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Scientific News
14th of October 2018
Sven Bulterijs

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Fourth Eurosymposium on Healthy Ageing

We envision a world free of age-related diseases

November 8-10, 2018
Muntpunt, Brussels (Belgium)

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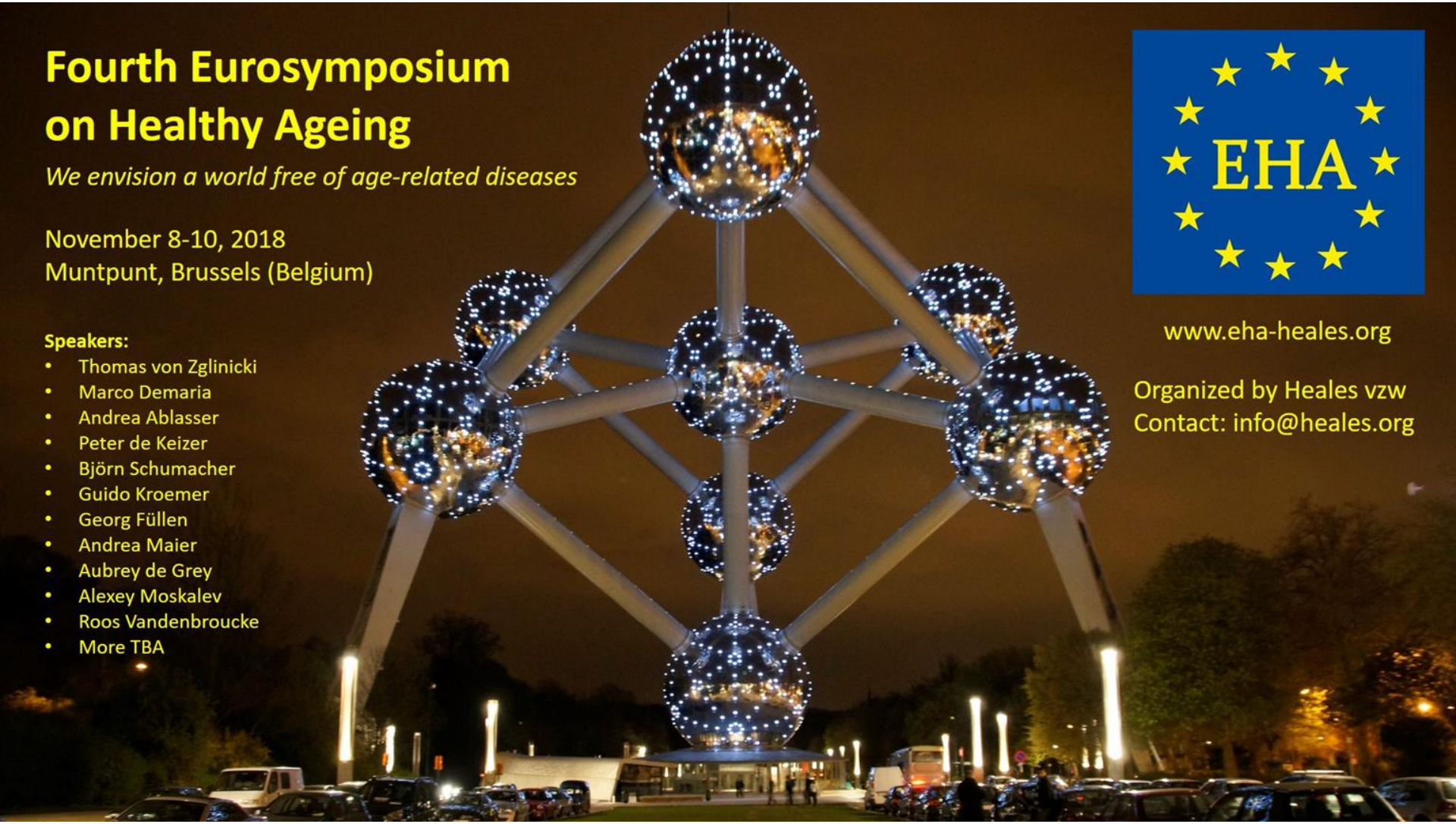
- Marco Demaria
- Andrea Ablasser
- Peter de Keizer
- Björn Schumacher
- Guido Kroemer
- Georg Füllen
- Andrea Maier
- Aubrey de Grey
- Alexey Moskalev
- Roos Vandenbroucke
- More TBA



