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## Scientific News
## 5th of March 2017
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# Future life expectancy in 35 industrialised countries: projections with a Bayesian model ensemble

## Findings

Life expectancy is projected to increase in all 35 countries with a probability of at least 65% for women and 85% for men. There is a 90% probability that life expectancy at birth among South Korean women in 2030 will be higher than 86·7 years, the same as the highest worldwide life expectancy in 2012, and a 57% probability that it will be higher than 90 years. Projected female life expectancy in South Korea is followed by those in France, Spain, and Japan. There is a greater than 95% probability that life expectancy at birth among men in South Korea, Australia, and Switzerland will surpass 80 years in 2030, and a greater than 27% probability that it will surpass 85 years. Of the countries studied, the USA, Japan, Sweden, Greece, Macedonia, and Serbia have some of the lowest projected life expectancy gains for both men and women. The female life expectancy advantage over men is likely to shrink by 2030 in every country except Mexico, where female life expectancy is predicted to increase more than male life expectancy, and in Chile, France, and Greece where the two sexes will see similar gains. More than half of the projected gains in life expectancy at birth in women will be due to enhanced longevity above age 65 years.

## Interpretation

There is more than a 50% probability that by 2030, national female life expectancy will break the 90 year barrier, a level that was deemed unattainable by some at the turn of the 21st century. Our projections show continued increases in longevity, and the need for careful planning for health and social services and pensions.

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# Life Expectancy Will Continue To Increase

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# Age-associated molecular changes are deleterious and may modulate life span through diet

Transition through life span is accompanied by numerous molecular changes, such as dysregulated gene expression, altered metabolite levels, and accumulated molecular damage. These changes are thought to be causal factors in aging; however, because they are numerous and are also influenced by genotype, environment, and other factors in addition to age, it is difficult to characterize the cumulative effect of these molecular changes on longevity. We reasoned that age-associated changes, such as molecular damage and tissue composition, may influence life span when used in the diet of organisms that are closely related to those that serve as a dietary source. To test this possibility, we used species-specific culture media and diets that incorporated molecular extracts of young and old organisms and compared the influence of these diets on the life span of yeast, fruitflies, and mice. In each case, the "old" diet or medium shortened the life span for one or both sexes. These findings suggest that age-associated molecular changes, such as cumulative damage and altered dietary composition, are deleterious and causally linked with aging and may affect life span through diet.

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# Intravenous administration of mitochondria for treating experimental Parkinson's disease

Mitochondrial dysfunction is associated with a large number of human diseases, including neurological and muscular degeneration, cardiovascular disorders, obesity, diabetes, aging and rare mitochondrial diseases. Replacement of dysfunctional mitochondria with functional exogenous mitochondria is proposed as a general principle to treat these diseases. Here we found that mitochondria isolated from human hepatoma cell could naturally enter human neuroblastoma SH-SY5Y cell line, and when the mitochondria were intravenously injected into mice, all of the mice were survived and no obvious abnormality appeared. The results of *in vivo* distribution suggested that the exogenous mitochondria distributed in various tissues including brain, liver, kidney, muscle and heart, which would benefit for multi-systemically mitochondrial diseases. In normal mice, mitochondrial supplement improved their endurance by increase of energy production in forced swimming test; and in experimental Parkinson's disease (PD) model mice induced by respiratory chain inhibitor MPTP, mitochondrial replacement prevented experimental PD progress through increasing the activity of electron transport chain, decreasing reactive oxygen species level, and preventing cell apoptosis and necrosis. Since effective drugs remain elusive to date for mitochondrial diseases, the strategy of mitochondrial replacement would provide an essential and innovative approach as mitochondrial therapy.

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# Senescent cells expose and secrete an oxidized form of membrane-bound vimentin as revealed by a natural polyreactive antibody

Studying the phenomenon of cellular senescence has been hindered by the lack of senescence-specific markers. As such, detection of proteins informally associated with senescence accompanies the use of senescence-associated β-galactosidase as a collection of semiselective markers to monitor the presence of senescent cells. To identify novel biomarkers of senescence, we immunized BALB/c mice with senescent mouse lung fibroblasts and screened for antibodies that recognized senescence-associated cell-surface antigens by FACS analysis and a newly developed cell-based ELISA. The majority of antibodies that we isolated, cloned, and sequenced belonged to the IgM isotype of the innate immune system. In-depth characterization of one of these monoclonal, polyreactive natural antibodies, the IgM clone 9H4, revealed its ability to recognize the intermediate filament vimentin. By using 9H4, we observed that senescent primary human fibroblasts express vimentin on their cell surface, and MS analysis revealed a posttranslational modification on cysteine 328 (C328) by the oxidative adduct malondialdehyde (MDA). Moreover, elevated levels of secreted MDA-modified vimentin were detected in the plasma of aged senescence-accelerated mouse prone 8 mice, which are known to have deregulated reactive oxygen species metabolism and accelerated aging. Based on these findings, we hypothesize that humoral innate immunity may recognize senescent cells by the presence of membrane-bound MDA-vimentin, presumably as part of a senescence eradication mechanism that may become impaired with age and result in senescent cell accumulation.

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# Cytosolic proteostasis through importing of misfolded proteins into mitochondria

Loss of proteostasis underlies ageing and neurodegeneration characterized by the accumulation of protein aggregates and mitochondrial dysfunction[1, 2, 3, 4, 5]. Although many neurodegenerative-disease-associated proteins can be found in mitochondria[4, 6], it remains unclear how mitochondrial dysfunction and protein aggregation could be related. In dividing yeast cells, protein aggregates that form under stress or during ageing are preferentially retained by the mother cell, in part through tethering to mitochondria, while the disaggregase Hsp104 helps to dissociate aggregates and thereby enables refolding or degradation of misfolded proteins[7, 8, 9, 10]. Here we show that, in yeast, cytosolic proteins prone to aggregation are imported into mitochondria for degradation. Protein aggregates that form under heat shock contain both cytosolic and mitochondrial proteins and interact with the mitochondrial import complex. Many aggregation-prone proteins enter the mitochondrial intermembrane space and matrix after heat shock, and some do so even without stress. Timely dissolution of cytosolic aggregates requires the mitochondrial import machinery and proteases. Blocking mitochondrial import but not proteasome activity causes a marked delay in the degradation of aggregated proteins. Defects in cytosolic Hsp70s leads to enhanced entry of misfolded proteins into mitochondria and elevated mitochondrial stress. We term this mitochondria-mediated proteostasis mechanism MAGIC (mitochondria as guardian in cytosol) and provide evidence that it may exist in human cells.

