as the primary driver of aging. Tumor-suppressive hallmarks like telomere shortening, cellular senescence and others restrain malignant transformation, and this benefit explains why they are extensively interconnected. The resulting stem cell exhaustion constitutes the proximate mechanism of aging, the store of biological age and characterizes the state of being old. Somatic mutations are therefore the (ultimate) reason why we age, but not the mechanism how we age, which is cellular depletion.

The idea that tumor suppression contributes to aging originated several decades ago, but was abandoned largely because caloric restriction (CR) and interventions into conserved aging pathways delay both aging and cancer, contradicting the idea. By explaining how CR slows aging through the regulation of cell proliferation rates, the tumor suppression theory of aging resolves this conundrum and identifies the instability of DNA giving rise to cancer as the ultimate reason why we age and the primary factor constraining the evolution of very long, if not indefinite, lifespans in larger animals.

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Culture-associated DNA methylation changes in T cells are associated with T cell activation

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Background

Culture expansion of T cells is important for production of cellular therapeutics. To facilitate *in vitro* proliferation, the cells need to be activated – for example by co-stimulatory signals of CD3 and CD28 agonists. However, this may also support to exhaustion and diminished cytotoxic functions. We have demonstrated that culture expansion of T cells and CAR T cells is also associated with specific changes in DNA methylation of CG dinucleotides (CpGs). In this study, we have analysed if the epigenetic changes are related to their activation. Furthermore, we have adopted the epigenetic signature that reflects culture expansion of T cells to a robust and cost-effective method based on digital PCR.

Results

We have cultured CD3 positive T cells for 21 days with or without TransAct. Proliferation was only observed with activation. Culture expansion resulted in decreased CD4/CD8 ratio only if the cells proliferated. We have subsequently compared genome wide DNA methylation profiles using Illumina's BeadChips (n = 3). Overall, the stimulated control samples recapitulated the culture-associated DNA methylation changes observed in our previous work. Notably, T cells that were cultured without stimulation did not reveal significant DNA methylation changes at day 21. We selected 6 culture-associated CpGs, which were previously shown to be associated with adverse outcome in CAR-T cell therapy, to develop targeted DNA methylation assays with digital PCR. This results clearly reflected the state of culture expansion in the activated T cells, whereas non-activated samples showed less DNA methylation at these sites.

Conclusions

Our results demonstrate that culture-associated changes in DNA methylation are associated with proliferation upon T cell activation. Furthermore, digital PCR successfully replicates the results obtained from Illumina's BeadChip, offering a more cost-effective and in-house method to determine the state of cellular aging in T cells.

Title:
Elevated levels of Ube2g1 in HSCs lead to segmental aging of the haematopoietic system

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Background
Aging is associated with functional decline in hematopoietic stem cells (HSCs), characterized by diminished self-renewal capacity, skewed lineage output, and compromised proteostasis. Proteasomal, ubiquitin-driven degradation pathways are likely critical for maintaining protein homeostasis in HSCs, especially upon aging. The role of distinct proteins regulating ubiquitin-driven degradation, like Ube2g1, an E2 ubiquitin-conjugating enzyme we previously found to give HSCs a positive clonal advantage through selection, remains poorly understood.

Aims
This study investigates whether age-related alterations in Ube2g1 expression contribute to HSC aging. We hypothesized that overexpression (OE) or knockdown (KD) of Ube2g1 in young and aged murine HSCs results in aging phenotypes in the hematopoietic system.

Results
Aged HSCs from both mice and humans exhibited a 10–20% increase in Ube2g1 protein levels. In transplantation experiments, mice receiving young Ube2g1-OE HSCs, thus mirroring aged Ube2g1 expression, showed a 3-fold increased myeloid over lymphoid cell frequency and old OE HSCs an 18-fold increased ratio. Notably, the age-associated decline in naïve $CD8^+$ T cells was exacerbated, with an almost complete loss in mice transplanted with young or old OE HSCs. Conversely, Ube2g1-KD resulted in a 2-fold decreased ratio in recipients of old OE HSCs.
Interestingly, overexpression of a catalytically inactive Ube2g1 mutant demonstrated that ubiquitination activity is not required to induce the age-associated phenotype following transplantation. Transcriptional profiling revealed enrichment of gene sets related to phosphorylation, immune regulation and TNF signaling. Together with a proteome analyses, we identified Ptpn11 (Shp2) as potential interactor of Ube2g1. Further analysis revealed a pronounced blockage in early thymic T cell maturation in animals transplanted with young Ube2g1-OE HSC. This was was accompanied by increased Tim-3 expression, a marker of exhaustion, on mature peripheral splenic T cells, suggesting an overall impaired T cell development following Ube2g1 overexpression.

Conclusion
Elevated Ube2g1 levels in aged HSCs correlate with disrupted proteostasis and aging-like hematopoietic imbalances. Ube2g1-OE exacerbates lineage skewing and T-cell depletion, while KD partially restores youthful hematopoietic output in aged HSCs. These findings underscore Ube2g1's role in HSC aging and early T cell development.

To SASP or NOT to SASP: DNA Damage and Oxidative Stress Induce Distinct Senescent Phenotypes in Osteoblastic Cells

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Background and aim: The aging population is experiencing a rising incidence of osteoporosis, an age-related skeletal disorder, characterized by an imbalance between bone formation and resorption, leading to fragility fractures. Cellular senescence, triggered by various stressors such as DNA damage or oxidative stress, contributes to bone loss. Senescent cells are marked by stable cell cycle arrest and a senescence-associated secretory phenotype (SASP), which includes pro-inflammatory factors that impair bone remodelling. The nature of the stressor determines the senescent phenotype. DNA damage-induced senescence elicits a robust SASP, while stress-induced premature senescence (SIPS), triggered by elevated reactive oxygen species (ROS) or certain chemotherapeutics, may cause a milder phenotype with minimal SASP. This study compares the senescence-inducing effects of DNA damage (via doxorubicin; Doxo) and ROS stress (via hydrogen peroxide; H_2O_2) in MC3T3-E1 cell line.

Methods: MC3T3 cells were treated with 0.1 µM Doxo daily for five days, with a double dose on day 5 to induce stable senescence. For ROS-induced stress, cells were treated twice with 100 µM H_2O_2, for 24 hours each, separated by a 24-hour recovery period. On day 8, mitochondrial membrane potential was assessed using JC-1 dye. Proliferation and senescence were evaluated via Ki-67 immunostaining and senescence-associated ß-galactosidase (SA-ß-gal) staining. RT-qPCR was used to analyse gene expression levels of *Cdkn1a*, *Cdkn2a*, *Il-6*, *Rankl*, and *Opg*.

Results: Both Doxo and H_2O_2 significantly disrupted mitochondrial membrane potential (Doxo: $p = 0.003$; H_2O_2: $p = 0.01$). SA-ß-gal staining showed 88 % positive cells after Doxo treatment and 15% after H_2O_2 treatment. Proliferation decreased by 68% in Doxo- and 40% in H_2O_2-treated cells. Doxo significantly upregulated *Cdkn1a* ($p = 0.0389$), *Cdkn2a* ($p = 0.0124$), *Il6* ($p = 0.0002$), *Rankl* ($p = 0.0313$), and *Opg* ($p = 0.0003$). In contrast, H_2O_2 reduced *Rankl* expression ($p = 0.01$), with no significant changes in other genes. The *Rankl/Opg* ratio increased 3.96-fold after Doxo and decreased to 0.82-fold after H_2O_2, though neither change was statistically significant.

Conclusion: Both Doxo and H_2O_2 induce a senescence-like phenotype in MC3T3-E1 cells. However, Doxo robustly induces cell cycle arrest and a pro-inflammatory SASP, while H_2O_2 triggers milder SIPS-like state with limited SASP expression. These findings suggest that Doxo is a potent inducer of senescence, while H_2O_2 represents a physiological stressor that elicits subtler cellular responses.

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Manipulations of Alzheimer proteins result in altered translational error rate of the ribosomes

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In previous studies, our lab identified disturbed ribosomal biogenesis and function in the premature aging diseases Cockayne syndrome and trichothiodystrophy. The subsequent loss of protein homeostasis (proteostasis) might be the driving force of the severe neurodegeneration characterizing these diseases. Loss of proteostasis is caused by an elevated error rate of ribosomal protein synthesis (translational infidelity) and results in an increased endoplasmic reticulum (ER) stress (Alupei et al. 2018, Phan et al. 2021, Khalid et al. 2023). Given the fact that a loss of proteostasis characterizes most neurodegenerative diseases of the aging body, we are asking the question where the misfolded proteins come from. Does the error rate of protein synthesis affect aging-associated diseases, such as Alzheimer's disease (AD)? We employed both CRISPR-Cas9 and short hairpin RNA (shRNA) technologies to knock down PSEN1 and amyloid beta precursor protein (APP) expression in human fibroblasts, creating a cellular model of AD. Surprisingly, knocking down PSEN1 significantly increased the error rate of protein translation whilst knocking down APP reduced translational errors of the ribosome. In line with these results, we find an increased protein aggregation in fibroblasts with PSEN1 deficiency and less protein aggregation in cells with reduced APP levels. Interestingly, manipulations of the Alzheimer proteins themselves led to altered ER stress signaling, a hallmark of Alzheimer`s disease. Future work will show if the loss of proteostasis in AD is causally related to ribosomal dysfunction.

