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Scientific News
2nd of March 2024
Sven Bulterijs

Business/Conferences/ General news

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New Journal club with me and others!

March 21st at 16:30 CET

Prophylactic and long-lasting efficacy of senolytic CAR T cells against age-related metabolic dysfunction

Corina Amor, Inés Fernández-Maestre, Saria Chowdhury, Yu-Jui Ho, Sandeep Nadella, Courtenay Graham, Sebastian E. Carrasco, Emmanuella Nnuji-John, Judith Feucht, Clemens Hinterleitner, Valentin J. A. Barthet, Jacob A. Boyer, Riccardo Mezzadra, Matthew G. Wereski, David A. Tuveson, Ross L. Levine, Lee W. Jones, Michel Sadelain & Scott W. Lowe

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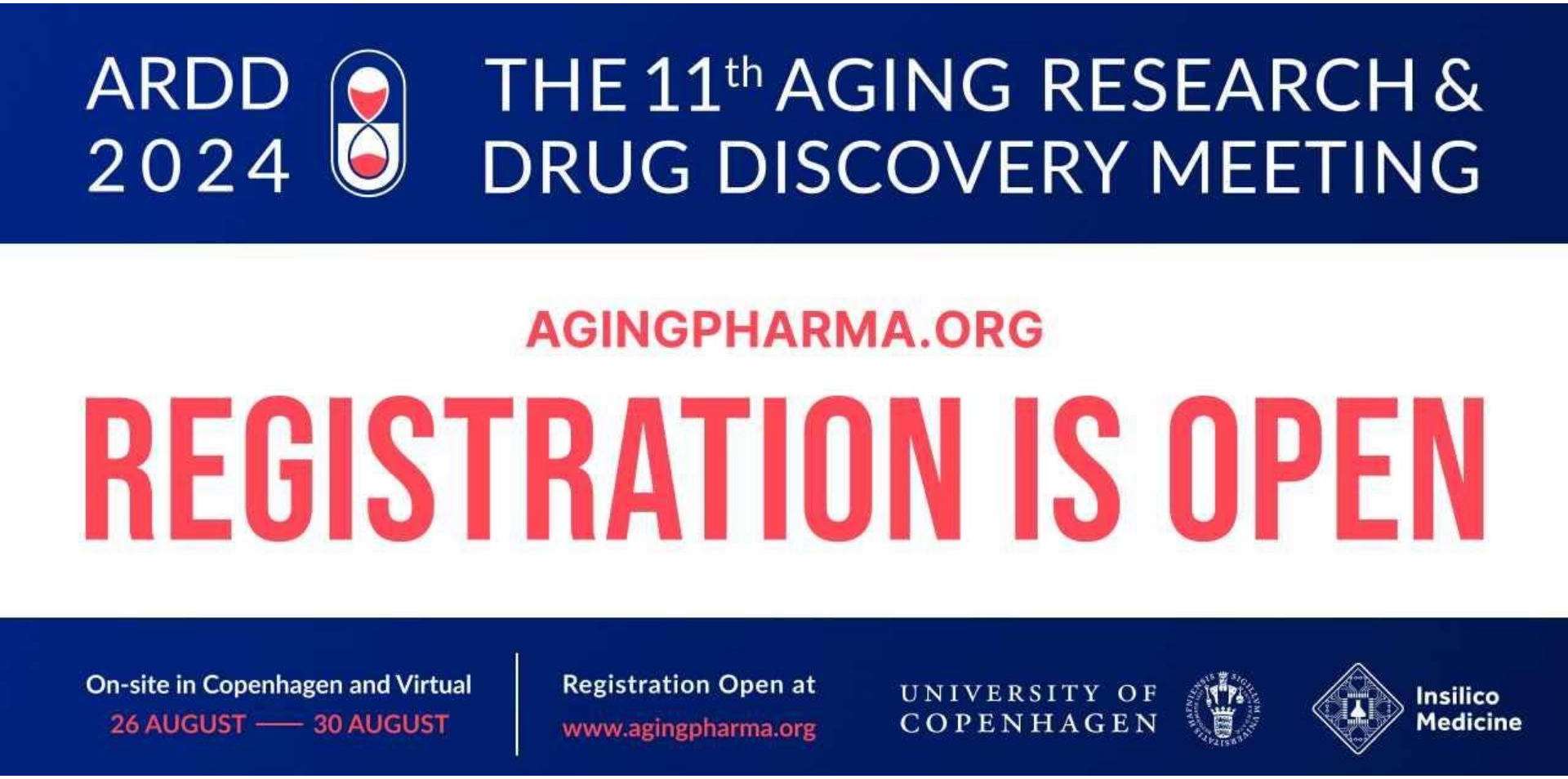

Aging research articles

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Press release

Rejuvenate Biomed's clinical trial demonstrates the therapeutic potential of RJx-01 in sarcopenia

Cutting-edge therapy shows meaningful improvements in muscle strength, function and fatigue resistance, offering hope for millions suffering from sarcopenia

Rapamycin rescues loss-of-function in blood-brain barrier-interacting regulatory T cells

Paulien Baeten,[1] Ibrahim Hamad,[1] Cindy Hoeks,[1] Michael Hiltensperger,[2] Bart Van Wijmeersch,[3] Veronica Popescu,[3] Lilian Aly,[2] Veerle Somers,[1] Thomas Korn,[2] Markus Kleinewietfeld,[1] Niels Hellings,[1] and Bieke Broux[1]

In autoimmunity, FOXP3⁺ regulatory T cells (Tregs) skew towards a pro-inflammatory, non-suppressive phenotype and are therefore unable to control the exaggerated autoimmune response. This largely impacts the success of autologous Treg therapy which is currently under investigation for autoimmune diseases, including multiple sclerosis (MS). There is a need to ensure *in vivo* Treg stability before successful application of Treg therapy. Using genetic fate-mapping mice, we demonstrate that inflammatory, cytokine-expressing exFOXP3 T cells accumulate in the central nervous system during experimental autoimmune encephalomyelitis. In a human *in vitro* model, we discovered that interaction with inflamed blood-brain barrier endothelial cells (BBB-ECs) induces loss-of-function by Tregs. Transcriptome and cytokine analysis revealed that *in vitro* migrated Tregs have disrupted regenerative potential, a pro-inflammatory Th1/17 signature and upregulate the mTORC1 signaling pathway. *In vitro* treatment of migrated human Tregs with the clinically-approved mTORC1 inhibitor rapamycin restored suppression. Finally, flow cytometric analysis indicated an enrichment of inflammatory, less suppressive CD49d⁺ Tregs in the cerebrospinal fluid of people with MS. In sum, interaction with BBB-ECs is sufficient to affect Treg function, and transmigration triggers an additive pro-inflammatory phenotype switch. These insights help improve the efficacy of autologous Treg therapy of MS.