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# Autophagy maintains the metabolism and function of young and old stem cells

With age, haematopoietic stem cells lose their ability to regenerate the blood system, and promote disease development. Autophagy is associated with health and longevity, and is critical for protecting haematopoietic stem cells from metabolic stress. Here we show that loss of autophagy in haematopoietic stem cells causes accumulation of mitochondria and an activated metabolic state, which drives accelerated myeloid differentiation mainly through epigenetic deregulations, and impairs haematopoietic stem-cell self-renewal activity and regenerative potential. Strikingly, most haematopoietic stem cells in aged mice share these altered metabolic and functional features. However, approximately one-third of aged haematopoietic stem cells exhibit high autophagy levels and maintain a low metabolic state with robust long-term regeneration potential similar to healthy young haematopoietic stem cells. Our results demonstrate that autophagy actively suppresses haematopoietic stem-cell metabolism by clearing active, healthy mitochondria to maintain quiescence and stemness, and becomes increasingly necessary with age to preserve the regenerative capacity of old haematopoietic stem cells.

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# Aging yeast gain a competitive advantage on non-optimal carbon sources

Stephen Frenk, Grazia Pizza, Rachael V. Walker, Jonathan Houseley

Animals, plants and fungi undergo an aging process with remarkable physiological and molecular similarities, suggesting that aging has long been a fact of life for eukaryotes and one to which our unicellular ancestors were subject. Key biochemical pathways that impact longevity evolved prior to multicellularity, and the interactions between these pathways and the aging process therefore emerged in ancient single-celled eukaryotes. Nevertheless, we do not fully understand how aging impacts the fitness of unicellular organisms, and whether such cells gain a benefit from modulating rather than simply suppressing the aging process. We hypothesized that age-related loss of fitness in single-celled eukaryotes may be counterbalanced, partly or wholly, by a transition from a specialist to a generalist life-history strategy that enhances adaptability to other environments. We tested this hypothesis in budding yeast using competition assays and found that while young cells are more successful in glucose, highly aged cells outcompete young cells on other carbon sources such as galactose. This occurs because aged yeast divide faster than young cells in galactose, reversing the normal association between age and fitness. The impact of aging on single-celled organisms is therefore complex and may be regulated in ways that anticipate changing nutrient availability. We propose that pathways connecting nutrient availability with aging arose in unicellular eukaryotes to capitalize on age-linked diversity in growth strategy and that individual cells in higher eukaryotes may similarly diversify during aging to the detriment of the organism as a whole.

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# Antiaging Effect of Metformin on Brain in Naturally Aged and Accelerated Senescence Model of Rat

Metformin, a biguanide, is a widely used antidiabetic drug, which inhibits gluconeogenesis and is used to treat hyperglycemia in type 2 diabetes. Through activation of AMPK (AMP-activated protein kinase) pathway, metformin also mimics caloric restriction health benefits. Aging causes substantial molecular to morphological changes in brain, the brain cells being more susceptible toward oxidative stress mediated damages due to the presence of high lipid content and higher oxygen consumption. Wistar rats (naturally aged and D-galactose induced rat model) were supplemented with metformin (300 mg/kg b.w. orally) for 6 weeks. The biomarkers of oxidative stress such as antioxidant capacity (ferric reducing antioxidant potential [FRAP]), malondialdehyde (MDA), reduced glutathione (GSH), protein carbonyl (PCO), reactive oxygen species (ROS), acetylcholinesterase (AChE) activity, and nitric oxide (NO) were measured in brain tissues of control and experimental groups. The results indicate that metformin treatment augmented the levels of FRAP and GSH in naturally aged, and D-gal induced aging model groups compared to the respective controls. In contrast, metformin treated groups exhibited significant reduction in MDA, PCO, ROS, and NO levels and a significant increase in AChE activity in induced aging rats. The administration of D-galactose upregulated the expression of sirtuin-2, interleukin-6 (*IL*-6), and tumor necrosis factor-alpha (*TNF-α*) and downregulated the expression of Beclin-1. Metformin supplementation downregulated the D-galactose induced expressions of sirtuin-2, *IL*-6, and *TNF-α* expression, whereas upregulated the Beclin-1 expression. Our data confirm that metformin restores the antioxidant status and improves healthy brain aging through the activation of autophagy and reduction in inflammation.

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# Metformin Alleviates Aging Cellular Phenotypes in Hutchinson-Gilford Progeria Syndrome Dermal Fibroblasts.

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Author information

## Abstract

Metformin is a popular antidiabetic biguanide, which has been considered as a candidate drug for cancer treatment and aging prevention. Hutchinson-Gilford progeria syndrome (HGPS) is a devastating disease characterized by premature aging and severe age-associated complications leading to death. The effects of metformin on HGPS dermal fibroblasts remain largely undefined. In this study, we investigated whether metformin could exert a beneficial effect on nuclear abnormalities and delay senescence in fibroblasts derived from HGPS patients. Metformin treatment partially restored normal nuclear phenotypes, delayed senescence, activated the phosphorylation of AMP-activated protein kinase, and decreased reactive oxygen species formation in HGPS dermal fibroblasts. Interestingly, metformin reduced the number of phosphorylated histone variant H2AX-positive DNA damage foci and suppressed progerin protein expression, compared to the control. Furthermore, metformin-supplemented aged mice showed higher splenocyte proliferation and mRNA expression of the antioxidant enzyme, superoxide dismutase 2 than the control mice. Collectively, our results show that metformin treatment alleviates the nuclear defects and premature aging phenotypes in HGPS fibroblasts. Thus, metformin can be considered a promising therapeutic approach for life extension in HGPS. This article is protected by copyright. All rights reserved.