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Establishment of a cellular system to manipulate ribosomal error-rate

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In childhood premature aging diseases (Cockayne syndrome and trichothiodystrophy) we could describe that an elevated ribosomal error-rate leads to ER-stress, an Unfolded Protein Response and a repression of protein synthesis and de-novo synthesis of ribosomes (Alupei et al, 2018, Phan et al, 2021, Khalid et al, 2023). As these progerias are characterized by severe neurodegeneration and neurodevelopmental abnormalities, we now ask if an elevated error rate of the ribosomes contribute to, or causes neurodegenerative diseases of the aging body. To investigate this hypothesis, we are establishing a tetracyclin-off system, where withdrawal of tetracyclin induces the expression of a mutant ribosomal protein. This protein, mRPS9, has already been shown to induce an error-prone protein synthesis in mammals (Shcherbakov et al, 2022). We can show that the wildtype and mutant genes are integrated in the vector backbone and when transfected in HEK cells are leading to the overexpression of the different RPS9 forms. When the transfected cells are tested with reporter plasmids encoding mutant nanoluciferase, the nanoluciferase is activated in the mRPS9, but not wtRPS9 cells, indicating an error-prone translation process. Moreover, we investigated ER-stress induction by the error-prone protein synthesis and could not detect significant differences in ER stress markers in HEK cells between the error-prone cell line and the cell line expressing the wildtype construct. Polysomal profiling experiments indicate that the expression of mutant, but not wildtype RPS9 influences the assembly of the 80S ribosomal monosome. Taken together we here present an inducible cell culture system in which we can assess the consequences of ribosomal errors for the cells. We plan to transfect fibroblasts from young human donors in comparison to old human donors and convert these fibroblasts direct into neuronal cells. These neuronal cells can then be further differentiated to neuronal cells that are affected in Amyotrophic lateral sclerosis (motoneurons) or Alzheimer disease (cortical neurons) and the outcome of ribosomal errors can be studied.

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The role of aging and environmental stress along the gut-lung axis in chronic inflammation

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Background:
Organismal aging is a major driver of chronic inflammatory diseases, including Chronic Obstructive Pulmonary Disease (COPD). COPD is a currently incurable age-related lung disease and a leading cause of morbidity and mortality worldwide. Its pathogenesis involves both chronic inflammation of the airways and systemic manifestations. Importantly, aging and environmental stress disrupt epithelial barrier function and impair immune responses, contributing to the onset and progression of COPD. The emerging concept of the *gut-lung axis* emphasizes that epithelial dysfunction is not limited to the lungs but interacts with the intestinal epithelium, where microbiota-derived signals and pro-inflammatory mediators can modulate lung inflammation and immunity.

Objective:
Thus, understanding the effects of aging and environmental stress on epithelial integrity and immune cell interaction properties across this axis and associated microbiome dynamics is critical for identifying early causes of systemic chronic inflammation and potential therapeutic targets.

Methods:
We used 3D organoid models of the respiratory tract and intestine to study epithelial homeostasis and stem cell regeneration capacity. Organoids from young, aged, and cigarette-smoke (CS)-exposed mice were analyzed for changes in the transcriptional landscape and DNA methylation patterns upon aging and longitudinal CS exposure. Simultaneously, gut microbiome dynamics were examined to assess epithelial-microbe interactions upon aging and environmental stress.

Results & Future Directions:
Airway organoids from aged mice showed reduced growth capacity, indicating exhaustion of progenitor cell potential and cellular senescence upon aging. Age-related transcriptional changes of the airway epithelium were linked to inflammation, senescence, and cholesterol biosynthesis, and were more pronounced than those caused by a two-month exposure to cigarette smoke.
Current investigations examine the effects of four months of CS exposure at the transcriptional and epigenetic level and their linkage to changes in microbiome dynamics. This will reveal potential candidates involved in the age-dependent vulnerability and environmental damage to the airway epithelium.
In a more holistic and systemic approach, we will assess epithelial interactions with the microbiome and immune cells upon aging and environmental stress to identify potential epithelial targets across the *gut-lung axis* for disease prevention and therapy.

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Glucocorticoid receptor in adipocytes mediates metabolic pathologies during aging

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Introduction: Glucocorticoids (GCs) are major stress hormones that are released during different stress conditions such as pathogen infection, metabolic or psychological stress, and act mainly via the glucocorticoid receptor (GR). GCs profoundly affect immune metabolism while having an effect on immune cells, but also modulate tissue homeostasis and energy in many cells including adipocytes and hepatocytes. This is important for an allostatic response, but in case it is exaggerated can lead to features of metabolic syndrome including diabetes. GC levels also increase during aging. Furthermore, aging is associated with different metabolic diseases such as diabetes and atherosclerosis, which may in part depend on GC and GR action. On the other hand, we showed previously that GCs may prevent insulin resistance when resolving adipose tissue inflammation.
Aim & Methods: To understand how GCs affect age-related metabolic diseases by their action in adipocytes, we investigated aged adipocyte specific GR knockout mice ($GR^{AdipoCre}$). Moreover, we characterized the binding behaviour of GR through single-molecule tracking in primary pre-adipocytes and macrophages.
Results: GR deletion in adipocytes of 80-week-old female mice did not result in significant alterations in insulin sensitivity or adipose tissue depot, or adipocyte size. However, a reduction in CD11c+ cells was observed in $GR^{AdipoCre}$ eWAT, indicating a potential decrease in visceral fat inflammation. Additionally, reduced hepatic lipid accumulation in $GR^{AdipoCre}$ was detected, suggesting an improvement in lipid metabolism. Through single-molecule tracking, we gained insight into the DNA binding kinetics of GR, including bound fractions, residence times and search times, *in cellula* across different cell types and age conditions. Between young and old cells, GR binding kinetics did not change.
Conclusion: During aging, GR is important for the maturation of adipocytes, but may lead to an increased visceral fat inflammation indicating a pro-inflammatory role of GCs during the aging process. GC resistance seems to be independent of the GR-DNA binding kinetics. We currently study the possibility that GR motility is affected by co-regulatory molecules.

Challenge the "Free Radical Theory of Ageing" and the "Aß peptide extracellular plaque hypothesis of Alzheimer's disease"

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We are searching for the common biology of health and diseases. If there is any, we might be able to understand and to manipulate those factors and mechanisms that determine e.g. human life-span and – even more important- health-span as well as age-associated diseases. There are a wealth of information and strong scientific data accumulated in recent years that biological membranes are this common link.

Do aging and calorie restriction modulate the mitochondrial proteome, the global protein oxidation profile and metabolism

Age-dependent changes in the cellular proteome (amount and interaction of proteins, post-translational modifications, enzymatic activities) are currently considered as targets and even triggers of ageing and of age-associated diseases. Therefore, we have investigated the role of the mitochondrial proteome in conserved mechanisms of ageing. The observed modulation of the mitochondrial proteome, OxPhos supercomplex (respirasome) architecture, and activity by age, reactive oxygen species (ROS), and nutrition gives insight into the involvement of metabolism on life- and health-span. In contradiction to the predictions of the "Free Radical Theory of Ageing", mitochondrial proteins of rat cortex exhibited less oxidative modifications (protein carbonylation) in aged rats compared to young (Monika Frenzel und Norbert A. Dencher, unpublished results). Supporting this initial observation, by our recent large-scale iTRAQ proteomics analysis no pronounced increase in protein oxidation during ageing both in brain and heart mitochondria was observed, challenging the "Mitochondrial Free Radical / Reactive Oxygen Species Theory of Ageing" (in the strict sense of elevated levels of oxidatively damaged proteins at advanced age as cause of cell death/impairment). However, beyond doubt free radicals, ROS, and RNS (and therefore antioxidants) are of uppermost importance for cell survival and disease onset.

Cell organelles and membranes as targets of Alzheimer's disease triggering amyloid beta peptides

Alzheimer's disease is one of many age-associated diseases, but the most common dementia in elderly (60-80% of all dementia in the currently about 47 million cases worldwide). The highest risk factor for AD is age. Contrary to the extracellular plaque hypothesis, still favoured by most researchers in the field, even Aß monomers are bioactive via insertion into membranes. In order to demonstrate cellular and organelle trafficking of Aß peptides, to identify their target(s), and to analyze their deleterious effects on cell and membrane function, in our studies $A\beta_{42}$ peptide monomers/small oligomers were externally applied to mammalian cells (human neuroblastoma cell line and rat oligodendroglia cell line). Monomeric/oligomeric peptides entered cells, as proven in our investigation by confocal fluorescence microscopy with $A\beta_{42}$ peptides labeled with different fluorophores employed. We were able to track in time and space the pathway of the Aß peptides from the outside of the cell across the plasma membrane to internal target membranes of specific organelles. In this way, for both cell lines, we did prove that fluorescently labeled Aβ peptides in minutes co-localized with the plasma membrane (Podolyak E.Y., unpublished results) and thereafter entered the cells and trafficked to organelles, e.g., predominantly to endosomes/lysosomes. All our results are in line with and do indicate at the molecular level that Aß peptides intercalate into membranes, perturb the structure of the lipid bilayer, modulate lipid dynamics, induce membrane fusion and in this manner will lead to malfunctioning and finally to death of cells. These data challenge the "Aß peptide extracellular plaque hypothesis of AD".

Old hematopoietic stem cells retain competence to reconstitute a youthful B cell system that is highly responsive to protein-based vaccination

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Ageing-associated remodeling of the murine B cell system is accompanied by a reduction of $CD19^+$ B cells such as follicular B cells (FOB) and an accumulation of age-associated B cells (ABC) or activated B cell subsets. This remodeling is thought to confer an attenuated antibody response, such as to SARS-CoV-2 spike (S) vaccines in both aged mice and humans. To gain insight into the *de novo* development and function of an old B cell system, we reconstituted young and old immune systems by transferring hematopoietic stem cells (HSCs) from immune-competent young (2–3 months) $CD45.1^+$ donors (DY-HSC) or old (20–24 months) donors (DO-HSC) into T and B cell-deficient young recipient $CD45.2^+$ $RAG1^{-/-}$ mice, followed by protein-based vaccination.
Using flow cytometry, we could show that in the same environment of young $RAG1^{-/-}$ mice, transplanted DO-HSCs compared to DY-HSCs reconstituted lower numbers of $CD19^+$ B cells and $CD45.1^+$ cells, though the engraftment of donor-derived HSCs in the young bone marrow (BM) was very similar. Furthermore, indicative for youthful and unchallenged B cell systems, and in contrast to aged mice, very low levels of antigen-experienced memory B cells or age-associated B cells (ABC) developed in both DY-HSC and DO-HSC hosts. The commercially available recombinant SARS-CoV-2 S vaccine (NVX-CoV2373) induced lower IgG^+ S-antibody titers and pseudovirus neutralization activity in old compared to young mice. In contrast, very similar high IgG^+ S-antibody titers were induced in DO-HSC and DY-HSC hosts, and pseudovirus neutralization activity was even enhanced in DO-HSC compared with DY-HSC hosts.
In summary, both DO-HSCs and DY-HSCs established in the young recipient BM to a similar extent, suggesting that the concomitant reduction in the *de novo* reconstitution of $CD19^+$ B cells in DO-HSC vs. DY-HSC transplanted animals is specifically related to old HSCs. DO-HSCs and DY-HSCs reconstitute very similar unchallenged B cell systems that efficiently elicit antigen-specific IgG antibodies by protein-based vaccination. Old HSCs thus retain competence to reconstitute a youthful and functional B cell system, at least in the young environment of transplanted $RAG1^{-/-}$ mice. This suggests that primarily age-related factors, and not HSCs per se, influence the composition and functionality of the old B cell system.