Intermittent rapamycin feeding recapitulates some effects of continuous treatment while maintaining lifespan extension

Methods

From 6 months of age, male and female C3B6F1 hybrid mice were either continuously fed with 42 mg/kg rapamycin, or intermittently fed by alternating weekly feeding of 42 mg/kg rapamycin food with weekly control feeding. Survival of these mice compared to control animals was measured. Furthermore, longitudinal phenotyping including metabolic (body composition, GTT, ITT, indirect calorimetry) and fitness phenotypes (treadmil, rotarod, electrocardiography and open field) was performed. Organ specific pathology was assessed at 24 months of age.

Results

Chronic rapamycin treatment induced glucose intolerance, which was partially ameliorated by intermittent treatment. Chronic and intermittent rapamycin treatments increased lifespan equally in males, while in females chronic treatment resulted in slightly higher survival. The two treatments had equivalent effects on testicular degeneration, heart fibrosis and liver lipidosis. In males, the two treatment regimes led to a similar increase in motor coordination, heart rate and Q-T interval, and reduction in spleen weight, while in females, they equally reduced BAT inflammation and spleen weight and maintained heart rate and Q-T interval. However, other health parameters, including age related pathologies, were better prevented by continuous treatment.

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PREPRINT

A Randomized, Controlled Clinical Trial Demonstrates Improved Cognitive Function in Senior Dogs Supplemented with a Senolytic and NAD+ Precursor Combination

Age-related decline in mobility and cognition are associated with cellular senescence and NAD+ depletion in dogs and people. A combination of a novel NAD+ precursor and senolytic, LY-D6/2 was examined in this randomized controlled trial. Seventy dogs were enrolled and allocated into placebo, low or full dose groups. Primary outcomes were change in cognitive impairment measured with the owner-reported Canine Cognitive Dysfunction Rating (CCDR) scale and change in activity measured with physical activity monitors. Fifty-nine dogs completed evaluations at the three-month primary endpoint, and 51 reached the six-month secondary endpoint. There was a significant difference in CCDR score across treatment groups from baseline to the primary endpoint (p=0.02) with the largest decrease in the full dose group. There were no significant differences between groups in changes in measured activity. However, the proportion of dogs that improved in frailty and owner-reported activity levels and happiness was higher in the full dose group than other groups. Adverse events occurred equally across groups. All groups showed improvement in cognition, frailty, and activity suggesting placebo effect and benefits of trial participation. We conclude that LY-D6/2 significantly improves owner-assessed cognitive function and may have broader effects on frailty, activity and happiness as reported by owners.

The use of a systems approach to increase NAD^+ in human participants

John D. Henderson, Sophia N. Z. Quigley, Shruti S. Chachra, Nichola Conlon & Dianne Ford

Reversal or mitigation against an age-related decline in NAD^+ has likely benefits, and this premise has driven academic and commercial endeavour to develop dietary supplements that achieve this outcome. We used a systems-based approach to improve on current supplements by targeting multiple points in the NAD^+ salvage pathway. In a double-blind, randomised, crossover trial, the supplement – Nuchido TIME+® (NT) - increased NAD^+ concentration in whole blood. This was associated with an increase in SIRT1 and an increase in nicotinamide phosphoribosyltransferase (NAMPT) in peripheral blood mononucleocytes, lower concentrations of pro-inflammatory cytokines in plasma, including a reduction in interleukin 2 (IL2), a reduction in glycated serum protein and a shift in the glycosylation profile of immunoglobulin G (IgG) toward a younger biological age, all of which are likely to promote a healthier ageing trajectory.

Gene Therapy-Mediated Partial Reprogramming Extends Lifespan and Reverses Age-Related Changes in Aged Mice

Carolina Cano Macip, Rokib Hasan, Victoria Hoznek, Jihyun Kim, Yuancheng Ryan Lu, Louis E. Metzger IV, Saumil Sethna, and Noah Davidsohn

Aging is a complex progression of changes best characterized as the chronic dysregulation of cellular processes leading to deteriorated tissue and organ function. Although aging cannot currently be prevented, its impact on life- and healthspan in the elderly can potentially be minimized by interventions that aim to return these cellular processes to optimal function. Recent studies have demonstrated that partial reprogramming using the Yamanaka factors (or a subset; *OCT4*, *SOX2*, and *KLF4; OSK)* can reverse age-related changes *in vitro* and *in vivo*. However, it is still unknown whether the Yamanaka factors (or a subset) are capable of extending the lifespan of aged wild-type (WT) mice. In this study, we show that systemically delivered adeno-associated viruses, encoding an inducible OSK system, in 124-week-old male mice extend the median remaining lifespan by 109% over WT controls and enhance several health parameters. Importantly, we observed a significant improvement in frailty scores indicating that we were able to improve the healthspan along with increasing the lifespan. Furthermore, in human keratinocytes expressing exogenous OSK, we observed significant epigenetic markers of age reversal, suggesting a potential reregulation of genetic networks to a younger potentially healthier state. Together, these results may have important implications for the development of partial reprogramming interventions to reverse age-associated diseases in the elderly.

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PREPRINT

Non-canonical Metabolic and Molecular Effects of Calorie Restriction Are Revealed by Varying Temporal Conditions

Calorie restriction (CR) extends lifespan and healthspan in diverse species. However, comparing ad libitum (AL) and CR-fed mice is challenging due to their significantly different feeding patterns. CR-fed mice consume their daily meal in approximately 2 hours, subjecting themselves to a prolonged self-imposed fast each day. To gain deeper insights into the effects of CR, we conducted a comprehensive examination of how AL and CR-fed mice respond to tests performed at various times relative to the completion of their once-daily CR meal. Our findings reveal that many well-known effects of CR, including its impact on insulin sensitivity, result from the specific temporal conditions. CR animals exhibit a divergent response to insulin, and this response varies based on the time elapsed since the CR-fed mice consumed their food. Utilizing an unbiased metabolomics approach, we discovered that the effects of CR on circulating metabolites are heavily dependent upon the time-of-day and feeding regimen. Finally, while it is widely believed that CR functions in part by reducing activity of the kinase mTORC1, our study suggests that the observed differences in mTORC1 activity between AL and CR-fed mice are dependent upon both fasting duration and the specific tissue examined. Furthermore, we find that the metabolic effects of CR are independent of hepatic mTORC1. Our results shed new light on the physiological, metabolic, and molecular effects of a CR diet, and highlight that much of our understanding of the effects of CR are related to when, relative to feeding, we choose to examine the mice.

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Reduction in metabolic noise reveals rejuvenation following transient severe caloric restriction

Among land vertebrates, the laying hen stands out due to its great reproductive efficiency: producing an egg daily all year long. This production rate makes the laying hen a special model animal to study the general process of reproduction and aging. One unique aspect of hens is their ability to undergo reproductive plasticity and to rejuvenate their reproductive tract during molting, a standard industrial feed restriction protocol for transiently pausing reproduction, followed by improved laying efficiency almost to peak production. Here we use longitudinal metabolomics, immunology, and physiological assays to show that molting promotes reproduction, compresses morbidity, and restores youthfulness when applied to old hens. We identified circulating metabolic biomarkers that quantitatively predict the reproduction and age of individuals. Lastly, we introduce metabolic noise, a robust, unitless, and quantifiable measure for heterogeneity of the complete metabolome as a general marker that can indicate the rate of aging of a population. Indeed, metabolic noise increased with age in control hens, whereas molted hens exhibited reduced noise following molting, indicating systemic rejuvenation. Our results suggest that metabolic noise can be used as a quick and universal proxy for assessing successful aging treatments, accelerating the timeline for drug development.