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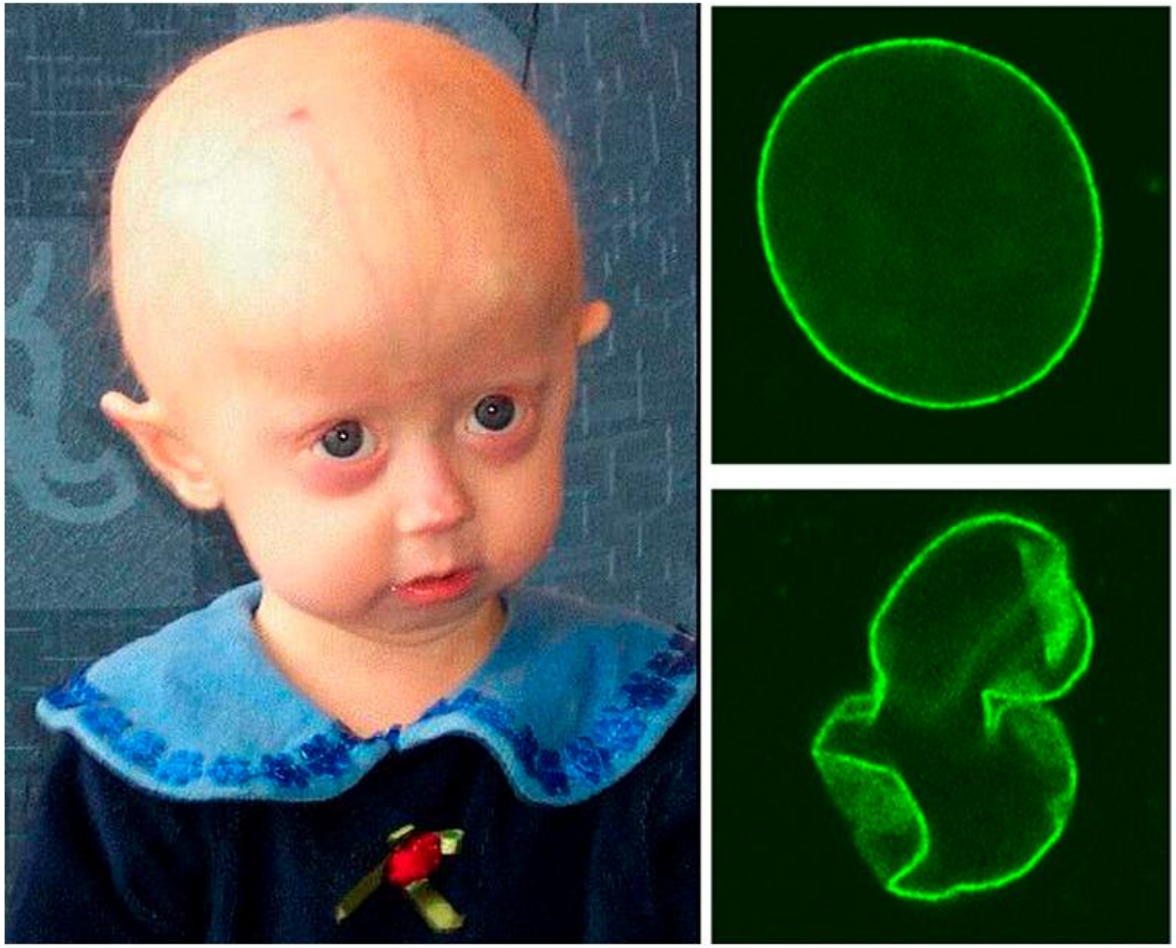
# Metformin Shows Promise As A New Treatment For The Premature Aging Disease Hutchinson-Gilford Progeria Syndrome



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# Combined statin and angiotensin-converting enzyme (ACE) inhibitor treatment increases the lifespan of long-lived F1 male mice

Statins, such as simvastatin, and ACE inhibitors (ACEis), such as ramipril, are standard therapies for the prevention and treatment of cardiovascular diseases. These types of drugs are commonly administered together. More recently, angiotensin II type 1 receptor (AT1R) antagonists, such as candesartan cilexetil (candesartan), have been used in the place of, or in combination with, ACEis. Here, we investigated the effects of simvastatin and ramipril single and combination therapy, and candesartan treatment on the lifespan of isocalorically fed, long-lived, B6C3F1 mice. Males were used for their relative endocrine simplicity and to minimize animal usage. The drugs were administered daily in food. The simvastatin and ramipril combination therapy significantly increased the mean and median lifespan by 9 %. In contrast, simvastatin, ramipril, or candesartan monotherapy was ineffective. All groups consumed the same number of calories. Simvastatin, alone or administered with ramipril, decreased body weight without changing caloric consumption, suggesting it may alter energy utilization in mice. Combination therapy elevated serum triglyceride and glucose levels, consistent with altered energy homeostasis. Few significant or consistent differences were found in mortality-associated pathologies among the groups. Simvastatin treatment did not reduce normal serum cholesterol or lipid levels in these mice, suggesting that the longevity effects may stem from the *pleiotropic*, non-cholesterol-related, effects of statins. Together, the results suggest that statins and ACEis together may enhance mouse longevity. Statins and ACE inhibitors are generally well-tolerated, and in combination, they have been shown to increase the lifespan of normotensive, normocholesterolemic humans.

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# Impact of genetic background and experimental reproducibility on identifying chemical compounds with robust longevity effects

Limiting the debilitating consequences of ageing is a major medical challenge of our time. Robust pharmacological interventions that promote healthy ageing across diverse genetic backgrounds may engage conserved longevity pathways. Here we report results from the *Caenorhabditis* Intervention Testing Program in assessing longevity variation across 22 *Caenorhabditis* strains spanning 3 species, using multiple replicates collected across three independent laboratories. Reproducibility between test sites is high, whereas individual trial reproducibility is relatively low. Of ten pro-longevity chemicals tested, six significantly extend lifespan in at least one strain. Three reported dietary restriction mimetics are mainly effective across *C. elegans* strains, indicating species and strain-specific responses. In contrast, the amyloid dye ThioflavinT is both potent and robust across the strains. Our results highlight promising pharmacological leads and demonstrate the importance of assessing lifespans of discrete cohorts across repeat studies to capture biological variation in the search for reproducible ageing interventions.