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Suppression of G-quadruplex DNA and interconnected genome instability to counteract aging in a cellular model of the Hutchinson-Gilford progeria syndrome

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Guanine quadruplexes (G-quadruplexes or G4s) are secondary, four-stranded structures formed by guanine-rich sequences in nucleic acids, stabilized through Hoogsteen hydrogen bonding. These structures are frequently found in regions of the genome associated with regulatory functions. Their presence can influence gene expression as well as genomic stability. It was shown that G4 structures lead to transcription- and replication-dependent DNA double strand breaks (DSBs), making them significant contributors to genomic instability and possibly aging. G4 helicases, such as the DEAD-box helicase 21 (DDX21), efficiently bind and unwind G4 structures and therefore prevent their accumulation in the genome. The functionality of DDX21 is influenced, among others, by sirtuin 7 (SIRT7), which deacetylates and therefore activates the enzyme. Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder, characterized by elevated levels of progerin, which destabilize SIRT7 by promoting proteasomal degradation. We hypothesize that progerin-mediated downregulation of SIRT7 results in the predominance of the acetylated, less active form of DDX21. The build-up of G4 structures and interconnected DNA damage, due to the impaired functionality of DDX21, is envisioned to promote the disease phenotype, as it might contribute to premature aging. To analyze this, I attempted to recover the molecular mechanism behind G4 unwinding in the cellular model of HGPS, via upregulation of DDX21 and SIRT7, respectively. Lentiviral transduction to achieve overexpression of the target proteins was performed in human HGPS-derived induced mesenchymal stem cells (HGPS-iMSCs). HGPS-iMSCs are ideal for disease remodeling, due to their high expression of the disease-causing protein progerin. CRISPR activation (CRISPRa) mediated upregulation of endogenous DDX21 or SIRT7 resulted in significant reduction of G4 structures and DNA damage in HGPS-iMSCs. Gene expression analysis via quantitative real-time PCR (qPCR) revealed a downregulation of the senescence marker p21 and an upregulation of the proliferation marker Ki67 in the CRISPRa transduced HGPS-iMSCs with DDX21 or SIRT7 overexpression in comparison to the Non-targeting Control, suggesting a possible rejuvenation effect *in vitro*. Therefore, CRISPRa mediated DDX21/SIRT7 overexpression partially rescued the disease phenotype in the cellular model of HGPS, possibly also slowing down aging in the iMSCs. These findings suggest a potential, future therapeutic application for premature aging disorders, such as HGPS, for the partial rescue of the disease phenotype and the deceleration of aging.

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HIV-1 activates an endogenous retrovirus regulating p53 signaling and senescence

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Transposable elements such as long interspersed nuclear elements (LINE-1) and endogenous retroviral long terminal repeats (LTRs) are typically silenced in somatic cells, but can be reactivated during aging and in response to viral infections. LINE-1 activation has been shown to contribute to aging by inducing interferon responses and sterile inflammation (De Cecco *et al*., 2019). We hypothesized that transposable elements not only promote inflammaging, but also function as direct regulators of cellular senescence pathways.

To investigate this, we analyzed the activation of transposable elements following HIV-1 infection and examined their downstream effects on cellular senescence. Using *ex vivo* infected primary CD4+ T cells, we show that HIV-1 infection results in the activation of a distinct subset of endogenous retroviral LTRs, specifically from the LTR12C and LTR12D subfamilies. Notably, one infection-induced LTR12D element serves as an alternative promoter for *DHRS2*, an NADPH-dependent dicarbonyl reductase known to activate the p53–p21 senescence pathway. Consistent with this, DHRS2 and p53 protein levels are elevated in HIV-1-infected primary $CD4^+$ T cells and associated with increased transcription of *CDKN1A*/*p21*. Boolean network simulations predicted that activation of the LTR12D-DHRS2-p53-p21 cascade results in the induction of cellular senescence upon HIV-1 infection. In agreement with these predictions, HIV-1-infected $CD4^+$ T cells show an increased activity of senescence-associated β-galactosidase and a senescence-associated secretory phenotype (SASP). Mechanistic studies further revealed that the LTR12D repeat upstream of the *DHRS2* gene is responsive to T cell factor 1 (TCF1) and heat shock factor 1 (HSF1), providing mechanistic insights into the phenomenon of HSF1 depletion-induced senescence (Oda *et al*., 2018). Finally, we found that the LTR12D-DHRS2-p53-p21 cascade is also activated in response to oxidative stress and ER stress.

In summary, our findings identify an HIV-1-induced, endogenous retroviral promoter that activates a p53 regulator and may potentially contribute to senescence and accelerated aging in people living with HIV.

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Blood-based Biomarkers for Frailty Detection beyond Geriatric Units

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Introduction Frail older adults require patient-centred treatment in all clinical settings. Thus, their efficient identification beyond geriatric units is crucial. We aimed to build sex-specific predictive models for frailty using blood-based biomarkers routinely available.

Methods We analysed baseline data from the ActiFE cohort study (Ulm, Germany, 2009/2010), an interdisciplinary project, which included 1188 community-dwelling adults aged 65 and older. Frailty was defined using a frailty index (FI) based on the concept of accumulation of deficits. Routine blood-based biomarkers associated with frailty were selected according to evidence in previous literature. Sex-specific logistic regression analyses were performed adjusting for age and statins.

Results Models for both sexes included haemoglobin, leucocytes, urea, gamma-glutamyl transferase (GGT), total and high-density lipoprotein cholesterol and C-reactive protein (CRP). In men, alanine-aminotransferase (ALT) was included additionally. Using a cut-off of 0.3 for the estimated probability of being frail (FI ≥ 0.2), the models showed low sensitivity (men: 0.508, women: 0.643) and low positive predictive value (PV) (men: 0.474, women: 0.522). In contrast, detection of non-frail individuals was accurate with high specificity (men: 0.873, women: 0.796) and high negative PV (men: 0.888, women: 0.865). Models AUC were 0.787 and 0.804 in men and women, respectively. Notably, GGT was particularly strong as a marker, an association previously poorly explored.

Conclusion With high specificity and negative PV, models including routine blood-based biomarkers offer a cost- and time-efficient method to identify non-frail individuals in different clinical settings. This could support the initial step of a serial frailty screening strategy aiming at an optimized patient-centred treatment.

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The effect of different adrenoreceptor blockers on fracture healing of non-osteoporotic and osteoporotic bone

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Osteoporosis is an age-related and the most common metabolic bone disease manifested by low bone mass and increased fracture risk. Fracture healing is significantly delayed in osteoporotic patients due to a more challenging fracture fixation in the weak osteoporotic bone and an intrinsic lack of healing capacity which is why new therapeutic strategies are urgently needed. In recent years, it became evident that the innervation of the bone with sensory and sympathetic nerve fibers plays a crucial role during fracture healing and particularly adrenergic signaling may play a role in the pathomechanisms of osteoporosis. In female mice ovariectomy (OVX) is a well-established procedure to induce postmenopausal osteoporosis by the removal of the ovaries resulting in estrogen deficiency, which allows to study this type of osteoporosis in vivo. Previous RNASeq results from our lab demonstrate that in the fracture callus from osteoporotic mice, adrenergic signaling was one of the most regulated pathways. In particular, the β_2-adrenoreceptor was significantly higher expressed. Other experiments showed that osteoporotic mice display increased numbers of neutrophils in the early fracture hematoma and that non-osteoporotic mice treated with the unspecific ß-adrenoreceptor blocker Propranolol immediately before fracture resulted in a significantly lower number of neutrophils in the fracture callus. These data led us to the hypothesis that adrenergic signaling might be involved in the misbalanced early immune response which seem to impair osteoporotic fracture healing.

Therefore, in this study we investigated the effect of different adrenoreceptor blockers (Propranolol = unspecific β-blocker; Butoxamine = specific β_2-blocker; Phentolamine = unspecific α-blocker) on fracture healing in non-osteoporotic and osteoporotic mice. The mice were subjected to OVX or sham surgery at the age of 12 weeks. Four weeks after, a standardized femur osteotomy stabilized with an external fixator was applied. Afterwards, the blockers were injected subcutaneously on the day of fracture and for three days after surgery to address the inflammatory healing phase. Fracture healing and bone phenotype was analyzed at day 1, day 3 and 21 days after fracture by FACS analysis, biomechanical testing and µCT.

Generally, the short-term administration of adrenoreceptor blockers had no effect on the development of osteoporosis itself. However, β-blocker treatment (Propranolol and Butoxamine) rather disturbed fracture healing in non-osteoporotic mice but ameliorated the negative effects of postmenopausal osteoporosis on fracture healing. In contrast α-blocker treatment did not ameliorate the effect of OVX on fracture healing. The reduced OVX effect in beta-blocker treated mice was most likely due to reduced neutrophil infiltration in the fracture hematoma. Further in-depth analysis is needed to conclude about a possible positive effect and the involved mechanisms of β-blockers specifically in postmenopausal osteoporotic mice.