Discovery of a Potent Nicotinamide Phosphoribosyltransferase Activator for Improving Aging-associated Dysfunctions

Sicheng Yang [1], Donghuan Sun [1], Ying Wu [1 2], Shuqiang Chen [1], Yuan Guo [3], Jian Li [4], Guoqiang Dong [1], Chunquan Sheng [1]

Nicotinamide adenine dinucleotide (NAD^+) plays a crucial role in the cellular energy metabolism pathway. Nicotinamide phosphoribosyltransferase (NAMPT) is a rate-limiting enzyme involved in the biosynthesis of NAD^+. Herein, a series of new NAMPT activators were designed to increase the NAD^+ levels and improve aging-associated dysfunctions. In particular, compound **C8** effectively activated NAMPT and promoted the biosynthesis of NAD^+. Furthermore, we demonstrated that NAMPT activator **C8** possessed excellent antiaging effects both *in vitro* and *in vivo*. Activator **C8** showed potent activity in delaying aging in senescent HL-7702 cells and extended the lifespan of *Caenorhabditis elegans*. In a naturally aging mouse model, compound **C8** effectively alleviated age-related dysfunctions and markers. Therefore, NAMPT activator **C8** represented a promising lead compound for the treatment of age-related diseases.

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PREPRINT

Aging disrupts blood-brain and blood-spinal cord barrier homeostasis, but does not increase paracellular permeability

Mitchell J Cummins, Ethan T Cresswell, Doug W Smith

Blood-CNS barriers protect the CNS from circulating immune cells and damaging molecules. It is thought barrier integrity becomes disrupted with aging, contributing to impaired CNS function. Using genome-wide and targeted molecular approaches, we found aging affected expression of predominantly immune invasion and pericyte-related genes in most CNS regions investigated, especially after middle age, with spinal cord being most impacted. We did not find significant perturbation of tight junction genes, nor were vascular density or pericyte coverage affected by aging. We evaluated barrier paracellular permeability using small molecular weight tracers, serum protein extravasation, CNS water content, and iron labelling measures. We found no evidence for age-related increased barrier permeability in any of these tests. We conclude that blood-brain (BBB) and blood-spinal cord barrier (BSCB) paracellular permeability does not increase with normal aging in mouse. Whilst expression changes were not associated with increased permeability, they may represent an age-related primed state whereby additional insults cause increased leakiness.

Longevity of companion dog breeds: those at risk from early death

Kirsten M. McMillan, Jon Bielby, Carys L. Williams, Melissa M. Upjohn, Rachel A. Casey & Robert M. Christley

The companion dog is one of the most phenotypically diverse species. Variability between breeds extends not only to morphology and aspects of behaviour, but also to longevity. Despite this fact, little research has been devoted to assessing variation in life expectancy between breeds or evaluating the potential for phylogenetic characterisation of longevity. Using a dataset of 584,734 unique dogs located within the UK, including 284,734 deceased, we present variation in longevity estimates within the following: parental lineage (purebred = 1 breed, crossbred ≥ 2 breeds), breed (n = 155), body size (large, medium, small), sex (male, female) and cephalic index (brachycephalic, mesocephalic, dolichocephalic). Survival estimates were then partitioned amongst phylogenetic clades: providing evidence that canine evolutionary history (via domestication and associated artificial selection) is associated with breed lifespan. This information provides evidence to inform discussions regarding pedigree health, whilst helping current/prospective owners, breeders, policy makers, funding bodies and welfare organisations improve decision making regarding canine welfare.

The immunity and redox clocks in mice, markers of lifespan

Judith Félix, Irene Martínez de Toda, Estefanía Díaz-Del Cerro, Fernando Gil-Agudo & Mónica De la Fuente

Immune function and redox markers are used for estimating the aging rate, namely biological age (BA). However, it is unknown if this BA and its changes can be reflected in longevity. Thus, we must quantify BA in experimental animals. In peritoneal immune cells of 202 female mice (ICR/CD1) in different ages, 10 immune and 6 redox parameters were evaluated to construct two mathematical models for BA quantification in mice by multiple linear regression. Immune and redox parameters were selected as independent variables and chronological age as dependent, developing two models: the Immunity and the Redox Clocks, reaching both an adjusted R^2 of 80.9% and a standard error of 6.38 and 8.57 weeks, respectively. Both models were validated in a different group of healthy mice obtaining a Pearson's correlation coefficient of 0.844 and 0.800 ($p < 0.001$) between chronological and BA. Furthermore, they were applied to adult prematurely aging mice, which showed a higher BA than non-prematurely aging mice. Moreover, after positive and negative lifestyle interventions, mice showed a lower and higher BA, respectively, than their age-matched controls. In conclusion, the Immunity and Redox Clocks allow BA quantification in mice and both the ImmunolAge and RedoxAge in mice relate to lifespan.

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An age classification model based on DNA methylation biomarkers of aging in human peripheral blood using random forest and artificial neural network

Recent epigenetic studies have revealed a strong association between DNA methylation and aging and lifespan, which changes (increases or decreases) with age. Based on these, the construction of age prediction models associated with DNA methylation levels can be used to infer biological ages closer to the functional state of the organism. We downloaded methylation data from the Gene Expression Omnibus (GEO) public database for normal peripheral blood samples from people of different ages. We grouped the samples according to age (18-35 years and >50 years), screened the methylation sites that differed between the two groups, identified 44 differentially methylated sites, and subsequently obtained 11 age-related characteristic methylation sites using the random forest method. Then, we constructed an age classification model with these 11 characteristic methylation sites using an artificial neural network and evaluated its efficacy. The age classification model was constructed by an artificial neural network and its efficacy was evaluated. The model predicted an area under the curve (AUC) of 0.97 in the validation set and accurately distinguished between those aged 18-35 and >50 years. Furthermore, the levels of these 11 characteristic methylation sites also differed significantly between the two sets of samples in the validation set, including six newly identified age-related methylation sites ($P<0.001$). Finally, we constructed a multifactor regulatory network based on the corresponding genes of age-related methylation sites to reveal the transcriptional and post-transcriptional regulation patterns. As a result of the increasing problem of aging, the age classification model we constructed allows us to accurately distinguish different age groups at the molecular level, which will be more predictive than chronological age for assessing individual aging and future health status.