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Elevian Launches to Develop Regenerative Medicines for Age-Related Diseases

Elevian
REIMAGINE AGING

Commercializing scientific breakthroughs from Elevian's scientific co-founders

NEWS PROVIDED BY
Elevian →
Sep 06, 2018, 11:00 ET

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SAN FRANCISCO, Sept. 6, 2018 /PRNewswire/ -- Elevian, a new company developing medicines that restore youthful regenerative capacity, with the potential to treat and prevent age-related disease, launches at TechCrunch Disrupt's Startup Battlefield. The company has secured $5.5M in seed funding to date from Bold Capital (led by founding Elevian investor Peter Diamandis), WTI, Stanford StartX fund, Longevity fund, Kizoo Ventures, Thynk Capital, and other investors.

"By therapeutically targeting a fundamental mechanism of aging, it may be possible to treat and prevent many diseases of aging with a single drug." – Mark Allen, MD, Co-founder, CEO

Elevian's founders, working at Stanford and then Harvard, discovered the regenerative effects of young blood. Using a model called parabiosis, they combined the circulatory systems of young and old mice, allowing blood to flow between the animals. After 30 days, they found that young blood regenerated many tissues and organs in the old mice.

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Ichor's Auctus Biologics Closes Quick $1.5MM

September 21, 2018 02:13 PM Eastern Daylight Time

LAFAYETTE, N.Y.--(BUSINESS WIRE)--Auctus Biologics, Inc., a new portfolio company of Ichor Therapeutics, Inc., announced today the closure of $1.5MM in seed funding. The company will develop RPtag, a hyper-stable antibody mimetic scaffold published earlier this year in the peer-reviewed journal ACS Biochemistry, to take on conventional clinical antibody therapy as an orally bioavailable formulation. New high priority immunosenescence and gastrointestinal targets will also be pursued.

"This technology was originally developed for protein expression applications. By following the data and affording the team an appropriate level of scientific freedom, we have created a robust therapeutic platform that can operate in environments where biologics are traditionally limited."

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"The RPtag platform opens up a new frontier in biological drug discovery and development," said Kelsey Moody, CEO at Auctus Biologics. "Although there may be significant opportunities to develop this platform as an oral formulation to replace the need for conventional intravenous infusions, we are also very excited about the prospect of deploying this technology to modulate gut micro-flora and to go after other gut targets that may drive age-associated disease and related processes."

"This program is a testament to the excellence of our research teams and their ability to identify unique value in all its manifestations," said Aaron Wolfe, COO at Auctus Biologics and co-inventor of the platform. "This technology was originally developed for protein expression applications. By following the data and affording the team an appropriate level of scientific freedom, we have created a robust therapeutic platform that can operate in environments where biologics are traditionally limited."

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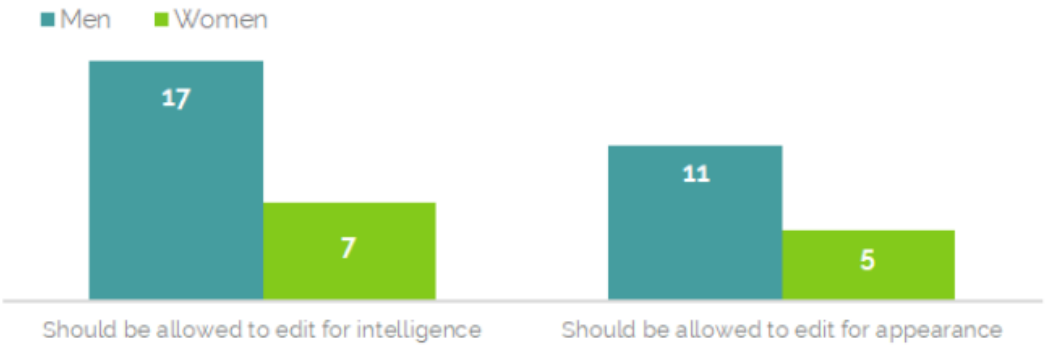
Most Brits say gene editing to reduce risk of disease should be allowed

Eight in ten Brits support gene editing to prevent passing on genetic disorders

If the technology was available, do you think gene editing should be allowed in order to prevent people from passing on hereditary genetic disorders, such as cystic fibrosis or Huntington's disease...? %

Men more than twice as likely as women to support gene editing for intelligence and appearance

If the technology was available, do you think gene editing should be allowed in order to change people's intelligence or appearance...? %