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GrandAge: A Deep-Learning Multi-Tissue Epigenetic Clock developed using Methylation Data from over 60,000 Samples

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Epigenetic clocks can estimate an individual's age with a median error of only a few years. Training these clocks requires large cohorts that provide both methylation data and annotated age. As with all machine learning models, the quantity and quality of this data can significantly impact the results. We curated a large dataset by combining more than 1,000 series with methylation data from the Gene Expression Omnibus (GEO) database. This combined dataset comprises over 60,000 non-malignant samples from 592 series and 20 tissue categories, to the best of our knowledge, is the largest unified methylation dataset. Based on extensive hyperparameter optimization, we developed a new deep-learning epigenetic clock, called GrandAge. GrandAge effectively leverages the large size of our dataset, achieving a significantly lower error (median absolute error of 0.84 years in cross-validation) compared to previously presented epigenetic clocks. We conducted in-depth analyses to investigate the impact of the number of training samples, sex, chronological age, and tissue type on GrandAge's performance. Comparison with other epigenetic clocks reveals that GrandAge consistently exhibits lower prediction error and bias across most ages and tissues. GrandAge predicts with a bias close to zero for ages up to 60 years. However, predictions for ages above 60 years show an increasing negative bias, although to a lesser extent than other epigenetic clocks. We demonstrate that this phenomenon can be reduced by either excluding CpGs that show signs of saturation for older ages, or by employing dataset resampling strategies to mitigate the dataset imbalance. Taken together, GrandAge increases the precision of estimation of chronological age combining a very large dataset and deep-learning – it will be interesting to explore if it still reflects aspects of the individual aging process.

Design of a targeted epigenetic clock to discriminate frailty status in the ActiFe cohort

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Epigenetic clocks have emerged as valuable tools for estimating biological age. For many clocks, it has been shown that the difference between the epigenetic age and chronological age (also called delta age) correlates with all-cause mortality.
We anticipate that delta age can also be indicative for frailty, which is characterized by a decline in biological function across various organ systems.
In this study, we designed a targeted epigenetic clock based on three CpGs for which methylation changes have previously been shown to be associated with all-cause mortality, to explore if this signature also reflects differences in frailty. We have preselected the CpGs with high mortality-association in the datasets of the Lothian Birth Cohorts of 1921 and 1936 and selected for the strongest age-association in a large cohort (GSE246337, n = 500). These selected sites were then utilized to construct a targeted multivariate regression model for predicting chronological age. Subsequently, we employed the EPICv2 methylation array to analyze DNA methylation profiles from whole blood samples of 40 donors (20 frail and 20 non-frail) from the ActiFe cohort at Ulm University.
Our results indicated that within this cohort of 40 samples, our new clock—which integrates sites that are both mortality- and age-associated—yielded significantly increased delta age for frail *versus* non-frail individuals (P = 0.03). In contrast, the traditional aging clock utilizing only age-associated sites did not demonstrate this difference (P = 0.43), suggesting that incorporating mortality-related CpG sites enhances the informative value of epigenetic clocks concerning individual health status.
In conclusion, our new targeted epigenetic clock may better capture aspects of frailty. The findings above are currently being further validated in approximately 400 blood donors from the ActiFe study using digital PCR for targeted assays.

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Replication stress responses in human lymphocytes change sex-specifically during aging

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The varying incidence of aging-related diseases such as cancer and autoimmune disorders as well as the gender gap in life expectancy suggest differences in the aging process between the sexes. However, little is known about sex-specific differences in genomic instability, a key hallmark of aging. In previous studies focusing on the repair of DNA double-strand breaks (DSB), we showed that peripheral blood lymphocytes (PBL) from older men feature upregulated activity of error-prone non-homologous end-joining (NHEJ). In contrast, in older women NHEJ, microhomology-mediated end joining (MMEJ) and single-strand annealing (SSA) are downregulated indicating a pathway-independent decline of DSB repair. Given that replication stress is a major source of DSB, we hypothesized that sex-specific regulation of DSB repair during aging is connected with differential replication stress response mechanisms. Therefore, we analyzed the DNA damage response (DDR) in cycling PBL and hematopoietic stem and progenitor cells (HSPC) from female and male donors of different age groups, thereby focusing on replication stress. RNA-Seq revealed striking sex-dependent transcriptional changes in DDR pathways during aging. Notably, several DDR components, involved in DNA repair and replication fork remodeling were upregulated with age in men but not women. On the other hand, functional analyses indicated reduced activity of the Fanconi Anemia pathway accompanied by increased pan-nuclear γH2AX signals in older women but not men. Analyses of replication dynamics (by DNA fiber spreading assay), proliferating cell nuclear antigen (PCNA) ubiquitination, translesion synthesis (TLS)-polymerase signals and sensitivities to TLS-polymerase inhibitors suggest a shift from fork remodeling to faster TLS inducing non-classical replication stress in older women. While replication dynamics were unaltered and replication stress rather reduced, PBL from older men exhibited a strong dependency on elevated poly (ADP-ribose) polymerase (PARP) activity. In conclusion, our findings revealed sex-specific strategies to cope with replication stress in PBL from older individuals, namely through DNA damage tolerance pathway switching in women and activation of PARP in men, differentially contributing to the increase of genomic instability with age. Our work provides insight into the mechanisms underlying sex- and age-specific decline of genomic stability paving the way for the development of measures protecting genome stability during life enabling healthy aging. Moreover, our findings have implications for cancer therapy of older patients involving PARP inhibitors or TLS inhibitors the latter of which emerge as promising drug candidates to delay treatment resistance.

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Hematopoietic stem cell size heterogeneity is not linked to changes in stem cell potential of aged HSCs

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Aging is associated with a decline in the function of hematopoietic stem cells (HSCs). This decline in HSC function results in reduced hematologic regenerative capacity and an increased incidence of hematologic disorders. In general, aged HSCs show on average an increase in cell size and a lower frequency of cells polar for protein polarity markers. The size of an HSCs has been proposed to be tightly linked to the potential of the HSCs, with small HSCs showing a higher potential compared to large HSCs. The increase in size of HSCs upon aging may be associated with the reduced potential of aged HSCs. HSCs are located within the bone marrow (BM) in distinct microenvironments called niches. These niches provide critical physical and molecular signals that are essential for HSC self-renewal, proliferation, migration and differentiation. There are multiple types of functional niches, and HSCs within these distinct types of niches show a distinct type of potential. Furthermore, the distribution of HSCs relative to niches changes upon aging. It is not known whether there is a correlation of HSCs size, HSCs polarity and the location of HSCs in distinct types of niches, as might be expected, as all three (size, polarity and position) have been linked to HSC potential. Here we show that in young mice smaller HSCs, which are more myeloid-biased, are preferentially located at central BM niches, including sinusoids and megakaryocytes. In contrast, larger HSCs, which show a bias toward B-lymphoid differentiation, are preferentially located in endosteal BM niches close to arterioles. However, in aged mice, which also contain HSCs of different sizes, there was no correlation between HSC size and localization and potential. Furthermore, within the hematopoietic stem and progenitor cell (HSPC) population, cell size increases as the cells become more limited in their capacity. Notably, we further report that changes in the level of polarity correlate with HSC potential even in aged mice.

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Polyamines sustain epithelial regeneration in aged intestines by modulating protein homeostasis

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Aging hampers the regenerative potential of intestinal epithelium across species including humans, yet the underlying causes remain elusive. Here, using proteomic and metabolomic profiling of intestinal tissues together with functional assays, we characterized the temporal dynamics of regeneration following injury induced by 5-fluorouracil, a commonly used chemotherapeutic agent. Comparison of regeneration dynamics in mice of different ages revealed the emergence of a proteostasis stress signature and increased levels of polyamines following injury exclusively in old epithelia. Mechanistically, we show that delayed regeneration is an intrinsic feature of aged epithelial cells that display reduced protein synthesis and accumulation of ubiquitylated proteins. Notably, dietary restriction followed by re-feeding prior to injury increases polyamine pathway activation, enhances protein synthesis, and restores the regenerative capacity of aged intestines. Our findings highlight promising epithelial targets for interventions aimed at tackling the decline in tissue repair mechanisms associated with aging.

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TET2 CLONAL HEMATOPOIESIS AS A MECHANISTIC DRIVER OF DIET-INDUCED LIVER CANCER

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Throughout life, somatic mutations accumulate in the hematopoietic system, which can lead to clonal expansion in hematopoietic stem cells and their progeny—a phenomenon known as clonal hematopoiesis (CH). CH is commonly observed in the elderly and is implicated in various age-related diseases, most likely through its association with enhanced inflammatory responses. However, despite inflammation being a well-established driver of solid tumor progression, there is a limited understanding of the link between CH and solid cancer development. Diet-induced hepatocellular carcinoma (HCC) is among the fastest-growing cancers worldwide. The progression from non-alcoholic fatty liver disease (NAFLD) to HCC is closely linked to chronic inflammation, but only a minority of NAFLD patients progress to HCC in their lifetime. Analysis of publicly accessible cancer patient datasets revealed an overrepresentation of CH with mutations in the TET2 gene within HCC patients compared to age-matched healthy individuals. To formally address if there was a cause-effect relationship between TET2 mutant CH and HCC, we generated a *Tet2* CH mouse model and combined it with administration of a high-fat, high sugar, high cholesterol “Western diet” (WD), which promotes evolution of NAFLD. We found that *Tet2* CH significantly increased the kinetics and penetrance of progression from NAFLD to HCC in both male and female mice. Notably, this effect was especially pronounced in females, who are highly resistant to developing HCC in the absence of *Tet2* CH, likely due to protective mechanisms such as hormonal factors. However, in the presence of *Tet2* CH, female mice exhibited an HCC risk nearly equal to that of males, suggesting that *Tet2* CH might override these protective mechanisms. These findings demonstrate that *Tet2* CH exacerbates NAFLD progression to HCC, through an as yet unknown mechanism involving interaction of mutant mature blood cells with the auto destructive inflammatory environment in the fatty liver to promote carcinogenesis. We would also suggest that TET2 CH may act a potential biomarker for early identification of high-risk individuals, particularly in the setting of female patients with NAFLD.