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Genome-wide profiles of DNA damage represent highly accurate predictors of mammalian age

Huifen Cao, Bolin Deng, Tianrong Song, Jiabian Lian, Lu Xia, Xiaojing Chu, Yufei Zhang, Fujian Yang, Chunlian Wang, Ye Cai, Yong Diao, Philipp Kapranov

The identification of novel age-related biomarkers represents an area of intense research interest. Despite multiple studies associating DNA damage with aging, there is a glaring paucity of DNA damage-based biomarkers of age, mainly due to the lack of precise methods for genome-wide surveys of different types of DNA damage. Recently, we developed two techniques for genome-wide mapping of the most prevalent types of DNA damage, single-strand breaks and abasic sites, with nucleotide-level resolution. Herein, we explored the potential of genomic patterns of DNA damage identified by these methods as a source of novel age-related biomarkers using mice as a model system. Strikingly, we found that models based on genomic patterns of either DNA lesion could accurately predict age with higher precision than the commonly used transcriptome analysis. Interestingly, the informative patterns were limited to relatively few genes and the DNA damage levels were positively or negatively correlated with age. These findings show that previously unexplored high-resolution genomic patterns of DNA damage contain useful information that can contribute significantly to both practical applications and basic science.

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PREPRINT

Nanopore-based DNA long-read sequencing analysis of the aged human brain

Paulino Ramirez, Wenyan Sun, Shiva Kazempour Dehkordi, Habil Zare, Bernard Fongang, Kevin F. Bieniek, Bess Frost

Aging disrupts cellular processes such as DNA repair and epigenetic control, leading to a gradual buildup of genomic alterations that can have detrimental effects in post-mitotic cells. Genomic alterations in regions of the genome that are rich in repetitive sequences, often termed "dark loci," are difficult to resolve using traditional sequencing approaches. New long-read technologies offer promising avenues for exploration of previously inaccessible regions of the genome. Using nanopore-based long-read whole-genome sequencing of DNA extracted from aged 18 human brains, we identify previously unreported structural variants and methylation patterns within repetitive DNA, focusing on transposable elements ("jumping genes") as crucial sources of variation, particularly in dark loci. Our analyses reveal potential somatic insertion variants and provides DNA methylation frequencies for many retrotransposon families. We further demonstrate the utility of this technology for the study of these challenging genomic regions in brains affected by Alzheimer's disease and identify significant differences in DNA methylation in pathologically normal brains versus those affected by Alzheimer's disease. Highlighting the power of this approach, we discover specific polymorphic retrotransposons with altered DNA methylation patterns. These retrotransposon loci have the potential to contribute to pathology, warranting further investigation in Alzheimer's disease research. Taken together, our study provides the first long-read DNA sequencing-based analysis of retrotransposon sequences, structural variants, and DNA methylation in the aging brain affected with Alzheimer's disease neuropathology.

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Spatially resolved transcriptome of the aging mouse brain

Cheng Wu [1], Tianxiang Tu [1], Mingzhe Xie [1], Yiting Wang [2], Biao Yan [2], Yajun Gong [1], Jiayi Zhang [2], Xiaolai Zhou [1], Zhi Xie [1]

Brain aging is associated with cognitive decline, memory loss and many neurodegenerative disorders. The mammalian brain has distinct structural regions that perform specific functions. However, our understanding in gene expression and cell types within the context of the spatial organization of the mammalian aging brain is limited. Here we generated spatial transcriptomic maps of young and old mouse brains. We identified 27 distinguished brain spatial domains, including layer-specific subregions that are difficult to dissect individually. We comprehensively characterized spatial-specific changes in gene expression in the aging brain, particularly for isocortex, the hippocampal formation, brainstem and fiber tracts, and validated some gene expression differences by qPCR and immunohistochemistry. We identified aging-related genes and pathways that vary in a coordinated manner across spatial regions and parsed the spatial features of aging-related signals, providing important clues to understand genes with specific functions in different brain regions during aging. Combined with single-cell transcriptomics data, we characterized the spatial distribution of brain cell types. The proportion of immature neurons decreased in the DG region with aging, indicating that the formation of new neurons is blocked. Finally, we detected changes in information interactions between regions and found specific pathways were deregulated with aging, including classic signaling WNT and layer-specific signaling COLLAGEN. In summary, we established a spatial molecular atlas of the aging mouse brain (http://sysbio.gzzoc.com/Mouse-Brain-Aging/), which provides important resources and novel insights into the molecular mechanism of brain aging.

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The cycling and aging mouse female reproductive tract at single-cell resolution

The female reproductive tract (FRT) undergoes extensive remodeling during reproductive cycling. This recurrent remodeling and how it shapes organ-specific aging remains poorly explored. Using single-cell and spatial transcriptomics, we systematically characterized morphological and gene expression changes occurring in ovary, oviduct, uterus, cervix, and vagina at each phase of the mouse estrous cycle, during decidualization, and into aging. These analyses reveal that fibroblasts play central—and highly organ-specific—roles in FRT remodeling by orchestrating extracellular matrix (ECM) reorganization and inflammation. Our results suggest a model wherein recurrent FRT remodeling over reproductive lifespan drives the gradual, age-related development of fibrosis and chronic inflammation. This hypothesis was directly tested using chemical ablation of cycling, which reduced fibrotic accumulation during aging. Our atlas provides extensive detail into how estrus, pregnancy, and aging shape the organs of the female reproductive tract and reveals the unexpected cost of the recurrent remodeling required for reproduction.

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PREPRINT

Analysis of protein levels and solubility in distinct brain regions reveals several elements of the protein homeostasis network that are impacted by aging

The onset of protein conformation diseases is inextricably linked to aging. During aging, cellular protein quality control declines which results in diminished protein homeostasis (proteostasis). In model organisms, such as C. elegans and killifish, proteostatic decline with age has been linked to the onset of aggregation of proteins in wild-type animals, observed through detergent-insoluble fractionation. Analysis of studies applying detergent-insoluble fractionation in mice revealed that the composition of detergent-insoluble proteins changes with age. However, these individual fractionation studies have generally been limited to small numbers of mice. Herein, we expand on our previous analysis by extending the experiments to a larger cohort of mice and to two brain regions implicated in neurodegenerative diseases, the cortex and hippocampus. These experiments unveil insights into alterations in the abundance and solubility of proteins involved in protein quality control and in inflammation. For example, ribosomal proteins and many chaperone proteins are downregulated with age. Consistent enrichment of subunits of the extracellular C1q complex was also observed in both brain regions alongside an increase in immunoglobulin signal indicating that markers of increased inflammation may also become insoluble during aging. More generally, insoluble proteins share features observed in datasets of impaired protein degradation indicating that the loss of activity of cellular protein degradation machinery may contribute to the specific aggregation of these proteins.