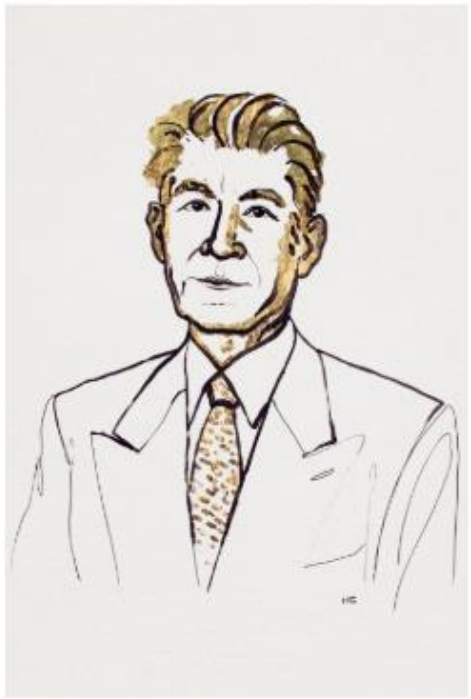
Nobel Prize for checkpoint inhibitors

The Nobel Prize in Physiology or Medicine 2018

Ill. Niklas Elmehed. © Nobel Media

James P. Allison

Prize share: 1/2



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Tasuku Honjo

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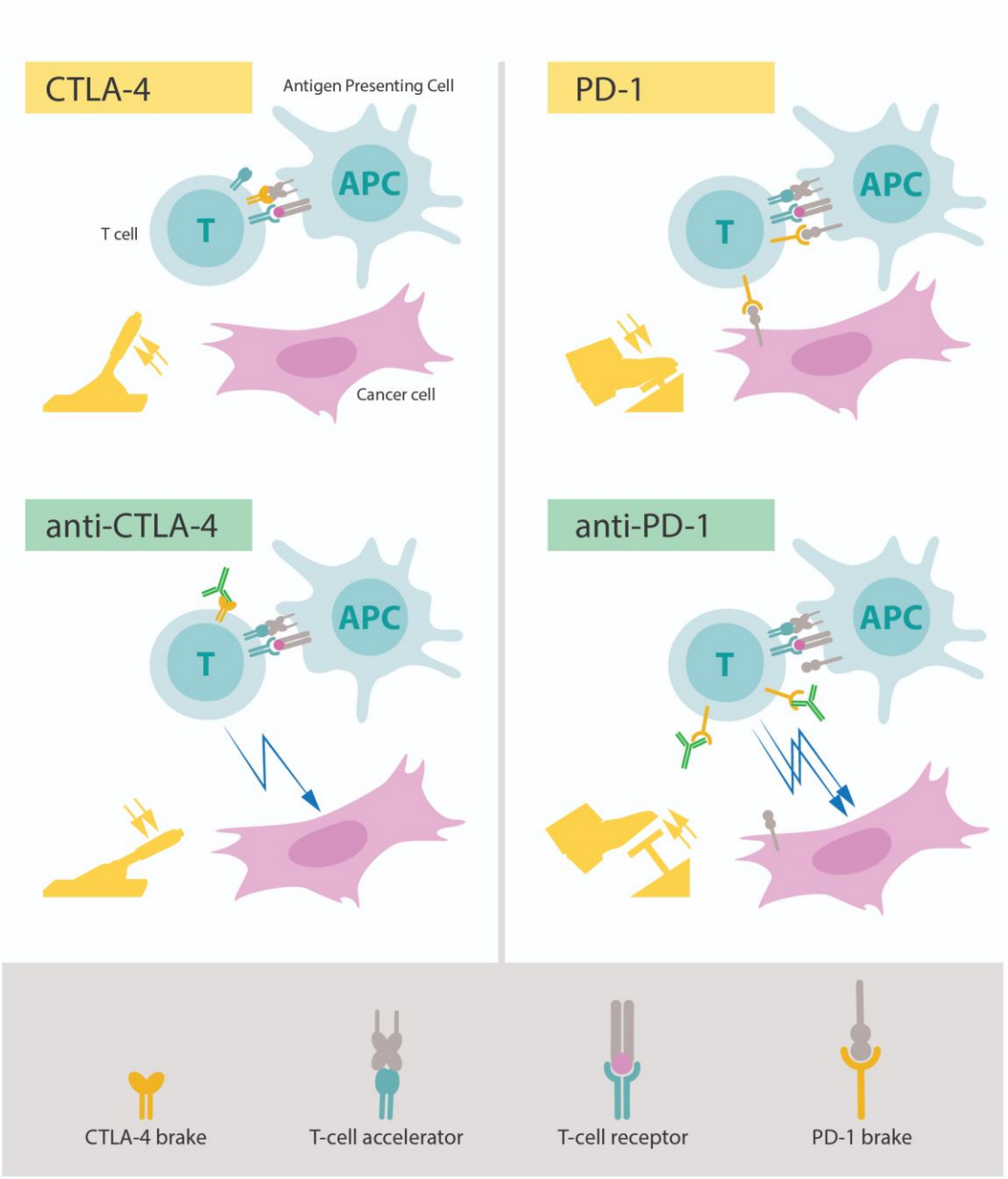