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Notch/NF-kB crosstalk signaling critically affects SASP gene expression in brain aging

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Brain aging is accompanied by chronic, low-grade inflammation, termed as inflammaging. In the CNS, this process is driven by senescent glial cells that secrete pro-inflammatory factors known as the senescence-associated secretory phenotype (SASP). The NF-κB pathway is a key regulator of SASP and neuroinflammation, while the Notch pathway contributes to glial cell homeostasis and immune modulation. Notch and NF-κB signaling are able to interact at multiple levels to regulate gene expression. However, the precise molecular mechanisms underlying the Notch/NF-κB crosstalk especially in the brain remain incompletely understood. Here, we established different mouse models to monitor and modulate Notch and NF-κB signaling in glia cells, revealing that brain aging is associated with NF-κB activation and Notch suppression (FACS, qRT-PCR, RNAseq). Interestingly, astrocytic NF-κB activation in young mice (3 month) is able to initiate a premature SASP gene signature in astrocytes and microglia cells of 5-month-old animals, while expression of Notch target genes gets downregulated in parallel (RNAseq). Importantly, we identified specific overlapping DNA motifs named crosstalk-(CT)-sites in the majority of SASP genes by comprehensive bioinformatic screening. These CT-sites are conserved between mice and men and occupied by Notch/RBPJ and NF-κB transcription factors *in vivo*. Multiple reporter gene assays demonstrated that such CT sites mediate combinatorial transcriptional responses to Notch and NF-κB signaling at which NF-κB driven activation preferentially gets suppressed in the presence of Notch signaling. Furthermore, we determined a physical interaction between NICD (Notch intracellular domain) and components of the NF-κB pathway (RelA, cRel, IKK1, IKK2) both *in vitro* and *in vivo*. In cell culture eperiments, we were also able to demonstrate their co-localization in the nucleus, indicating a previously undescribed to poorly described direct mechanistic interaction between Notch and NF-κB pathway components.
These findings provide new insights into the transcriptional integration of Notch and NF-κB signaling in regulating SASP genes during brain aging. Overall, our data implies that aging-dependent SASP gene expression in glia cells can be counteracted by Notch signaling. Ongoing work will validate these regulatory relationships in human postmortem brain tissue and primary mouse glia cells. Also, the distinction of beneficial (‚eat me') versus detrimental (paracrine expansion of senescence), glia-derived SASP factors in the brain will be addressed.

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DETRIMENTAL INTERPLAY BETWEEN HIV-1 AND AMYLOID FIBRILS ASSOCIATED WITH NEURODEGENERATIVE DISEASES

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HIV-1 accelerates aging-associated diseases, and infected individuals may develop HIV-associated neurocognitive disorders (HAND) even under effective antiretroviral therapy (ART). In this study, we analyzed the interplay between HIV-1 and brain amyloids, which are known to be associated with neurological disorders such as Parkinson's and Alzheimer's disease. We found that α-synuclein and (to a lesser extent) Aβ fibrils, significantly enhance HIV-1 entry and replication in human macrophages and microglia, the major viral target cells in the brain (Olari, Liu et al., Nat. Comm. 2025). Mechanistically, these amyloid fibrils facilitate viral attachment and fusion, likely by bridging viral and cellular membranes despite their overall negative charge. Furthermore, we demonstrate that amyloidogenic fragments of the HIV-1 Envelope protein can cross-seed and accelerate the formation of α-synuclein and Aβ fibrils. Extracts from human brains enhanced HIV-1 infection, and notably, the magnitude of the effect correlated with the levels of binding Thioflavin T, a dye commonly used to stain amyloid fibrils. Our results suggest that HIV-1 infection may exacerbate age-related microglial senescence through amyloid-driven enhancement of viral replication. Currently, we are investigating potential synergies between HIV-1 and brain amyloids in inducing senescence pathways and inflammatory responses. Our preliminary data show that HIV-1 replicates in cerebral organoids containing innately developed microglia and triggers an immune response that may drive accelerated aging. Moreover, exogenous α-synuclein and Aβ fibrils seem to enhance HIV-1 infection in this organoid model. Understanding the interplay between HIV-1 and brain amyloids may open new avenues for anti-amyloid or senolytic therapies as adjuvants to ART for treating HIV-associated neurodegenerative disorders.

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Biological Age Estimation based on blood biomarkers – the ActiFE study

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Background: Biological age (BA) estimation based on blood-based biomarkers can be time-efficient. Due to blood-based biomarkers changes observed with ageing, it does not seem appropriate to estimate and validate BA for older adults in younger populations. Therefore, we aim to estimate BA using blood-based biomarkers in a cohort of community-dwelling older adults over 65 years old evaluating BA performance on all-cause mortality compared to chronological age (CA).
Method: We used data from 1506 participants of the ActiFE study, excluding those with a history of cancer (n=320, 21.3%). BA was estimated with the Klemera-Doubal method from a selection of 35 blood-based biomarkers linked to aging in the literature. Sex-specific blood-based biomarkers panels as well as a common panel (biomarkers are the same across sex), were used for BA estimation. BA was evaluated on 10-year all-cause mortality against CA using integrated brior scores (iBS) and area under the curve (iAUC). A secondary analysis explored if the addition of functional measurements (i.e., grip strength, chair rise test, daily steps) or clinical parameters (i.e., systolic blood pressure (BP) and pulse pressure (systolic minus diastolic BP)) improved BA estimation.
Results: BA estimation in the dataset with complete cases (median age 74.2 years (Q1 70.1, Q2 81.45), 533 (44.9%) women, 653 (55.1%) men) resulted in sex-specific models with leucocytes, iron, cystatin C, albumin, dehydroepiandrosterone (DHEA), sex hormone-binding globulin (SHBG), N-terminal prohormone of brain natriuretic peptide (NT-proBNP), growth differentiation Factor-15 (GDF15) for females (**Model 1f**) and hemoglobin, transferrin, cystatin C, albumin, alanine aminotransferase (ALT), parathyroid hormone (PTH), interleukin 6 (IL-6), DHEA, SHBG, NT-proBNP, GDF15 for males (**Model 1m**). The overall model included relevant biomarkers in both sexes: cystatin C, albumin, DHEA, SHBG, NT-proBNP, and GDF15 (**Model 2**). In the sex-specific approach, the overall Model 2 suggests better performance for females (**Model 2f** $iAUC_{women}$ 0.850, iBS_{women} 0.035; vs. **Model 1f** $iAUC_{women}$ 0.847, iBS_{women} 0.042) and males (**Model 2m** $iAUC_{men}$ 0.780; iBS_{men} 0.075 vs. **Model 1m** $iAUC_{men}$ 0.782; iBS_{men} 0.080). When using Model 2, an overall approach with a BA estimation trained on the whole dataset led to similar results compared to a sex-specific approach in women (**Model 2** $iAUC_{women}$ 0.845, iBS_{women} 0.044 vs. **Model 2f** $iAUC_{women}$ 0.850, iBS_{women} 0.035) and men (**Model 2** $iAUC_{men}$ 0.786; iBS_{men} 0.080 vs. **Model 2m** $iAUC_{men}$ 0.780; iBS_{men} 0.075). For CA following metrics were obtained in women: CA $iAUC_{women}$ 0.785; iBS_{women} 0.043 and in men: CA $iAUC_{men}$ 0.748; iBS_{men} 0.083. Adding functional or clinical markers to the overall model did not improve model performance.
Conclusion: Our results show a promising time-efficient way of estimating BA in older adults. Although blood biomarkers have sex-specific variations in their distribution and association with CA, BA estimation can be performed using a model containing shared biomarkers trained in the whole population. Overall, BA outperformed CA in all-cause mortality analysis, with better discriminatory properties in women compared to men. Adding functional and clinical parameters did not lead to further model improvement.

Aging Restricts the Function of Human Colonic Stem Cells

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The human colon is an organ particularly affected by age-related diseases. Despite the obvious relationship between aging and age-related diseases, our knowledge of the parameters and mechanisms of aging of the human colon is limited. In a tissue with high cell turnover, such as the colon, tissue homeostasis is ensured by epithelial stem cells (ESCs). The function of ESCs can be assessed in an in vitro colon-organoid model (colonoids).

We analyzed primary human colonoid cultures from old (older than 75 years) and young (younger than 30 years) donors. Organoid proliferation was quantified over 72 h by the integrated organoid area (“biomass”) in bright-field images. Colonoids derived from young donors accumulated significantly more biomass than those from aged donors over the entire observation periods (significant differences at 48 h ($p = 9.3 \times 10^{-4}$) and 72 h ($p = 1.8 \times 10^{-4}$)). By day 3, mean biomass in the young group was about two-fold higher than in the old group (10.73 ± 0.92 M px² vs. 5.01 ± 1.02 M px²; mean ± SEM).

To investigate likely mechanisms by which donor age affects the long-term expansion capacity of colonic stem cells, colonoids from both cohorts (young/aged) were serially passaged until they could no longer re-establish growth (defined as < 5 viable organoids per well, 14 days after splitting). Colonoids from young donors displayed a markedly prolonged replicative lifespan, with a median of 19 passages (95 % CI 17–20) compared with 9 passages for aged colonoids (95 % CI 8–16, Kaplan-Meier statistics). Survival curves diverged early and remained significantly separated (log-rank $\chi^2 = 8.4$, $p = 0.0037$; hazard ratio = 0.58, 95 % CI 0.23–1.46), indicating that cultures derived from young tissue survived in culture for roughly twice as many passages before reaching exhaustion.

We next determined the extent of expansion of colonoid cultures under varying concentrations of activators of canonical Wnt or EGFR signaling to test the role of these well-known colonic signaling cascades for restricting growth in aged colonoid cultures. Interestingly, the magnitude of the differences between the young and aged organoids was not significantly affected by modulation of these pathways, suggesting that mechanisms of aging of human colonoids are not primarily affected by Wnt or EGFR signaling.