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A CRISPR base editing approach for the functional assessment of telomere biology disorder-related genes in human health and aging

Telomere Biology Disorders (TBDs) are a group of rare diseases characterized by the presence of short and/or dysfunctional telomeres. They comprise a group of bone marrow failure syndromes, idiopathic pulmonary fibrosis, and liver disease, among other diseases. Genetic alterations (variants) in the genes responsible for telomere homeostasis have been linked to TBDs. Despite the number of variants already identified as pathogenic, an even more significant number must be better understood. The study of TBDs is challenging since identifying these variants is difficult due to their rareness, it is hard to predict their impact on the disease onset, and there are not enough samples to study. Most of our knowledge about pathogenic variants comes from assessing telomerase activity from patients and their relatives affected by a TBD. However, we still lack a cell-based model to identify new variants and to study the long-term impact of such variants on the genes involved in TBDs. Herein, we present a cell-based model using CRISPR base editing to mutagenize the endogenous alleles of 21 genes involved in telomere biology. We identified key residues in the genes encoding 17 different proteins impacting cell growth. We provide functional evidence for variants of uncertain significance in patients with TBDs. We also identified variants resistant to telomerase inhibition that, similar to cells expressing wild-type telomerase, exhibited increased tumorigenic potential using an in vitro tumour growth assay. We believe that such cell-based approaches will significantly advance our understanding of the biology of TBDs and may contribute to the development of new therapies for this group of diseases.

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Titan mice as a model to test interventions that attenuate frailty and increase longevity

Wild-type murine models for aging research have lifespans of several years, which results in long experimental duration and late output. Here we explore the short-lived non-inbred Titan mouse (DU6) as a mouse model to test longevity interventions. We show that Titan mice exhibit increased frailty and senescence-associated beta-galactosidase activity at an early age. Dietary intervention attenuates the frailty progression of Titan mice. Additionally, cyclic administration of the senolytic drug Navitoclax at an early age increases the lifespan and reduces senescence-associated beta-galactosidase activity. Our data suggests that Titan mice can serve as a cost-effective and timely model for longevity interventions in mammals.

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PREPRINT

Mitochondrial DNA copy number reduction via *in vitro TFAM* knockout remodels the nuclear epigenome and transcriptome

Julia Nguyen, Phyo W. Win, Tyler Shin Nagano, Elly H. Shin, Charles Newcomb, Dan E. Arking, Christina A. Castellani

Mitochondrial DNA copy number (mtDNA-CN) is associated with several age-related chronic diseases and is a predictor of all-cause mortality. Here, we examine site-specific differential nuclear DNA (nDNA) methylation and differential gene expression resulting from *in vitro* reduction of mtDNA-CN to uncover shared genes and biological pathways mediating the effect of mtDNA-CN on disease. Epigenome and transcriptome profiles were generated for three independent human embryonic kidney (HEK293T) cell lines harbouring a mitochondrial transcription factor A (*TFAM*) heterozygous knockout generated via CRISPR-Cas9, and matched control lines. We identified 4,242 differentially methylated sites, 228 differentially methylated regions, and 179 differentially expressed genes associated with mtDNA-CN. Integrated analysis uncovered 381 Gene-CpG pairs. $GABA_A$ receptor genes and related pathways, the neuroactive ligand receptor interaction pathway, ABCD1/2 gene activity, and cell signalling processes were overrepresented, providing insight into the underlying biological mechanisms facilitating these associations. We also report evidence implicating chromatin state regulatory mechanisms as modulators of mtDNA-CN effect on gene expression. We demonstrate that mitochondrial DNA variation signals to the nuclear DNA epigenome and transcriptome and may lead to nuclear remodelling relevant to development, aging, and complex disease.

Restricting bioenergetic efficiency enhances longevity and mitochondrial redox capacity in *Drosophila melanogaster*

Analisa L. Taylor, Olga Dubuisson, Pritika Pandey, Elizabeth R. M. Zunica, Bolormaa Vandanmagsar, Wagner S. Dantas, Alyssa Johnson, Christopher L. Axelrod, John P. Kirwan

Mitochondria are essential for survival and as such, impairments in organelle homeostasis significantly accelerate age-related morbidity and mortality. Here, we determined the contribution of bioenergetic efficiency to life span and health span in *Drosophila melanogaster* utilizing the mitochondrial uncoupler BAM15. Life span was determined in flies fed a normal diet (ND) or high fat diet (HFD) supplemented with vehicle or BAM15. Locomotor function was determined by negative geotaxis assay in middle-aged flies fed vehicle or BAM15 under ND or HFD conditions. Redox capacity (high-resolution respirometry/fluorometry), citrate synthase (enzyme activity), mtDNA content (qPCR), gene expression (qPCR), and protein expression (western blot) were assessed in flight muscle homogenates of middle-aged flies fed vehicle or BAM15 ND. The molar ratio of H_2O_2 and O_2 (H_2O_2:O_2) in a defined respiratory state was calculated as a measure of redox balance. BAM15 extended life span by 9% on ND and 25% on HFD and improved locomotor activity by 125% on ND and 53% on HFD. Additionally, BAM15 enhanced oxidative phosphorylation capacity supported by pyruvate + malate, proline, and glycerol 3-phosphate. Concurrently, BAM15 enhanced the mitochondrial H_2O_2 production rate, reverse electron flow from mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) to Complex I, mGPDH, and Complex I without altering the H_2O_2:O_2 ratio. BAM15 upregulated transcriptional signatures associated with mitochondrial function and fitness as well as antioxidant defense. BAM15-mediated restriction of bioenergetic efficiency prolongs life span and health span in *Drosophila* fed a ND or HFD. Improvements in life span and health span in ND were supported by synergistic enhancement of muscular redox capacity.

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A CRISPR base editing approach for the functional assessment of telomere biology disorder-related genes in human health and aging

Telomere Biology Disorders (TBDs) are a group of rare diseases characterized by the presence of short and/or dysfunctional telomeres. They comprise a group of bone marrow failure syndromes, idiopathic pulmonary fibrosis, and liver disease, among other diseases. Genetic alterations (variants) in the genes responsible for telomere homeostasis have been linked to TBDs. Despite the number of variants already identified as pathogenic, an even more significant number must be better understood. The study of TBDs is challenging since identifying these variants is difficult due to their rareness, it is hard to predict their impact on the disease onset, and there are not enough samples to study. Most of our knowledge about pathogenic variants comes from assessing telomerase activity from patients and their relatives affected by a TBD. However, we still lack a cell-based model to identify new variants and to study the long-term impact of such variants on the genes involved in TBDs. Herein, we present a cell-based model using CRISPR base editing to mutagenize the endogenous alleles of 21 genes involved in telomere biology. We id[Geen titel]l key residues in the genes encoding 17 different proteins impacting cell growth. We provide functional evidence for variants of uncertain significance in patients with TBDs. We also identified variants resistant to telomerase inhibition that, similar to cells expressing wild-type telomerase, exhibited increased tumorigenic potential using an in vitro tumour growth assay. We believe that such cell-based approaches will significantly advance our understanding of the biology of TBDs and may contribute to the development of new therapies for this group of diseases.