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The Nobel Prize in Chemistry 2018

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Frances H. Arnold

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George P. Smith

Prize share: 1/4

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Sir Gregory P. Winter

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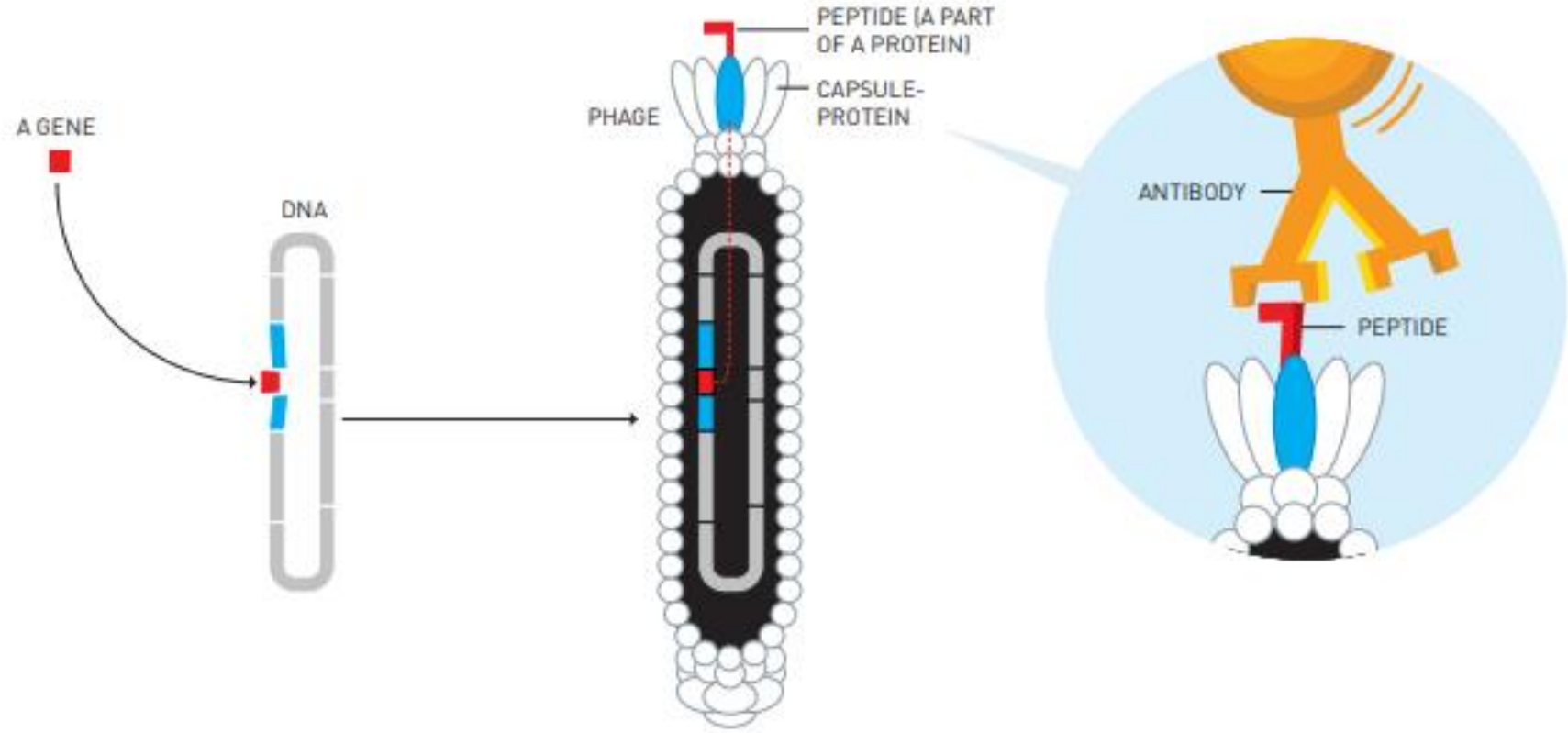

1 Smith introduced a gene into the gene for a protein in the phage's capsule. The phage DNA was then inserted into bacteria that produced phages.