In summary, aged human colonic ESCs show reduced function. The human colonoid model further provides a basis for testing age-modifying interventions and for more in-depth exploration of the mechanisms underlying ageing of colonic ESCs.

SASP-Panel as a biomarker for ageing: GDF-15 identified as key driver of age-related disease

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The accumulation of senescent cells promotes organ dysfunction and chronic diseases. Senescent cells secrete pro- and anti-inflammatory factors, collectively referred to as the senescence-associated secretory phenotype (SASP), which can influence the tissue microenvironment.
Previous studies identified a robust SASP core proteome, suggesting that circulating SASP components may serve as biomarkers of biological ageing and predictors of age-related diseases.

In this study, we established a SASP panel comprising 11 proteins - CCL3, TNFRSF6, IGFBP-1, Total Inhibin, MMP-1, FABP4, GDF-15, IL-15, IL-6, Leptin, and Osteopontin - as markers for biological ageing. These biomarkers were quantified using multiplex immunoassays in the CARLA epidemiological cohort from Halle (Saale), which includes 1,779 participants aged 45 to 83 years at baseline. All analyses were cross-sectional and based solely on baseline data. The cohort provides up to 20 years of follow-up data, enabling robust longitudinal analyses of ageing and disease incidence in future studies.

Multivariate logistic regression analyses identified GDF-15 as the primary risk factor among the SASP proteins. Elevated serum levels of GDF-15 were consistently associated with a higher prevalence of stroke, hypertension, type 2 diabetes, chronic kidney disease, and heart failure. These associations were independent of chronological age and showed no significant sex differences.

Focusing on type 2 diabetes, a common age-related condition, we observed beside the strong positive association with GDF-15, an additional risk linked to FABP4, and a protective association with IGFBP-1. CCL3 and Leptin demonstrated sex-specific correlation patterns with diabetes. Notably, sex-specific differences were not limited to diabetes but were also evident in the associations with stroke, hypertension, kidney disease, and heart failure.

Our findings identify GDF-15 as a central biomarker associated with multiple age-related diseases, independent of chronological age and sex. Moreover, the observed sex-specific associations of other SASP components — particularly in the context of diabetes — highlight the complexity of ageing biology. These results support previous findings on the role of GDF-15 in ageing and age-related pathophysiology. Future work will include longitudinal analyses to investigate temporal relationships and improve disease prediction models.

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ABCB5^{+}MSCs From Old Adults Fail to Elicit Multilayered Microbicidal Functions to Control Gram Negative Bacteria

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Previously, we showed that skin-derived ABCB5^{+}mesenchymal stem cells (MSCs) upon exposure to infection mimicking lipopolysaccharide (LPS) fundamentally shift their transcriptome with high expression and the release of neutrophil activating chemokines. This adaptive response also resulted in a significant increase in neutrophil expulsed DNA traps (NETs) and proteolytic enzymes which guarantees the defense from bacterial attack. With age the propensity for severe skin infections dramatically increases. We here set out to address the question whether MSCs from old healthy donors (> 60 years) unlike young healthy donors (< 30 years) may change their adaptive response upon LPS exposure towards a reduced microbicidal response. We found that co-cultures of LPS primed MSCs from old donors with activated neutrophils revealed a significant reduction in NET formation, phagocytosis of FITC labelled *E.coli* and a severely reduced killing ability (3 log phases) of either *E. coli*, *P. aeruginosa* or *Staph. aureus* when compared to young MSCs. To explore the underlying mechanisms, we subjected young and old donor MSCs non-primed or LPS primed MSCs to bulk RNA seq analysis. Enrichment analysis showed that NF-κB and Wnt signaling, among others, are highly upregulated, while innate immune signaling and genes encoding antimicrobial peptides like CAMP (cathelicidin/LL-37) were suppressed in LPS primed old MSCs. In fact, NF kappa B activation as depicted by Western blots and immunostaining with enhanced phosphorylation and nuclear translocation of p65 occurred in LPS primed old MSCs vs lesser NF-κB activation in LPS primed young MSCs. IL-6, a major target gene of NF-κB, was significantly induced with a prolonged high IL-6 release from old MSCs compared to young MSCs. By contrast to the high release of cathelicidin from young MSCs, very low cathelicidin concentrations were released from old MSCs. As IL-6 suppresses cathelicidin, we studied whether silencing of the CAMP/cathelicidin gene in young MSCs may affect the microbicidal neutrophil functions in co-culture. In fact, NET formation was significantly reduced in CAMP/cathelicidin silenced young MSCs compared to non-silenced or scramble RNA treated young MSCs. The degree of cathelicidin inhibition by CAMP silencing in LPS primed young MSCs now reflects the low cathelicidin concentrations occurring in LPS primed old MSCs. Of note, cathelicidin degradation in supernatants from LPS primed young MSCs co-cultured with neutrophils abrogates the strong microbicidal effects. We furthermore employed full thickness skin samples with standardized epidermal and dermal wounds (Genoskin®) which were infected with a standardized load of *E. coli* in the absence and presence of supernatants from MSCs from old adults and young adults co-cultured with neutrophils. Our preliminary data show a reduced transcriptomic antibacterial defense gene signature in *E.coli* infected human skin wounds when exposed to supernatants from old adults and neutrophils as opposed to a much stronger skin response of supernatants from young MSCs and neutrophil co-cultures. These data confirm our *in vitro* data in human skin *in situ*. Collectively, we here uncovered a previously unreported, dysregulated anti-bacterial adaptive response in LPS-primed MSCs from old individuals with an impressively reduced killing ability of gram-negative bacteria. This is likely clinically relevant and highlight that aging dysregulate the adaptive response of MSCs. This may contribute to the higher susceptibility for severe local and systemic infections in elderly.

Preliminary Establishment of Hippocampal and Hypothalamic Neurospheres Derived from Mice: First Steps Toward Functional Investigation and Cryopreservation.

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Introduction: Brain aging is a complex process that contributes to the onset of neurodegenerative and metabolic conditions. The hippocampus, important for memory and learning, and the hypothalamus, involved in metabolic and neuroendocrine regulation, are particularly vulnerable to age-related dysfunctions. Changes in these regions are associated with diseases like Alzheimer's Disease (AD) and Type 2 Diabetes Mellitus (T2DM). Neurospheres derived from these regions provide a 3D in vitro model to explore brain aging and test therapeutic strategies, although their ability to reflect age-related changes is not fully established. **Aims:** This study aims to establish hippocampal and hypothalamic neurospheres from mice and evaluate their potential as models for studying the functions of these cells, as well as the effectiveness of cryopreservation. **Methodology:** Tissue samples from the hippocampus and hypothalamus of 5xFAD mice were enzymatically digested using TrypLE, followed by mechanical dissociation and filtration through 40 µm cell strainers. A total of 200,000 cells were seeded in each well of a 24-well plate and cultivated for 7 days. Subsequently, neurospheres were cryopreserved using two different protocols: (1) 10% DMSO + 20% FBS + 70% neurosphere medium and (2) 10% DMSO + 90% FBS. After one month, neurospheres were thawed and cultured for an additional 7 days. Bright-field images were acquired throughout the entire process to monitor growth. **Results:** Images recorded between days 0 and 7 confirmed the formation of neurospheres from both brain regions after the cryopreservation process using both tested protocols. However, images of the neurospheres cryopreserved with 10% DMSO and 90% FBS showed preserved morphology, growth, cellular integrity and formation of new neurospheres, compared to those derived from cryopreservation with 10% DMSO + 20% FBS + 70% medium. **Study Implications:** Although additional analyses are currently underway to assess the model's efficiency, the results obtained so far are promising, given the limited data available in the literature on neurospheres, particularly from the hippocampal region. While this model does not directly represent the aging process, it provides a relevant starting point for studying the hippocampus and hypothalamus — regions that undergo significant changes with aging. The successful cryopreservation of neurospheres is an important advantage, as it allows for long-term storage and the creation of a larger cell bank for experimentation. This reduces the need for a large number of animals, facilitating long-term studies. **Conclusion:** Consequently, this model holds potential for pharmacological testing, biomarker identification, and the investigation of mechanisms related to cognitive decline and metabolic dysfunction in aging.

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Enteric neuron transcriptomics in accelerated and diseased aging

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Abstract

Aging leads to structural and functional alterations in the enteric nervous system (ENS), but detailed insights into its molecular signatures remain largely undiscovered. Therefore, this study aims to unravel how aging affects the transcriptomic profile of the ENS – specifically enteric neurons – in both accelerated aging and the diseased aging associated with Alzheimer's disease (AD).

For that purpose, colonic longitudinal muscle/myenteric plexus (LMMP) specimens were derived from two animal settings: 1) 3- and 10-month-old senescence-accelerated mouse model (SAMP8) and its control (SAMR1 mice resistant to aging); 2) 1-, 3-, and 8-month-old Alzheimer mouse model (5xFAD) and wild-type (WT) littermates. Specimen were collected and analyzed using bulk RNA sequencing. Due to the cellular complexity of LMMP tissue, an RNA deconvolution approach using the BayesPrism algorithm was applied to selectively analyze the transcriptomic profile of enteric neurons on the background of contaminating of other cell types.

An increase in the neuronal cell fractions was – astonishingly - observed in female 10-month-old SAMP8 mice compared to SAM1 mice and with age in both WT and 5xFAD mice. Moreover, transcriptomic analysis of enteric neurons revealed that the SAMP8 strain exhibited a high number of differentially expressed genes (DEGs) – over 160 per comparison – altered across both age and sex, relative to SAMR1. These genes are broadly associated with aging phenotypes and showed sex-specific enrichment: upregulated genes in males were linked to protein folding and processing in endoplasmic reticulum, while in females they were related to lipid metabolism. Downregulated genes were mainly associated with synaptic functions in both sexes, including several solute carrier (SLC) family members. Additionally, many DEGs in SAMP8 vs. SAMR1 comparisons were associated with increased neurological disease risk, especially AD, e.g., *App*, *Fkbp4*, and *Prkn*. Transcriptomic analysis of enteric neurons in 5xFAD mice compared to WT, however, showed only few DEGs in males but a high number in 3-month-old females. Specifically, the number of DEGs in females increased from 21 at 1 month of age to 309 at 3 months (shortly after disease onset). This dropped to just three by 8 months, when pathology becomes more evident. The DEG dataset from 3-month-old females revealed genes linked to abnormal neuronal function (including SLC members), with 41% overlapping with known AD risk genes derived from brain and blood datasets, e.g., *Mapt* and *Ttbk1*.