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PREPRINT

Humanization of the mouse telomerase gene reset telomeres to human length

Telomeres undergo shortening with each cell division, serving as biomarkers of human aging, which is characterized by short telomeres and restricted telomerase expression in adult tissues. Contrarily, mice, featuring their longer telomeres and widespread telomerase activity, present limitations as models for understanding telomere-related human biology and diseases. To bridge this gap, we engineered a mouse strain with a humanized *mTert* gene, *hmTert*, wherein specific non-coding sequences were replaced with their human counterparts. The *hmTert* gene, encoding the wildtype mTert protein, was repressed in adult tissues beyond the gonads and thymus, closely resembling the regulatory pattern of the human *TERT* gene. Remarkably, the *hmTert* gene rescued telomere dysfunction in late generations of *mTert*-knockout mice. Through successive intercrosses of $Tert^{h/-}$ mice, telomere length progressively declined, stabilizing below 10-kb. $Tert^{h/h}$ mice achieved a human-like average telomere length of 10-12 kb, contrasting with the 50-kb length in wildtype C57BL/6J mice. Despite shortened telomeres, $Tert^{h/h}$ mice maintained normal body weight and cell homeostasis in highly proliferative tissues. Notably, colonocyte proliferation decreased significantly in Terth/h mice during dextran sodium sulfate-induced ulcerative colitis-like pathology, suggesting limitations on cellular renewal due to short telomeres. Our findings underscore the genetic determination of telomere homeostasis in mice by the *Tert* gene. These mice, exhibiting humanized telomere homeostasis, serve as a valuable model for exploring fundamental questions related to human aging and cancer.

C. elegans aging research

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PREPRINT

Developmental disruption of the mitochondrial fission gene *drp-1* extends the longevity of *daf-2* insulin/IGF-1 receptor mutant

Annika Traa, Jeremy M. Van Raamsdonk

The dynamic nature of the mitochondrial network is regulated by mitochondrial fission and fusion, allowing for re-organization of mitochondria to adapt to the cell's ever-changing needs. As organisms age, mitochondrial fission and fusion become dysregulated and mitochondrial networks become increasingly fragmented. Modulation of mitochondrial dynamics has been shown to affect longevity in fungi, yeast, *Drosophila* and *C. elegans*. While disruption of the mitochondrial fission gene *drp-1* only mildly increases wild-type lifespan, it drastically increases the already long lifespan of *daf-2* insulin/IGF-1 signaling (IIS) mutants. In this work, we determined the conditions required for *drp-1* disruption to extend *daf-2* longevity and explored the molecular mechanisms involved. We found that knockdown of *drp-1* during development is sufficient to extend *daf-2* lifespan, while tissue-specific knockdown of *drp-1* in neurons, intestine or muscle failed to increase *daf-2* longevity. Disruption of other genes involved in mitochondrial fission also increased *daf-2* lifespan as did treatment with a number of different RNAi clones that decrease mitochondrial fragmentation. In exploring potential mechanisms involved, we found that deletion of *drp-1* increases resistance to chronic stresses and slows physiologic rates in *daf-2* worms. In addition, we found that disruption of *drp-1* increased mitochondrial and peroxisomal connectedness in *daf-2* worms, increased oxidative phosphorylation and ATP levels, and increased mitophagy in *daf-2* worms, but did not affect their ROS levels or mitochondrial membrane potential. Overall, this work defined the conditions under which *drp-1* disruption increases *daf-2* lifespan and has identified multiple changes in *daf-2;drp-1* mutants that may contribute to their lifespan extension.

REVIEWS/COMMENTS/ METHODS/EDITORIALS

The information theory of aging has not been tested

James A. Timmons • Charles Brenner

A recent article in *Cell* claimed that

using inducible changes to the epigenome, we find that the act of faithful DNA repair advances aging at physiological, cognitive, and molecular levels, including erosion of the epigenetic landscape, cellular exdifferentiation, senescence, and advancement of the DNA methylation clock, which can be reversed by OSK-mediated rejuvenation. These data are consistent with the information theory of aging, which states that a loss of epigenetic information is a reversible cause of aging. [1]

In this work, Dr. Sinclair and colleagues induced whole-mouse expression of homing endonuclease I-*Ppo*I to prematurely age mice. The corresponding author published two papers—neither cited—showing that I-*Ppo*I targeted to specific cell types is mutagenic, cytotoxic, and progeric, thereby accounting for the progeric effects that they attributed to faithful DNA repair. The corresponding author declined to provide requested data [2] on mice in the 30 days after I-*Ppo*I treatment was concluded or to provide data in support of the statement that "fortunately, it is now apparent that mammals retain a back-up copy of youthful epigenetic information that can safely restore the function of old tissues, akin to reinstalling software."

Cellular reprogramming as a tool to model human aging in a dish

Patricia R. Pitrez, Luis M. Monteiro, Oliver Borgogno, Xavier Nissan, Jerome Mertens & Lino Ferreira

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The design of human model systems is highly relevant to unveil the underlying mechanisms of aging and to provide insights on potential interventions to extend human health and life span. In this perspective, we explore the potential of 2D or 3D culture models comprising human induced pluripotent stem cells and transdifferentiated cells obtained from aged or age-related disorder-affected donors to enhance our understanding of human aging and to catalyze the discovery of anti-aging interventions.

A unified framework for evolutionary genetic and physiological theories of aging

Jean-François Lemaître, Jacob Moorad, Jean-Michel Gaillard, Alexei A. Maklakov, Daniel H. Nussey

Why and how we age are 2 intertwined questions that have fascinated scientists for many decades. However, attempts to answer these questions remain compartmentalized, preventing a comprehensive understanding of the aging process. We argue that the current lack of knowledge about the evolution of aging mechanisms is due to a lack of clarity regarding evolutionary theories of aging that explicitly involve physiological processes: the disposable soma theory (DST) and the developmental theory of aging (DTA). In this Essay, we propose a new hierarchical model linking genes to vital rates, enabling us to critically reevaluate the DST and DTA in terms of their relationship to evolutionary genetic theories of aging (mutation accumulation (MA) and antagonistic pleiotropy (AP)). We also demonstrate how these 2 theories can be incorporated in a unified hierarchical framework. The new framework will help to generate testable hypotheses of how the hallmarks of aging are shaped by natural selection.

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Validation of biomarkers of aging

Mahdi Moqri, Chiara Herzog, Jesse R. Poganik, Kejun Ying, Jamie N. Justice, Daniel W. Belsky, Albert T. Higgins-Chen, Brian H. Chen, Alan A. Cohen, Georg Fuellen, Sara Hägg, Riccardo E. Marioni, Martin Widschwendter, Kristen Fortney, Peter O. Fedichev, Alex Zhavoronkov, Nir Barzilai, Jessica Lasky-Su, Douglas P. Kiel, Brian K. Kennedy, Steven Cummings, P. Eline Slagboom, Eric Verdin, Andrea B. Maier, ... Luigi Ferrucci + Show authors

The search for biomarkers that quantify biological aging (particularly 'omic'-based biomarkers) has intensified in recent years. Such biomarkers could predict aging-related outcomes and could serve as surrogate endpoints for the evaluation of interventions promoting healthy aging and longevity. However, no consensus exists on how biomarkers of aging should be validated before their translation to the clinic. Here, we review current efforts to evaluate the predictive validity of omic biomarkers of aging in population studies, discuss challenges in comparability and generalizability and provide recommendations to facilitate future validation of biomarkers of aging. Finally, we discuss how systematic validation can accelerate clinical translation of biomarkers of aging and their use in gerotherapeutic clinical trials.