# On the Pathogenesis of Alzheimer's Disease: The MAM Hypothesis

The pathogenesis of Alzheimer's disease (AD) is currently unclear and is the subject of much debate. The most widely accepted hypothesis designed to explain AD pathogenesis is the amyloid cascade, which invokes the accumulation of extracellular plaques and intracellular tangles as playing a fundamental role in the course and progression of the disease. However, besides plaques and tangles, other biochemical and morphological features are also present in AD, often manifesting early in the course of the disease before the accumulation of plaques and tangles. These include altered calcium, cholesterol, and phospholipid metabolism; altered mitochondrial dynamics; and reduced bioenergetic function. Notably, these other features of AD are associated with functions localized to a subdomain of the endoplasmic reticulum (ER), known as mitochondria-associated ER membranes (MAMs). The MAM region of the ER is a lipid raft–like domain closely apposed to mitochondria in such a way that the 2 organelles are able to communicate with each other, both physically and biochemically, thereby facilitating the functions of this region. We have found that MAM-localized functions are increased significantly in cellular and animal models of AD and in cells from patients with AD in a manner consistent with the biochemical findings noted above. Based on these and other observations, we propose that increased ER–mitochondrial apposition and perturbed MAM function lie at the heart of AD pathogenesis.—Area-Gomez, E., Schon, E. A. On the pathogenesis of Alzheimer's disease: the MAM hypothesis.

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# Familial Alzheimer's Disease Mutations within the Amyloid Precursor Protein Alter the Aggregation and Conformation of the Amyloid-β Peptide*

Most cases of Alzheimer's disease (AD) are sporadic, but a small percentage of AD cases, called familial AD (FAD), are associated with mutations in presenilin 1, presenilin 2, or the amyloid precursor protein. Amyloid precursor protein mutations falling within the amyloid-β (Aβ) sequence lead to a wide range of disease phenotypes. There is increasing evidence that distinct amyloid structures distinguished by amyloid conformation-dependent monoclonal antibodies have similarly distinct roles in pathology. It is possible that this phenotypic diversity of FAD associated with mutations within the Aβ sequence is due to differences in the conformations adopted by mutant Aβ peptides, but the effects of FAD mutations on aggregation kinetics and conformational and morphological changes of the Aβ peptide are poorly defined. To gain more insight into this possibility, we therefore investigated the effects of 11 FAD mutations on the aggregation kinetics of Aβ, as well as its ability to form distinct conformations recognized by a panel of amyloid conformation-specific monoclonal antibodies. We found that most FAD mutations increased the rate of aggregation of Aβ. The FAD mutations also led to the adoption of alternative amyloid conformations distinguished by monoclonal antibodies and resulted in the formation of distinct aggregate morphologies as determined by transmission electron microscopy. In addition, several of the mutant peptides displayed a large reduction in thioflavin T fluorescence, despite forming abundant fibrils indicating that thioflavin T is a probe of conformational polymorphisms rather than a reliable indicator of fibrillization. Taken together, these results indicate that FAD mutations falling within the Aβ sequence lead to dramatic changes in aggregation kinetics and influence the ability of Aβ to form immunologically and morphologically distinct amyloid structures.

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# Association of blood lipids with Alzheimer's disease: A comprehensive lipidomics analysis


## Introduction

The aim of this study was to (1) replicate previous associations between six blood lipids and Alzheimer's disease (AD) (Proitsi et al 2015) and (2) identify novel associations between lipids, clinical AD diagnosis, disease progression and brain atrophy (left/right hippocampus/entorhinal cortex).

## Methods

We performed untargeted lipidomic analysis on 148 AD and 152 elderly control plasma samples and used univariate and multivariate analysis methods.

## Results

We replicated our previous lipids associations and reported novel associations between lipids molecules and all phenotypes. A combination of 24 molecules classified AD patients with >70% accuracy in a test and a validation data set, and we identified lipid signatures that predicted disease progression ($R^2$ = 0.10, test data set) and brain atrophy ($R^2 \geq 0.14$, all test data sets except left entorhinal cortex). We putatively identified a number of metabolic features including cholesteryl esters/triglycerides and phosphatidylcholines.

## Discussion

Blood lipids are promising AD biomarkers that may lead to new treatment strategies.

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# Macrophage Migration Inhibitory Factor is subjected to glucose modification and oxidation in Alzheimer's Disease

Glucose and glucose metabolites are able to adversely modify proteins through a non-enzymatic reaction called glycation, which is associated with the pathology of Alzheimer's Disease (AD) and is a characteristic of the hyperglycaemia induced by diabetes. However, the precise protein glycation profile that characterises AD is poorly defined and the molecular link between hyperglycaemia and AD is unknown. In this study, we define an early glycation profile of human brain using fluorescent phenylboronate gel electrophoresis and identify early glycation and oxidation of macrophage migration inhibitory factor (MIF) in AD brain. This modification inhibits MIF enzyme activity and ability to stimulate glial cells. MIF is involved in immune response and insulin regulation, hyperglycaemia, oxidative stress and glycation are all implicated in AD. Our study indicates that glucose modified and oxidised MIF could be a molecular link between hyperglycaemia and the dysregulation of the innate immune system in AD.

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# Hallmarks of Alzheimer's Disease in Stem-Cell-Derived Human Neurons Transplanted into Mouse Brain

Human pluripotent stem cells (PSCs) provide a unique entry to study species-specific aspects of human disorders such as Alzheimer's disease (AD). However, in vitro culture of neurons deprives them of their natural environment. Here we transplanted human PSC-derived cortical neuronal precursors into the brain of a murine AD model. Human neurons differentiate and integrate into the brain, express 3R/4R Tau splice forms, show abnormal phosphorylation and conformational Tau changes, and undergo neurodegeneration. Remarkably, cell death was dissociated from tangle formation in this natural 3D model of AD. Using genome-wide expression analysis, we observed upregulation of genes involved in myelination and downregulation of genes related to memory and cognition, synaptic transmission, and neuron projection. This novel chimeric model for AD displays human-specific pathological features and allows the analysis of different genetic backgrounds and mutations during the course of the disease.