2 The peptide produced from the introduced gene ended up as part of the capsule protein on the surface of the phage.

3 Smith was able to fish out the phage using an antibody designed to attach to the peptide. As a bonus, he got the gene for the peptide.

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Comprehensive transcriptome profiling in elderly cancer patients reveals aging-altered immune cells and immune checkpoints

Aging is the single most significant risk factor for cancer development. However, the potential impact of aging on cancer microenvironment remains poorly understood. Here, we performed a pan-cancer transcriptome analysis to identify aging-specific molecular patterns across 18 cancer types. Strikingly, aging-specific molecular features define human cancers into two types, including the strong and weak aging-effect groups. Significant aging associated molecular signature was observed in 16 cancer types (strong aging-effect group) such as breast invasive carcinoma and acute myeloid leukemia. In such 16 cancer types, old patients showed obvious poor survival compared to young patients, but this observation was not found in the weak aging-effect cancers. Aging-associated cancer-relevant molecules significantly enriched in 23 pathways including EMT and KRAS signaling. More interestingly, in cancer microenvironment, aging significantly restrains adaptive immunity, but strikingly, increases the number of infiltrated innate immune cells. Further analysis shows that the expression of immune checkpoints including PD-1, PD-L1, PD-L2, and CTLA-4 are mostly correlated with age. In general, cancer cells in elderly patients show a more aggressive phenotype and their surrounding microenvironment is under a more immune suppression status compared with young patients. Our study provides a systematic understanding of aging-associated molecular features in pan-cancer and indicates a clinical requirement to develop aging-specific therapeutic strategies in a majority of cancer types. Furthermore, aging-altered immune cells and immune checkpoints should be considered in cancer immunotherapy.

Metformin Promotes Antitumor Immunity via Endoplasmic-Reticulum-Associated Degradation of PD-L1

Metformin has been reported to possess antitumor activity and maintain high cytotoxic T lymphocyte (CTL) immune surveillance. However, the functions and detailed mechanisms of metformin's role in cancer immunity are not fully understood. Here, we show that metformin increases CTL activity by reducing the stability and membrane localization of programmed death ligand-1 (PD-L1). Furthermore, we discover that AMP-activated protein kinase (AMPK) activated by metformin directly phosphorylates S195 of PD-L1. S195 phosphorylation induces abnormal PD-L1 glycosylation, resulting in its ER accumulation and ER-associated protein degradation (ERAD). Consistently, tumor tissues from metformin-treated breast cancer patients exhibit reduced PD-L1 levels with AMPK activation. Blocking the inhibitory signal of PD-L1 by metformin enhances CTL activity against cancer cells. Our findings identify a new regulatory mechanism of PD-L1 expression through the ERAD pathway and suggest that the metformin-CTLA4 blockade combination has the potential to increase the efficacy of immunotherapy.

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Effects of metformin use on total mortality in patients with type 2 diabetes and chronic obstructive pulmonary disease: A matched-subject design

Methods

We conducted a retrospective cohort study for patients with T2DM and COPD who were enrolled between January 1, 2000 and June 30, 2012. Individuals with exacerbated symptoms who were hospitalized or sent to the emergency department (ED) were identified as having exacerbated COPD; outpatient claims were identified as having stable COPD. A total of 40,597 metformin users and 39,529 nonusers comprised the cohort of stable COPD; 14,001 metformin users and 21,613 nonusers comprised the cohort of exacerbated COPD. Users and nonusers were matched using propensity score (1:1). Our primary outcome was all-cause mortality.

Results

A total of 19,505 metformin users were matched to 19,505 nonusers in the cohort of diabetes with stable COPD. The mean follow-up time was 3.91 years. All-cause mortality was reported in 1326 and 1609 metformin users and nonusers, respectively. After multivariate adjustment, metformin users had lower risk of mortality (adjusted hazard ratio [aHR] = 0.84, $p < 0.0001$). Metformin users had significantly lower risk of noncardiovascular death (aHR = 0.86, $p = 0.0008$). A total of 7721 metformin users were matched to 7721 nonusers in the cohort of diabetes with exacerbated COPD. The mean follow-up time was 3.18 years. All-cause mortality was reported in 1567 and 1865 metformin users and nonusers, respectively. After multivariate adjustment, metformin users had significantly lower risk of mortality (aHR = 0.89, $p = 0.002$) and cardiovascular death (aHR = 0.70, $p = 0.01$).