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Targeting ageing with rapamycin and its derivatives in humans: a systematic review

Deborah J W Lee, MD † • Ajla Hodzic Kuerec, MD † • Prof Andrea B Maier, MD • Show footnotes

Rapamycin and its derivatives (rapalogs) are inhibitors of mTOR, a major regulator of the ageing process. We aimed to summarise the effects of rapamycin and its derivatives on the severity of ageing-related physiological changes and disease in adults. A search across five databases yielded 18 400 unique articles, resulting in 19 included studies. Rapamycin and its derivatives improved physiological parameters associated with ageing in the immune, cardiovascular, and integumentary systems of healthy individuals or individuals with ageing-related diseases. Overall, no significant effects on the endocrine, muscular, or neurological systems were found. The effects of rapamycin or its derivatives on the respiratory, digestive, renal, and reproductive systems were not assessed. No serious adverse events attributed to rapamycin and its derivatives were reported in healthy individuals; however, there were increased numbers of infections and increases in total cholesterol, LDL cholesterol, and triglycerides in individuals with ageing-related diseases. Future studies should assess the remaining unexamined systems and test the effects of long-term exposure to rapamycin and its derivatives.

Pharmacological interventions in human aging

Michael Angelo Petr, Frida Matiyevskaya, Brenna Osborne, Magnus Berglind, Simon Reves, Bin Zhang, Michael Ben Ezra, Lina Maria Carmona-Marin, Muhammad Farraz Syadzha, Marta Cortés Mediavilla, Guido Keijzers, Daniela Bakula, Garik V Mkrtchyan, Morten Scheibye-Knudsen

Pharmacological interventions are emerging as potential avenues of alleviating age-related disease. However, the knowledge of ongoing clinical trials as they relate to aging and pharmacological interventions is dispersed across a variety of mediums. In this review we summarize 136 age-related clinical trials that have been completed or are ongoing. Furthermore, we establish a database that describe the trials (AgingDB, www.agingdb.com) keeping track of the previous and ongoing clinical trials, alongside their outcomes. The aim of this review and database is to give people the ability to easily query for their trial of interest and stay up to date on the latest results. In sum, herein we give an overview of the current pharmacological strategies that have been applied to target human aging.

Therapeutic effect of dietary ingredients on cellular senescence in animals and humans: A systematic review

Lihuan Guan [a b 1], Anna Eisenmenger [a b 1], Karen C. Crasta [a b c d e], Elena Sandalova [a b], Andrea B. Maier [a b f 2 3]

Methods

This systematic review was registered at PROSPERO International prospective register of systematic reviews (Reg #: CRD42022338885). The databases PubMed and Embase were systematically searched for key terms related to cellular senescence, senescence markers, diets, nutrients and bioactive compounds. Intervention and observational studies on human and animals investigating the effects of dietary ingredients via oral administration on cellular senescence load were included. The SYRCLE's risk of bias tool and Cochrane risk of bias tool v2.0 were used to assess the risk of bias for animal and human studies respectively.

Results

Out of 5707 identified articles, 83 articles consisting of 78 animal studies and 5 human studies aimed to reduce cellular senescence load using dietary ingredients. In animal studies, the most-frequently used senescence model was normative ageing (26 studies), followed by D-galactose-induced models (17 studies). Resveratrol (8 studies), vitamin E (4 studies) and soy protein isolate (3 studies) showed positive effects on reducing the level of senescence markers such as p53, p21, p16 and senescence-associated ß-galactosidase in various tissues of physiological systems. In three out of five human studies, ginsenoside Rg1 had no positive effect on reducing senescence in muscle tissues after exercise. The risk of bias for both animal and human studies was largely unclear.

Conclusion

Resveratrol, vitamin E and soy protein isolate are promising senotherapeutics studied in animal models. Studies testing dietary ingredients with senotherapeutic potential in humans are limited and translation is highly warranted.

Senotherapeutics to Counteract Senescent Cells Are Prominent Topics in the Context of Anti-Ageing Strategies

by Anna Calabrò [1], Giulia Accardi [1], Anna Aiello [1], Calogero Caruso [1,2,*], Damiano Galimberti [2] and Giuseppina Candore [1]

Cellular senescence is implicated in ageing and associated with a broad spectrum of age-related diseases. Importantly, a cell can initiate the senescence program irrespective of the organism's age. Various stress signals, including those defined as ageing hallmarks and alterations leading to cancer development, oncogene activation, or loss of cancer-suppressive functions, can trigger cellular senescence. The primary outcome of these alterations is the activation of nuclear factor (NF)-κB, thereby inducing the senescence-associated secretory phenotype (SASP). Proinflammatory cytokines and chemokines, components of this phenotype, contribute to chronic systemic sterile inflammation, commonly referred to as inflamm-ageing. This inflammation is linked to age-related diseases (ARDs), frailty, and increased mortality in older individuals. Additionally, senescent cells (SCs) accumulate in multiple tissues with age and are believed to underlie the organism functional decline, as demonstrated by models. An escalating effort has been dedicated to identify senotherapeutics that selectively target SCs by inducing apoptosis; these drugs are termed senolytics. Concurrently, small molecules that suppress senescent phenotypes without causing cell death are known as senomorphics. Both natural and synthetic senotherapeutics, along with immunotherapies employing immune cell-mediated clearance of SCs, currently represent the most promising strategies to combat ageing and ARDs. Indeed, it is fascinating to observe that information regarding the immune reaction to SCs indicates that regulation by specific lymphocyte subsets, elevated in the oldest centenarians, plays a role in attaining extreme longevity. Regardless, the application of methods already utilized in cancer treatment, such as CAR cells and monoclonal antibodies, broadens the spectrum of potential approaches to be utilized.

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Magnesium and the Hallmarks of Aging

by Ligia J. Dominguez [1,2], Nicola Veronese [2] and Mario Barbagallo [2,*]

Magnesium is an essential ion in the human body that regulates numerous physiological and pathological processes. Magnesium deficiency is very common in old age. Age-related chronic diseases and the aging process itself are frequently associated with low-grade chronic inflammation, called 'inflammaging'. Because chronic magnesium insufficiency has been linked to excessive generation of inflammatory markers and free radicals, inducing a chronic inflammatory state, we formerly hypothesized that magnesium inadequacy may be considered among the intermediaries helping us explain the link between inflammaging and aging-associated diseases. We show in this review evidence of the relationship of magnesium with all the hallmarks of aging (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, disabled autophagy, dysbiosis, and chronic inflammation), which may positively affect the human healthspan. It is feasible to hypothesize that maintaining an optimal balance of magnesium during one's life course may turn out to be a safe and economical strategy contributing to the promotion of healthy aging. Future well-designed studies are necessary to further explore this hypothesis.