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# The amino acid metabolite homocysteine activates mTORC1 to inhibit autophagy and form abnormal proteins in human neurons and mice

The molecular mechanisms leading to and responsible for age-related, sporadic Alzheimer's disease (AD) remain largely unknown. It is well documented that aging patients with elevated levels of the amino acid metabolite homocysteine (Hcy) are at high risk of developing AD. We investigated the impact of Hcy on molecular clearance pathways in mammalian cells, including *in vitro* cultured induced pluripotent stem cell-derived forebrain neurons and *in vivo* neurons in mouse brains. Exposure to Hcy resulted in up-regulation of the mechanistic target of rapamycin complex 1 (mTORC1) activity, one of the major kinases in cells that is tightly linked to anabolic and catabolic pathways. Hcy is sensed by a constitutive protein complex composed of leucyl-tRNA-synthetase and folliculin, which regulates mTOR tethering to lysosomal membranes. In hyperhomocysteinemic human cells and cystathionine β-synthase-deficient mouse brains, we find an acute and chronic inhibition of the molecular clearance of protein products resulting in a buildup of abnormal proteins, including β-amyloid and phospho-Tau. Formation of these protein aggregates leads to AD-like neurodegeneration. This pathology can be prevented by inhibition of mTORC1 or by induction of autophagy. We conclude that an increase of intracellular Hcy levels predisposes neurons to develop abnormal protein aggregates, which are hallmarks of AD and its associated onset and pathophysiology with age.—Khayati, K., Antikainen, H., Bonder, E. M., Weber, G. F., Kruger, W. D., Jakubowski, H., Dobrowolski, R. The amino acid metabolite homocysteine activates mTORC1 to inhibit autophagy and form abnormal proteins in human neurons and mice.

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# Loss of Glyoxalase 1 Induces Compensatory Mechanism to Achieve Dicarbonyl Detoxification in Mammalian Schwann Cells*

The glyoxalase system is a highly specific enzyme system existing in all mammalian cells that is responsible for the detoxification of dicarbonyl species, primarily methylglyoxal (MG). It has been implicated to play an essential role in preventing the increased formation of advanced glycation end products under certain pathological conditions. We have established the first glyoxalase 1 knock-out model ($GLO1^{-/-}$) in mammalian Schwann cells using the CRISPR/Cas9 technique to investigate compensatory mechanisms. Neither elevated concentrations of MG nor associated protein modifications were observed in $GLO1^{-/-}$ cells. Alternative detoxification of MG in $GLO1^{-/-}$ is achieved by increased catalytic efficiency of aldose reductase toward hemithioacetal (product of glutathione and MG), which is most likely caused by *S*-nitrosylation of aldose reductase. The hemithioacetal is mainly converted into lactaldehyde, which is paralleled by a loss of reduced glutathione. Inhibition of aldose reductase in $GLO1^{-/-}$ cells is associated with an increased sensitivity against MG, elevated intracellular MG levels, associated modifications, as well as increased oxidative stress. Our data suggest that aldose reductase can compensate for the loss of GLO1. This might be of clinical importance within the context of neuronal diseases caused by an impaired glyoxalase system and elevated levels of dicarbonyl species, such as MG.

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# The Substantial Loss of Nephrons in Healthy Human Kidneys with Aging

Nephron number may be an important determinant of kidney health but has been difficult to study in living humans. We evaluated 1638 living kidney donors at Mayo Clinic (MN and AZ sites) and Cleveland Clinic. We obtained cortical volumes of both kidneys from predonation computed tomography scans. At the time of kidney transplant, we obtained and analyzed the sections of a biopsy specimen of the cortex to determine the density of both nonsclerotic and globally sclerotic glomeruli; the total number of glomeruli was estimated from cortical volume×glomerular density. Donors 18–29 years old had a mean 990,661 nonsclerotic glomeruli and 16,614 globally sclerotic glomeruli per kidney, which progressively decreased to 520,410 nonsclerotic glomeruli per kidney and increased to 141,714 globally sclerotic glomeruli per kidney in donors 70–75 years old. Between the youngest and oldest age groups, the number of nonsclerotic glomeruli decreased by 48%, whereas cortical volume decreased by only 16% and the proportion of globally sclerotic glomeruli on biopsy increased by only 15%. Clinical characteristics that independently associated with fewer nonsclerotic glomeruli were older age, shorter height, family history of ESRD, higher serum uric acid level, and lower measured GFR. The incomplete representation of nephron loss with aging by either increased glomerulosclerosis or by cortical volume decline is consistent with atrophy and reabsorption of globally sclerotic glomeruli and hypertrophy of remaining nephrons. In conclusion, lower nephron number in healthy adults associates with characteristics reflective of both lower nephron endowment at birth and subsequent loss of nephrons.

# IL-10 prevents aging-associated inflammation and insulin resistance in skeletal muscle

Altered energy balance and insulin resistance are important characteristics of aging. Skeletal muscle is a major site of glucose disposal, and the role of aging-associated inflammation in skeletal muscle insulin resistance remains unclear. To investigate, we examined glucose metabolism in 18-mo-old transgenic mice with muscle-specific overexpression of IL-10 ($M^{IL10}$) and in wild-type mice during hyperinsulinemic-euglycemic clamping. Despite similar fat mass and energy balance, $M^{IL10}$ mice were protected from aging-associated insulin resistance with significant increases in glucose infusion rates, whole-body glucose turnover, and skeletal muscle glucose uptake (~60%; $P < 0.05$), as compared to age-matched WT mice. This protective effect was associated with decreased muscle inflammation, but no changes in adipose tissue inflammation in aging $M^{IL10}$ mice. These results demonstrate the importance of skeletal muscle inflammation in aging-mediated insulin resistance, and our findings further implicate a potential therapeutic role of anti-inflammatory cytokine in the treatment of aging-mediated insulin resistance. —Dagdeviren, S., Jung, D. Y., Friedline, R. H., Noh, H. L., Kim, J. H., Patel, P. R., Tsitsilianos, N., Inashima, K., Tran, D. A., Hu, X., Loubato, M. M., Craige, S. M., Kwon, J. Y., Lee, K. W., Kim, J. K. IL-10 prevents aging-associated inflammation and insulin resistance in skeletal muscle.

# The atheroma plaque secretome stimulates the mobilization of endothelial progenitor cells *ex vivo*

Endothelial progenitor cells (EPCs) constitute a promising alternative in cardiovascular regenerative medicine due to their assigned role in angiogenesis and vascular repair. In response to injury, EPCs promote vascular remodeling by replacement of damaged endothelial cells and/or by secreting angiogenic factors over the damaged tissue. Nevertheless, such mechanisms need to be further characterized. In the current approach we have evaluated the initial response of early EPCs (eEPCs) from healthy individuals after direct contact with the factors released by carotid arteries complicated with atherosclerotic plaques (AP), in order to understand the mechanisms underlying the neovascularization and remodeling properties assigned to these cells. Herein, we found that the AP secretome stimulated eEPCs proliferation and mobilization ***ex vivo***, and such increase was accompanied by augmented permeability, cell contraction and also an increase of cell-cell adhesion in association with raised vinculin levels. Furthermore, a comparative mass spectrometry analysis of control *versus* stimulated eEPCs revealed a differential expression of proteins in the AP treated cells, mostly involved in cell migration, proliferation and vascular remodeling. Some of these protein changes were also detected in the eEPCs isolated from atherosclerotic patients compared to eEPCs from healthy donors.