Our study shows that aging might affect enteric neurons differently when investigating healthy and diseased status regarding sex-difference. Enteric neurons in the SAMP8 mouse model when comparing with its control SAMR1 exhibit aging phenotypes and elevated neurological diseases-related risk, especially AD, while those of 5xFAD recapitulate to a certain extent AD pathology but not accelerated aging. Future studies will be conducted to validate the findings as well as to investigate the transcriptomic profile of the other major component of the ENS, glial cells, which have already been separated by the convolution strategy.

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Characterization of two transgenic mouse lines harbouring rare protein-altering variants in a gene involved in insulin/insulin-like growth factor-1 signalling identified in long-lived individuals.

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Healthspan and lifespan are primarily influenced by lifestyle, yet the genetic background stipulates an individual's potential to become long-lived. The heritable component of longevity is strongest in families that contain exceptionally long-lived individuals in multiple generations. Interestingly, longevity is often accompanied by an escape from or, at least, a compression of late-life morbidity. Understanding the underlying mechanisms encoded in the genome of long-lived individuals could thus elucidate general aspects of living a long and healthy life. Multiple studies have shown that the heritable component of longevity cannot be explained by the presence of common protective genetic variants or the absence of disease-causing variants. Hence, long-lived individuals may thus harbour (multiple) rare variants that assert a protective effect.

Therefore, we have focused on studying the functional effects of rare genetic variants identified in long-lived family members from the Leiden Longevity Study. We identified two promising genetic variants in a gene that plays an important role in the insulin/insulin-like growth factor-1 signalling (IIS) pathway. We subsequently generated transgenic mouse lines for these variants using CRISPR-Cas9 and assessed their effects on lifespan, general health, and metabolic parameters by comparing the transgenic homozygous (HOZ) and heterozygous (HEZ) mice with their wild-type littermates.

We have completed the lifespan and phenotypic assessment for the first mouse line and observed a significant effect of the genetic variants on some of the evaluated parameters. In young female HOZ mice, weight is lower, and motor coordination is improved. Both male and female HOZ mice display a slower increase in frailty with age. Additionally, Insulin resistance is increased in middle-aged male HEZ mice, and the breeding of HEZ mice resulted in a significant increase in the number of pups born per litter.

The lifespan assessment of the second mouse line is still ongoing, but the preliminary analysis of the phenotypic assessment showed a significant decrease in grip strength in male HEZ mice at a young age and HOZ mice at middle age. Additionally, the body weight of HOZ male mice was significantly lower at middle age and remained lower at old age.

Preliminary assessment of the tissues of the two transgenic mouse lines suggests that tissue-specific phenotypes and protein levels are differentially affected in a genotype, age and sex-dependent manner.

In conclusion, we created two transgenic mouse lines harbouring rare genetic variants in a gene involved in IIS identified in long-lived individuals. The preliminary data of these mouse lines support a favourable effect of at least one of these variants on general health.

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Standard-of-Care vs. Expert-Recommended Discharge Destinations for Geriatric Surgical Inpatients: A Prospective Observational Cohort Study

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Introduction: Discharge planning is important to ensure optimal postoperative outcomes for older surgical inpatients. As part of the Supporting SURgery with GEriatric co-management and AI (SURGE-Ahead) project, this study investigates how congruence between standard of care discharge decisions and geriatric expert recommendations affects functional outcomes in older surgical inpatients.

Methods: A prospective observational cohort study was conducted across three surgical departments at Ulm University Medical Center (Trauma, Visceral, Urology). Patients aged 70 years or older with an Identification of Seniors at Risk score ≥ 2 were enrolled. The congruence between the standard of care discharge decisions (actual discharge destination) and recommendations made by expert geriatricians (unknown to clinicians) was determined across four discharge options: home, acute geriatric care unit, rehabilitation facility or nursing home. Multivariable logistic regression was employed to examine how the match (congruence) between recommended and actual discharge destinations related to functional outcomes and readmission rates post-discharge.

Results: Among the 169 enrolled participants, a discrepancy of 27% was observed between the standard of care and expert recommendations. Patients with discharge decisions incongruent to geriatric expert recommendations showed higher frailty scores, more dependence in activities of daily living, and reduced mobility pre-operatively. Mismatch between expert recommendations and standard of care was associated with decline in Barthel Index and Charité Mobility Index scores, and higher 3-month readmission rates.

Conclusion: Optimizing discharge destinations may prevent functional decline and reduce readmissions. Closing the gap in discharge decisions that are incongruent with geriatric expert recommendations could enhance functional outcomes for older surgical patients.

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Role of B cells in chronic inflammation and their implication in Inflammaging:

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Aging is associated with inflammaging, a chronic, low-grade inflammatory state that compromises immune function and contributes to the development of age-related diseases. Inflammaging is attributed to dysregulation of the immune system with age and an exaggerated response to minor triggers, leading to persistent inflammation over extended periods. Additionally, long-term exposure to stress, harmful chemicals, and hazardous radiation contributes to chronic inflammation, further burdening the immune system. Moreover, old and damaged cells that are not promptly eliminated can secrete pro-inflammatory cytokines hence contributing to chronic inflammation. So far, macrophages and T cells have been identified as the major contributors to inflammaging. Among B cells, age-associated B cells (ABCs) have been shown to secrete inflammatory cytokines, thereby contributing to inflammaging, though to a lesser extent. However, whether other B-cell subpopulations also contribute to chronic inflammation and inflammaging remains unclear.

In this study, we analyzed peripheral blood mononuclear cells (PBMCs) from young and aged donors to investigate age-related changes in different B-cell populations and their role in inflammaging. Using magnetic-activated cell sorting (MACS), we observed that among various B-cell subpopulations, memory B cells from aged donors were the main contributors to cytokine secretion, exhibiting elevated levels of TNF-α and IL-6 compared to those from young donors. Flow cytometric analysis further dissected the expression patterns of these inflammatory cytokines, revealing that unswitched IgM^+ memory B cells predominantly expressed IL-6, while switched IgG^+ memory B cells expressed TNF-α. Additionally, flow cytometry analysis revealed an age-related decline in naïve B cells, accompanied by a relative increase in memory B cells. Cytokine expression also correlated with the senescence marker p16, indicating a potential link between the memory B-cell phenotype, cytokine secretion, and cellular aging.

These findings highlight the contribution of B cells to the elevated cytokine levels observed during aging, suggesting their role in inflammaging and pointing toward potential new therapeutic targets to address the underlying causes of this age-related disorder.

Keywords: Inflammaging, Chronic inflammation, B cells.

Abbreviations: TNF-α: Tumor necrosis factor-α, IL-6: interleukin-6.

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Barrier to Aging: Enhancing Intestinal Barrier Function to Prevent Biological Decline.

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Systemic inflammation is a hallmark of aging, yet its main trigger—the breakdown of the intestinal barrier—remains underexplored. We observed that human centenarians have unique gut microbiomes, and a lower incidence of leaky gut compared to the general population, suggesting that microbiome factors may help preserve intestinal integrity in long-lived individuals. To identify these factors, we conducted an in-silico analysis of centenarian microbiomes from Italy, China, and Japan, seeking consistently enriched microbial species. The in-silico study was coupled with survival, molecular, and functional screens in *C. elegans* and human cells, testing individual strains. This led to the identification of two non-pathogenic *Pseudomonas* strains and a metabolite they produce, SEAL1, which improve intestinal integrity by over 100%, preventing functional decline and extending lifespan. SEAL1 modulates the conserved Wnt-GATA axis to enhance barrier function and is structurally identical to an existing drug approved for another indication in some European countries. Based on these findings, we are preparing a clinical study to evaluate the efficacy of SEAL1 in preventing intestinal leakage in humans.

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The Three-Body Problem: Host, Microbiome, and Diet interactions in regulating proteostasis and longevity

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Abstract

The dynamic interaction between the microbiome, diet, and host proteostasis plays a pivotal role in regulating health-span and longevity. While proteostasis—largely governed by the ubiquitin-proteasome system (UPS)—is widely recognized as a hallmark of healthy aging, the contribution of microbiota-derived signals in modulating this system remains incompletely understood. In this study, we utilize *Caenorhabditis elegans* as a model organism to investigate how microbial diet influences host proteostasis and lifespan. *Escherichia coli*, serving as both a nutritional source and simplified microbiota for *C. elegans*, provides a tractable platform to dissect host–microbe–diet interactions. We compared the effects of two widely used *E. coli* laboratory strains, OP50 (B derivative) and HT115 (K-12 derivative), on UPS activity and longevity in the worm. Strikingly, *C. elegans* fed HT115 exhibited a significant extension in lifespan despite showing reduced UPS activity, in contrast to those fed OP50. These results challenge the conventional view that improved proteostatic capacity uniformly promotes longevity and highlights the complexity of host–microbiome–diet interaction. Furthermore, our metabolomic analysis revealed that the OP50 strain is enriched in branched-chain amino acids (BCAAs), leading us to hypothesize that BCAA metabolism may play a critical role in modulating UPS function. Together, our results underscore the complexity of diet–microbiome–host interactions and suggest that longevity can be uncoupled from classical proteostasis pathways under specific microbial dietary conditions.