Considering *Caenorhabditis elegans* Aging on a Temporal and Tissue Scale: The Case of Insulin/IGF-1 Signaling

The aging process is inherently complex, involving multiple mechanisms that interact at different biological scales. The nematode *Caenorhabditis elegans* is a simple model organism that has played a pivotal role in aging research following the discovery of mutations extending lifespan. Longevity pathways identified in *C. elegans* were subsequently found to be conserved and regulate lifespan in multiple species. These pathways intersect with fundamental hallmarks of aging that include nutrient sensing, epigenetic alterations, proteostasis loss, and mitochondrial dysfunction. Here we summarize recent data obtained in *C. elegans* highlighting the importance of studying aging at both the tissue and temporal scale. We then focus on the neuromuscular system to illustrate the kinetics of changes that take place with age. We describe recently developed tools that enabled the dissection of the contribution of the insulin/IGF-1 receptor ortholog DAF-2 to the regulation of worm mobility in specific tissues and at different ages. We also discuss guidelines and potential pitfalls in the use of these new tools. We further highlight the opportunities that they present, especially when combined with recent transcriptomic data, to address and resolve the inherent complexity of aging. Understanding how different aging processes interact within and between tissues at different life stages could ultimately suggest potential intervention points for age-related diseases.

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Emerging epigenetic insights into aging mechanisms and interventions

Zeming Wu • Weiqi Zhang • Jing Qu • Guang-Hui Liu

Epigenetic dysregulation emerges as a critical hallmark and driving force of aging. Although still an evolving field with much to explore, it has rapidly gained significance by providing valuable insights into the mechanisms of aging and potential therapeutic opportunities for age-related diseases. Recent years have witnessed remarkable strides in our understanding of the epigenetic landscape of aging, encompassing pivotal elements, such as DNA methylation, histone modifications, RNA modifications, and noncoding (nc) RNAs. Here, we review the latest discoveries that shed light on new epigenetic mechanisms and critical targets for predicting and intervening in aging and related disorders. Furthermore, we explore burgeoning interventions and exemplary clinical trials explicitly designed to foster healthy aging, while contemplating the potential ramifications of epigenetic influences.

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A high-resolution view of the heterogeneous aging endothelium

Vascular endothelial cell (EC) aging has a strong impact on tissue perfusion and overall cardiovascular health. While studies confined to the investigation of aging-associated vascular readouts in one or a few tissues have already drastically expanded our understanding of EC aging, single-cell omics and other high-resolution profiling technologies have started to illuminate the intricate molecular changes underlying endothelial aging across diverse tissues and vascular beds at scale. In this review, we provide an overview of recent insights into the heterogeneous adaptations of the aging vascular endothelium. We address critical questions regarding tissue-specific and universal responses of the endothelium to the aging process, EC turnover dynamics throughout lifespan, and the differential susceptibility of ECs to acquiring aging-associated traits. In doing so, we underscore the transformative potential of single-cell approaches in advancing our comprehension of endothelial aging, essential to foster the development of future innovative therapeutic strategies for aging-associated vascular conditions.

OTHER RESEARCH & REVIEWS

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Ultra-processed food exposure and adverse health outcomes: umbrella review of epidemiological meta-analyses

Results The search identified 45 unique pooled analyses, including 13 dose-response associations and 32 non-dose-response associations (n=9 888 373). Overall, direct associations were found between exposure to ultra-processed foods and 32 (71%) health parameters spanning mortality, cancer, and mental, respiratory, cardiovascular, gastrointestinal, and metabolic health outcomes. Based on the pre-specified evidence classification criteria, convincing evidence (class I) supported direct associations between greater ultra-processed food exposure and higher risks of incident cardiovascular disease related mortality (risk ratio 1.50, 95% confidence interval 1.37 to 1.63; GRADE=very low) and type 2 diabetes (dose-response risk ratio 1.12, 1.11 to 1.13; moderate), as well as higher risks of prevalent anxiety outcomes (odds ratio 1.48, 1.37 to 1.59; low) and combined common mental disorder outcomes (odds ratio 1.53, 1.43 to 1.63; low). Highly suggestive (class II) evidence indicated that greater exposure to ultra-processed foods was directly associated with higher risks of incident all cause mortality (risk ratio 1.21, 1.15 to 1.27; low), heart disease related mortality (hazard ratio 1.66, 1.51 to 1.84; low), type 2 diabetes (odds ratio 1.40, 1.23 to 1.59; very low), and depressive outcomes (hazard ratio 1.22, 1.16 to 1.28; low), together with higher risks of prevalent adverse sleep related outcomes (odds ratio 1.41, 1.24 to 1.61; low), wheezing (risk ratio 1.40, 1.27 to 1.55; low), and obesity (odds ratio 1.55, 1.36 to 1.77; low). Of the remaining 34 pooled analyses, 21 were graded as suggestive or weak strength (class III-IV) and 13 were graded as no evidence (class V). Overall, using the GRADE framework, 22 pooled analyses were rated as low quality, with 19 rated as very low quality and four rated as moderate quality.

Conclusions Greater exposure to ultra-processed food was associated with a higher risk of adverse health outcomes, especially cardiometabolic, common mental disorder, and mortality outcomes. These findings provide a rationale to develop and evaluate the effectiveness of using population based and public health measures to target and reduce dietary exposure to ultra-processed foods for improved human health. They also inform and provide support for urgent mechanistic research.

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Durable and efficient gene silencing in vivo by hit-and-run epigenome editing

Martino Alfredo Cappelluti, Valeria Mollica Poeta, Sara Valsoni, Piergiuseppe Quarato, Simone Merlin, Ivan Merelli & Angelo Lombardo

Permanent epigenetic silencing using programmable editors equipped with transcriptional repressors holds great promise for the treatment of human diseases[1,2,3]. However, to unlock its full therapeutic potential, an experimental confirmation of durable epigenetic silencing after the delivery of transient delivery of editors in vivo is needed. To this end, here we targeted *Pcsk9*, a gene expressed in hepatocytes that is involved in cholesterol homeostasis. In vitro screening of different editor designs indicated that zinc-finger proteins were the best-performing DNA-binding platform for efficient silencing of mouse *Pcsk9*. A single administration of lipid nanoparticles loaded with the editors' mRNAs almost halved the circulating levels of PCSK9 for nearly one year in mice. Notably, *Pcsk9* silencing and accompanying epigenetic repressive marks also persisted after forced liver regeneration, further corroborating the heritability of the newly installed epigenetic state. Improvements in construct design resulted in the development of an all-in-one configuration that we term evolved engineered transcriptional repressor (EvoETR). This design, which is characterized by a high specificity profile, further reduced the circulating levels of PCSK9 in mice with an efficiency comparable with that obtained through conventional gene editing, but without causing DNA breaks. Our study lays the foundation for the development of in vivo therapeutics that are based on epigenetic silencing.