We have shown, for the first time, that the AP released factors activate eEPCs ***ex vivo*** by inducing their mobilization together with the expression of vasculogenic related markers. The present approach could be taken as a ***ex vivo*** model to study the initial activation of vascular cells in atherosclerosis and also to evaluate strategies looking to potentiate the mobilization of EPCs prior to clinical applications.

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# Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue

Concurrent with a progressive loss of regenerative capacity, connective tissue aging is characterized by a progressive accumulation of Advanced Glycation End-products (AGEs). Besides being part of the typical aging process, type II diabetics are particularly affected by AGE accumulation due to abnormally high levels of systemic glucose that increases the glycation rate of long-lived proteins such as collagen. Although AGEs are associated with a wide range of clinical disorders, the mechanisms by which AGEs contribute to connective tissue disease in aging and diabetes are still poorly understood. The present study harnesses advanced multiscale imaging techniques to characterize a widely employed *in vitro* model of ribose induced collagen aging and further benchmarks these data against experiments on native human tissues from donors of different age. These efforts yield unprecedented insight into the mechanical changes in collagen tissues across hierarchical scales from molecular, to fiber, to tissue-levels. We observed a linear increase in molecular spacing (from 1.45 nm to 1.5 nm) and a decrease in the D-period length (from 67.5 nm to 67.1 nm) in aged tissues, both using the ribose model of *in vitro* glycation and in native human probes. Multiscale mechanical analysis of *in vitro* glycated tendons strongly suggests that AGEs reduce tissue viscoelasticity by severely limiting fiber–fiber and fibril–fibril sliding. This study lays an important foundation for interpreting the functional and biological effects of AGEs in collagen connective tissues, by exploiting experimental models of AGEs crosslinking and benchmarking them for the first time against endogenous AGEs in native tissue.

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# Extracellular matrix downregulation in the *Drosophila* heart preserves contractile function and improves lifespan

Aging is associated with extensive remodeling of the heart, including basement membrane (BM) components that surround cardiomyocytes. Remodeling is thought to impair cardiac mechanotransduction, but the contribution of specific BM components to age-related lateral communication between cardiomyocytes is unclear. Using a genetically tractable, rapidly aging model with sufficient cardiac genetic homology and morphology, e.g. *Drosophila melanogaster*, we observed differential regulation of BM collagens between laboratory strains, correlating with changes in muscle physiology leading to cardiac dysfunction. Therefore, we sought to understand the extent to which BM proteins modulate contractile function during aging. Cardiac-restricted knockdown of ECM genes *Pericardin*, *Laminin A*, and *Viking* in *Drosophila* prevented age-associated heart tube restriction and increased contractility, even under viscous load. Most notably, reduction of *Laminin A* expression correlated with an overall preservation of contractile velocity with age and extension of organismal lifespan. Global heterozygous knockdown confirmed these data, which provides new evidence of a direct link between BM homeostasis, contractility, and maintenance of lifespan.

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# Tissue Transglutaminase Modulates Vascular Stiffness and Function Through Crosslinking-Dependent and Crosslinking-Independent Functions


**Background** The structural elements of the vascular wall, namely, extracellular matrix and smooth muscle cells (SMCs), contribute to the overall stiffness of the vessel. In this study, we examined the crosslinking-dependent and crosslinking-independent roles of tissue transglutaminase (TG2) in vascular function and stiffness.

**Methods and Results** SMCs were isolated from the aortae of TG2−/− and wild-type (WT) mice. Cell adhesion was examined by using electrical cell–substrate impedance sensing and PicoGreen assay. Cell motility was examined using a Boyden chamber assay. Cell proliferation was examined by electrical cell–substrate impedance sensing and EdU incorporation assays. Cell micromechanics were studied using magnetic torsion cytometry and spontaneous nanobead tracer motions. Aortic mechanics were examined by tensile testing. Vasoreactivity was studied by wire myography. SMCs from TG2−/− mice had delayed adhesion, reduced motility, and accelerated de-adhesion and proliferation rates compared with those from WT. TG2−/− SMCs were stiffer and displayed fewer cytoskeletal remodeling events than WT. Collagen assembly was delayed in TG2−/− SMCs and recovered with adenoviral transduction of TG2. Aortic rings from TG2−/− mice were less stiff than those from WT; stiffness was partly recovered by incubation with guinea pig liver TG2 independent of crosslinking function. TG2−/− rings showed augmented response to phenylephrine-mediated vasoconstriction when compared with WT. In human coronary arteries, vascular media and plaque, high abundance of fibronectin expression, and colocalization with TG2 were observed.

**Conclusions** TG2 modulates vascular function/tone by altering SMC contractility independent of its crosslinking function and contributes to vascular stiffness by regulating SMC proliferation and matrix remodeling.

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# Chronic Treatment with Minoxidil Induces Elastic Fiber Neosynthesis and Functional Improvement in the Aorta of Aged Mice

Normal arterial aging processes involve vascular cell dysfunction associated with wall stiffening, the latter being due to progressive elastin and elastic fiber degradation, and elastin and collagen cross-linking by advanced glycation end products (AGEs). These processes progressively lead to cardiovascular dysfunction during aging. Elastin is only synthesized during late gestation and childhood, and further degradation occurring throughout adulthood cannot be physiologically compensated by replacement of altered material. However, the ATP-dependent $K^+$ channel opener minoxidil has been shown to stimulate elastin expression *in vitro* and *in vivo* in the aorta of young adult rats. Therefore, we have studied the effect of a 10-week chronic oral treatment with minoxidil (120 mg/L in drinking water) on the aortic structure and function in aged 24-month-old mice. Minoxidil treatment increased tropoelastin, fibulin-5, and lysyl-oxidase messenger RNA levels, reinduced a moderate expression of elastin, and lowered the levels of AGE-related molecules. This was accompanied by the formation of newly synthesized elastic fibers, which had diverse orientations in the wall. A decrease in the glycation capacity of aortic elastin was also produced by minoxidil treatment. The ascending aorta also underwent a minoxidil-induced increase in diameter and decrease in wall thickness, which partly reversed the age-associated thickening and returned the wall thickness value and strain-stress relation closer to those of younger adult animals. In conclusion, our results suggest that minoxidil presents an interesting potential for arterial remodeling in an antiaging perspective, even when treating already aged animals.