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The assessment of histones single amino acid exchanges *in silico* to mimic aging-related glycation sites for *in vitro* and *in vivo* studies

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Introduction: Glycation as a non-enzymatic reaction produces posttranslational modifications (PTMs) of amino acids like Carboxymethyl lysine (CML) or Methylglyoxal-derived hydroimidazolone (MG-H1) in case of lysine or arginine. In histones, several CML and MGH1 adducts were identified in aging cell models and human heart tissues. To understand the effects of single glycation sites, an amino acid exchange that mimics the glycated residue is proposed using Glutamine(Q) to mimic CML and Tyrosine(Y) to mimic MGH1. The study aims to evaluate (*in silico)* Glutamine and Tyrosine amino acid exchange as a model to resemble CML and MGH1 glycation sites.
Methods: Analysis of the histone glycation sites [H2B(K/CML/Q)43, H4(K/CML/Q)31, and H3(R/MGH/Y)42] was performed using the nucleosome core particle (NCP) 5AV6 from RCSB Protein Data Bank. CML and MG-H1 adducts were built using PyMOL. Forcefield parameters were generated using PyRED. Three molecular dynamics simulations for each system for 100 nanoseconds were performed by the Amber Molecular Dynamics Package using Amber ff14SB forcefield supplemented with Parmbsc1 for DNA and TIP3P water model with DNA ends fraying restrained during the simulation. Separate 1µs simulations were performed for NCPs with all glycation sites, all mutation sites or the original sites. For short simulations. Root Mean Square Deviation (RMSD) was measured. hydrogen bonds, salt bridges, DNA & protein contact fraction scores within 5.5 Angstroms, Root-Mean-Square Fluctuations (RMSF) and residue surface area were evaluated for each glycation site. For long simulations, RMSD was calculated, protein and DNA contact scores within 5.5 Angstroms of residues of each glycation site were calculated. Hydrogen bonds and salt bridges between the double stranded DNA (dsDNA) and the histone octamer were assessed. Average RMSF for the dsDNA residues was calculated.
Results: Glutamine and TYR residues had a similar trend as CML and MGH adducts in the change of Hydrogen bonds fraction, total protein contact, RMSF (With an exception for Histone H3 Chain A), and solvent accessible surface in comparison with lysine and Arginine residues, respectively.
MGH residue had more DNA contact while TYR had about 40-50% less contact with DNA in comparison with ARG.
RMSD values for the 1µs simulation showed big changes for the mutation NCP peaking at 313ns while being consistent for the glycation and WT NCPs.
Hydrogen bonds, salt bridges accumulative fractions and RMSF values for the double stranded DNA show Loss in the bonds for the mutation NCP at the terminal base pairs in addition to higher flexibility, especially in the proximity of Histone H3 Chain E with the Tyrosine mutation.
Conclusion: GLN seems to be a good choice to represent CML modifications at the sites H2B-43 and H4-31 for *in vitro* experiments. While TYR at H3-42 is not well-suited for mimicking MG-H1 adduct. Arginine 42 of Histone H3 seems to have an important role in DNA stability and its modification might facilitate DNA unwrapping and increase DNA availability.

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DISRUPTED HARMONY: INFLAMMATORY REMODELING OF THE BONE MARROW NICHE IN CLONAL HEMATOPOIESIS AND MYELODYSPLASIA

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Abstract: Aging impacts the bone marrow (BM) niche at multiple levels, including altered stromal cell composition, increased inflammatory signaling, and skewed differentiation of hematopoietic stem and progenitor cells (HSPCs). One striking age-associated phenomenon is clonal hematopoiesis of indeterminate potential (CHIP), in which somatic mutations in HSPCs lead to clonal expansion and an increased risk of hematologic malignancies such as myelodysplastic syndromes (MDS). While the genetic and cell-intrinsic drivers of clonal evolution are increasingly well-understood, how the aging BM microenvironment contributes to or is shaped by this process remains poorly defined.

To address this, we investigated the BM niche remodeling within 84 elderly individuals, including healthy donors, CHIP carriers, and MDS patients, using BM aspirates and bone biopsies. We analyzed the remodeling of T cell and stromal compartments in the BM niche through a multi-modal approach combining single-cell transcriptomics, mutation calling, imaging, and proteomics. These *in situ* analyses were complemented with a *Dnmt3a*-driven CHIP mouse model and *in vitro* co-culture systems.

Our data reveal a progressive inflammatory transformation of the BM niche, which is already evident at the CHIP stage. We observed a loss of adipogenic CXCL12-abundant reticular (CAR) stromal cells and the emergence of an inflammatory mesenchymal stromal cell (iMSC) population, which further expanded in MDS. These iMSCs exhibited early pro-inflammatory signatures in CHIP, and further acquired angiogenic potential in MDS while retaining residual HSPC-supportive functions. This shift correlated with increased BM microvasculature in MDS. In parallel, we identified an IFN-responsive T cell subset with cytotoxic features that was predicted to interact with iMSCs. Imaging confirmed proximity between these immune and stromal cells, suggesting active immune-stromal crosstalk that may sustain niche inflammation.

To dissect whether mutated HSPCs can actively instruct an inflammatory phenotype in the stroma, we co-cultured primary human BM stromal cells and CD34+ HSPCs from healthy, CHIP, and MDS donors. Ongoing analyses include transcriptional profiling of the MSCs and secretome analysis to uncover the molecular mechanisms of cell-cell communications between clonal HSPCs and stromal cells.
Together, our findings highlight the BM niche as an active participant, rather than a passive bystander, in age-related clonal hematopoiesis. By uncovering early inflammatory remodeling processes, our work advances the understanding of BM pathophysiology in CHIP and MDS, and paves the way for future therapeutic strategies to intercept early inflammatory remodeling in the aging hematopoietic BM niche.

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Machine Learning-Based Mining for Potential Age Markers in Human-Derived Hematopoietic Stem Cells

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Single-cell RNA sequencing (scRNA-seq) allows high-resolution transcriptomic analysis at the individual cell level, making it essential for studying cellular heterogeneity. Early studies highlighted its ability to reveal unexpected diversity in areas such as embryonic development and immune cells, helping to identify rare cell types and unique gene expression patterns. Ratliff and colleagues provided a scRNA-seq database comprising 729 samples of isolated peripheral blood long-term hematopoietic stem cells (LT-HSCs) from four young (19, 21, 37, 40 years) and four aged (61, 66, 68, 70 years) human subjects, with two females and two males per age group. The publicly available data (NCBI Gene Expression Omnibus, GSE138544) comprise log-normalized counts of 45,858 genes or gene products per sample. We utilized this data in a binary age classification scenario, combining it with a linear support vector machine (SVM) and the leave-one-subject-out cross-validation (LOSO-CV) evaluation protocol. Our analysis led to the following outcomes. First, using the entire feature space resulted in a remarkable accuracy of 91.6%. Second, 93.4 % of the misclassifications were obtained for two individuals, the youngest aged person and the oldest from the young group. Third, including semantic knowledge, i.e., focusing on genes or gene products related to ageing, did not improve the classification performance, nor did it yield results close to those obtained with the complete gene set. In fact, solely focusing on the 61 genes available (out of 62 genes in total) from the KEGG ageing pathway (longevity regulation pathway, hsa04213) led to an accuracy below 50 %, which is worse than random guessing. Fourth, by applying a systematic random selection method, we were able to identify a set of 385 genes or gene products that resulted in an error-free classification. Fifth, none of the 385 genes could be found in any KEGG or REACTOME pathways. Sixth, an additional leave-one-sample-out cross-validation evaluation showed that the complete gene set was able to further outperform the LOSO-CV accuracy of 91.6%, leading to 97.0%. In contrast, the performance of the reduced set of 385 genes decreased drastically to 63.5 % in this sample-specific (not subject-specific) setting. In summary, the identification of these 385 efficient genes or gene products highlights their potential importance for further analysis. Future research could focus on validating this gene set using diverse, independent scRNA-seq datasets to confirm its generalizability.

Foxa transcription and aging-associated limits to dietary restriction responses

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Dietary restriction (DR) has long been recognized as an effective strategy for extending health span and lifespan across various species. However, its efficacy diminishes when initiated later in life due to unknown mechanisms. We found that food supplementation with nicotinamide riboside (NR) ‐ a bioavailable precursor of nicotinamide adenine dinucleotide (NAD) - enables late-life DR (70% of overall food intake) to enhance hematopoietic stem cell function and doubles its efficacy to extend the total lifespan of already old mice by 14.3%. The combined NRDR treatment shows synergistic effects to enhance transcriptional responses to DR. Studies in C. Elegans indicate that the PHA4/Foxa transcription factor mediates transcription responses that are essential for DR-mediated lifespan elongation. The role in DR-mediated response in mammals is not clear. Here we present experimental evidence that Foxa-target genes are efficiently induced by DR in young adult mice but greatly attenuated during aging.

MAPPING THE GENETIC ARCHITECTURE OF BRAIN AGING IN KILLIFISH

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Laboratory model organisms have demonstrated that single-gene mutation can dramatically impact aging and lifespan. However, such artificially induced mutations may not reflect how aging evolves in natural populations. Studies in wild vertebrates reveal a complex genetic architecture, where lifespan is shaped by numerous naturally occurring variants with varying effects. The turquoise killifish (Nothobranchius furzeri) has provided key insights into the polygenic basis of lifespan differences across populations and species, largely driven by the accumulation of slightly deleterious germline mutations due to drift and relaxed purifying selection on late-acting genes. To uncover the genetic underpinnings of spontaneous brain aging in a natural vertebrate model, we conducted a forward-genetic study using a cross between two killifish species (N. furzeri and N. kadleci) with divergent neurodegenerative phenotypes. By integrating QTL mapping with brain transcriptomics and proteomics, we identify key genomic regions and molecular signatures associated with fast or slow brain aging. This approach reveals novel, naturally occurring variants and chromosomal changes that influence neurodegeneration, highlighting pathways distinct from those identified through traditional candidate-gene approaches. Our findings demonstrate the power of unbiased genetic approaches, such as forward genetics, in uncovering the true complexity of aging evolution. By leveraging QTL mapping and genome-wide analyses, we move beyond reductionist single-gene paradigms to identify the naturally occurring genetic variation that shapes lifespan and aging in wild populations— offering a more realistic and comprehensive view of aging biology.