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# Suspected non Alzheimer's pathology – Is it non-Alzheimer's or non-amyloid?

Neurodegeneration, the progressive loss of neurons, is a major process involved in dementia and age-related cognitive impairment. It can be detected clinically using currently available biomarker tests. Suspected Non Alzheimer Pathology (SNAP) is a biomarker-based concept that encompasses a group of individuals with neurodegeneration, but no evidence of amyloid deposition (thereby distinguishing it from Alzheimer's disease (AD)). These individuals may often have a clinical diagnosis of AD, but their clinical features, genetic susceptibility and progression can differ significantly, carrying crucial implications for precise diagnostics, clinical management, and efficacy of clinical drug trials.

SNAP has caused wide interest in the dementia research community, because it is still unclear whether it represents distinct pathology separate from AD, or whether in some individuals, it could represent the earliest stage of AD. This debate has raised pertinent questions about the pathways to AD, the need for biomarkers, and the sensitivity of current biomarker tests.

In this review, we discuss the biomarker and imaging trials that first recognised SNAP. We describe the pathological correlates of SNAP and comment on the different causes of neurodegeneration. Finally, we discuss the debate around the concept of SNAP, and further unanswered questions that are emerging.

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# Restricted access: spatial sequestration of damaged proteins during stress and aging

The accumulation of damaged and aggregated proteins is a hallmark of aging and increased proteotoxic stress. To limit the toxicity of damaged and aggregated proteins and to ensure that the damage is not inherited by succeeding cell generations, a system of spatial quality control operates to sequester damaged/aggregated proteins into inclusions at specific protective sites. Such spatial sequestration and asymmetric segregation of damaged proteins have emerged as key processes required for cellular rejuvenation. In this review, we summarize findings on the nature of the different quality control sites identified in yeast, on genetic determinants required for spatial quality control, and on how aggregates are recognized depending on the stress generating them. We also briefly compare the yeast system to spatial quality control in other organisms. The data accumulated demonstrate that spatial quality control involves factors beyond the canonical quality control factors, such as chaperones and proteases, and opens up new venues in approaching how proteotoxicity might be mitigated, or delayed, upon aging.

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# Does transcription-associated DNA damage limit lifespan?

Small mammals undergo an aging process similar to that of larger mammals, but aging occurs at a dramatically faster rate. This phenomenon is often assumed to be the result of damage caused by reactive oxygen species generated in mitochondria. An alternative explanation for the phenomenon is suggested here. The rate of RNA synthesis is dramatically elevated in small mammals and correlates quantitatively with the rate of aging among different mammalian species. The rate of RNA synthesis is reduced by caloric restriction and inhibition of TOR pathway signaling, two perturbations that increase lifespan in multiple metazoan species. From bacteria to man, the transcription of a gene has been found to increase the rate at which it is damaged, and a number of lines of evidence suggest that DNA damage is sufficient to induce multiple symptoms associated with normal aging. Thus, the correlations frequently found between the rate of RNA synthesis and the rate of aging could potentially reflect an important role for transcription-associated DNA damage in the aging process.

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# Evidence that a mitochondrial death spiral underlies antagonistic pleiotropy

The antagonistic pleiotropy (AP) theory posits that aging occurs because alleles that are detrimental in older organisms are beneficial to growth early in life and thus are maintained in populations. Although genes of the insulin signaling pathway likely participate in AP, the insulin-regulated cellular correlates of AP have not been identified. The mitochondrial quality control process called mitochondrial autophagy (mitophagy), which is inhibited by insulin signaling, might represent a cellular correlate of AP. In this view, rapidly growing cells are limited by ATP production; these cells thus actively inhibit mitophagy to maximize mitochondrial ATP production and compete successfully for scarce nutrients. This process maximizes early growth and reproduction, but by permitting the persistence of damaged mitochondria with mitochondrial DNA mutations, becomes detrimental in the longer term. I suggest that as mitochondrial ATP output drops, cells respond by further inhibiting mitophagy, leading to a further decrease in ATP output in a classic death spiral. I suggest that this increasing ATP deficit is communicated by progressive increases in mitochondrial ROS generation, which signals inhibition of mitophagy via ROS-dependent activation of insulin signaling. This hypothesis clarifies a role for ROS in aging, explains why insulin signaling inhibits autophagy, and why cells become progressively more oxidized during aging with increased levels of insulin signaling and decreased levels of autophagy. I suggest that the mitochondrial death spiral is not an error in cell physiology but rather a rational approach to the problem of enabling successful growth and reproduction in a competitive world of scarce nutrients.

# REVIEWS/COMMENTS/EDITORIALS

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# Animal and human models to understand ageing

Human ageing is the gradual decline in organ and tissue function with increasing chronological time, leading eventually to loss of function and death. To study the processes involved over research-relevant timescales requires the use of accessible model systems that share significant similarities with humans. In this review, we assess the usefulness of various models, including unicellular yeasts, invertebrate worms and flies, mice and primates including humans, and highlight the benefits and possible drawbacks of each model system in its ability to illuminate human ageing mechanisms. We describe the strong evolutionary conservation of molecular pathways that govern cell responses to extracellular and intracellular signals and which are strongly implicated in ageing. Such pathways centre around insulin-like growth factor signalling and integration of stress and nutritional signals through mTOR kinase. The process of cellular senescence is evaluated as a possible underlying cause for many of the frailties and diseases of human ageing. Also considered is ageing arising from systemic changes that cannot be modelled in lower organisms and instead require studies either in small mammals or in primates. We also touch briefly on novel therapeutic options arising from a better understanding of the biology of ageing.

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Medical News & Perspectives

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# Alzheimer Outlook Far From Bleak

Jeff Lyon

Article Information

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Author Interview

Last summer, deep disappointment befell the Alzheimer disease (AD) community when study results showed that the widely heralded experimental drug LMTX had failed to help AD patients. In November, another promising drug, solanezumab, also dashed hopes. Because these drugs target either amyloid β (solanezumab) or tau (LMTX), proteins that aggregate into the plaques and tangles in brain tissue characteristic of AD, some have suggested that researchers are following the wrong path by attacking these proteins and that AD research is back to square one after decades of work.

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# Calcium Hypothesis of Alzheimer's disease and brain aging: A framework for integrating new evidence into a comprehensive theory of pathogenesis

This article updates the *Calcium Hypothesis of Alzheimer's disease and brain aging* on the basis of emerging evidence since 1994 (The present article, with the subtitle "New evidence for a central role of $Ca^{2+}$ in neurodegeneration," includes three appendices that provide context and further explanations for the rationale for the revisions in the updated hypothesis—the three appendices are as follows: Appendix I "Emerging concepts on potential pathogenic roles of $[Ca^{2+}]$," Appendix II "Future studies to validate the central role of dysregulated $[Ca^{2+}]$ in neurodegeneration," and Appendix III "Epilogue: towards a comprehensive hypothesis.") (Marx J. Fresh evidence points to an old suspect: calcium. Science 2007; 318:384–385). The aim is not only to re-evaluate the original key claims of the hypothesis with a critical eye but also to identify gaps in knowledge required to validate relevant claims and delineate additional studies and/or data that are needed. Some of the key challenges for this effort included examination of questions regarding (1) the temporal and spatial relationships of molecular mechanisms that regulate neuronal *calcium ion* ($Ca^{2+}$), (2) the role of changes in *concentration of calcium ion* $[Ca^{2+}]$ in various subcellular compartments of neurons, (3) how alterations in $Ca^{2+}$ signaling affect the performance of neurons under various conditions, ranging from optimal functioning in a healthy state to conditions of decline and deterioration in performance during aging and in disease, and (4) new ideas about the contributions of aging, genetic, and environmental factors to the causal relationships between dysregulation of $[Ca^{2+}]$ and the functioning of neurons (see Appendices I and II). The updated *Calcium Hypothesis* also includes revised postulates that are intended to promote further crucial experiments to confirm or reject the various predictions of the hypothesis (see Appendix III).

# GH and ageing: Pitfalls and new insights

The interrelationships of growth hormone (GH) actions and aging are complex and incompletely understood. The very pronounced age-related decline in GH secretion together with benefits of GH therapy in individuals with congenital or adult GH deficiency (GHD) prompted interest in GH as an anti-aging agent. However, the benefits of treatment of normal elderly subjects with GH appear to be marginal and counterbalanced by worrisome side effects.

In laboratory mice, genetic GH deficiency or resistance leads to a remarkable extension of longevity accompanied by signs of delayed and/or slower aging. Mechanisms believed to contribute to extended longevity of GH-related mutants include improved anti-oxidant defenses, enhanced insulin sensitivity and reduced insulin levels, reduced inflammation and cell senescence, major shifts in mitochondrial function and energy metabolism, and greater stress resistance. Negative association of the somatotropic signaling and GH/insulin-like growth factor 1 (IGF-1)-dependent traits with longevity has also been shown in other mammalian species. In humans, syndromes of GH resistance or deficiency have no consistent effect on longevity, but can provide striking protection from cancer, diabetes and atherosclerosis. More subtle alterations in various steps of GH and IGF-1 signaling are associated with reduced old-age mortality, particularly in women and with improved chances of attaining extremes of lifespan. Epidemiological studies raise a possibility that the relationship of IGF-1 and perhaps also GH levels with human healthy aging and longevity may be biphasic. However, the impact of somatotropic signaling on neoplastic disease is difficult to separate from its impact on aging, and IGF-1 levels exhibit opposite associations with different chronic, age-related diseases.

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Insights & Perspectives

# Targeting the SASP to combat ageing: Mitochondria as possible intracellular allies?

Jodie Birch, João F. Passos

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## Abstract

Anti-senescence therapies, such as drugs that specifically kill senescent cells, to stave off ageing are currently under investigation. While these interventions show promise, their potential pitfalls are discussed herein. We have shown that the mitochondria are essential for development of senescence and many of the associated phenotypes, including the often detrimental senescence-associated secretory phenotype (SASP). Here, we disentangle many ways in which the mitochondria may influence senescence and development of the SASP and focus on possible pathways that could be exploited for future generation of anti-senescence therapies with a clear aim; to specifically eliminate the problematic features of senescent cells, while maintaining their beneficial characteristics.

Review

# The gut microbiome in human neurological disease: A review

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## Abstract

Almost half the cells and 1% of the unique genes found in our bodies are human, the rest are from microbes; predominantly bacteria, archaea, fungi, and viruses. These microorganisms collectively form the human microbiota, with most colonizing the gut. Recent technological advances, open access data-libraries, and application of high throughput sequencing have allowed these microbes to be identified and their contribution to neurological health examined. Emerging evidence links perturbations in the gut microbiota to neurological disease, including disease risk, activity, and progression. This review provides an overview of the recent advances in microbiome research in relation to neuro(auto)immune and neurodegenerative conditions affecting humans such as multiple sclerosis, neuromyelitis optica spectrum disorders, Parkinson's, Alzheimer's, Huntington's, and amyotrophic lateral sclerosis. Study design and terminology used in this rapidly evolving, highly multi-disciplinary field are summarized to empower and engage the neurology community in this 'newly discovered organ.' This article is protected by copyright. All rights reserved.

# OTHER RESEARCH

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Viewpoint

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# Acknowledging and Overcoming Nonreproducibility in Basic and Preclinical Research

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» Author Affiliations | Article Information

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The evidence for nonreproducibility in basic and preclinical biomedical research is compelling. Accumulating data from diverse subdisciplines and types of experimentation suggest numerous problems that can create a fertile ground for nonreproducibility.[1] For example, most raw data and protocols are often not available for in-depth scrutiny and use by other scientists. The current incentive system rewards selective reporting of success stories. There is poor use of statistical methods, and study designs are often suboptimal. Simple laboratory flaws —eg, contamination or incorrect identification of widely used cell lines—occur with some frequency.

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Users' Guides to the Medical Literature

February 21, 2017

# Adjusted Analyses in Studies Addressing Therapy and Harm

## Users' Guides to the Medical Literature

Thomas Agoritsas, MD, PhD[1,2]; Arnaud Merglen, MD, MSc[2,3,4]; Nilay D. Shah, PhD[5]; et al

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