Conclusion

This large-series, nationwide cohort study demonstrated that metformin use could significantly lower the risk of all-cause mortality in patients with T2DM and either stable or exacerbated COPD.

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Modulation of dietary methionine intake elicits potent, yet distinct, anticancer effects on primary versus metastatic tumors

Methionine dependency describes the characteristic rapid *in vitro* death of most tumor cells in the absence of methionine. Combining chemotherapy with dietary methionine deprivation [methionine-deficient diet (MDD)] at tolerable levels has vast potential in tumor treatment; however, it is limited by MDD-induced toxicity during extended deprivation. Recent advances in imaging and irradiation delivery have created the field of stereotactic body radiotherapy (SBRT), where fewer large-dose fractions delivered in less time result in increased local-tumor control, which could be maximally synergistic with an MDD short course. Identification of the lowest effective methionine dietary intake not associated with toxicity will further enhance the cancer therapy potential. In this study, we investigated the effects of MDD and methionine-restricted diet (MRD) in primary and metastatic melanoma models in combination with radiotherapy (RT). *In vitro*, MDD dose-dependently sensitized mouse and human melanoma cell lines to RT. *In vivo* in mice, MDD substantially potentiated the effects of RT by a significant delay in tumor growth, in comparison with administering MDD or RT alone. The antitumor effects of an MDD/RT approach were due to effects on one-carbon metabolism, resulting in impaired methionine biotransformation via downregulation of *Mat2a*, which encodes methionine adenosyltransferase 2A. Furthermore, and probably most importantly, MDD and MRD substantially diminished metastatic potential; the antitumor MRD effects were not associated with toxicity to normal tissue. Our findings suggest that modulation of methionine intake holds substantial promise for use with short-course SBRT for cancer treatment.

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iScience. 2018 Sep 28;7:96-109. doi: 10.1016/j.isci.2018.08.011. Epub 2018 Aug 17.

Fundamental Characteristics of Single-Cell Aging in Diploid Yeast.

Sarnoski EA[1], Song R[2], Ertekin E[1], Koonce N[1], Acar M[3].

Author information

Abstract

Single-cell-level experimentation can elucidate key biological insights about cellular aging that are masked in population-level studies. However, the extensive time requirement of tracking single cells has historically prevented their long-term longitudinal observation. Using a microfluidic device that automates microscopic monitoring of diploid Saccharomyces cerevisiae cells throughout their replicative lifespan, here we report the fundamental characteristics of single-cell aging for diploid yeast. We find that proteins with short versus long half-lives exhibit distinct dynamics as cells age and that the intercellular gene expression noise increases during aging, whereas the intracellular noise stays unchanged. A stochastic model provides quantitative mechanistic insights into the observed noise dynamics and sheds light on the age-dependent intracellular noise differences between diploid and haploid yeast. Our work elucidates how a set of canonical phenotypes dynamically change while the host cells are aging in real time, providing essential insights for a comprehensive understanding on and control of lifespan at the single-cell level.

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Cell Rep. 2018 Oct 2;25(1):199-211.e6. doi: 10.1016/j.celrep.2018.09.009.

Maintenance of Proteostasis by P Body-Mediated Regulation of eIF4E Availability during Aging in Caenorhabditis elegans.

Rieckher M[1], Markaki M[2], Princz A[2], Schumacher B[3], Tavernarakis N[4].

⊕ Author information

Abstract

Aging is accompanied by a pervasive collapse of proteostasis, while reducing general protein synthesis promotes longevity across taxa. Here, we show that the eIF4E isoform IFE-2 is increasingly sequestered in mRNA processing (P) bodies during aging and upon stress in Caenorhabditis elegans. Loss of the enhancer of mRNA decapping EDC-3 causes further entrapment of IFE-2 in P bodies and lowers protein synthesis rates in somatic tissues. Animals lacking EDC-3 are long lived and stress resistant, congruent with IFE-2-deficient mutants. Notably, neuron-specific expression of EDC-3 is sufficient to reverse lifespan extension, while sequestration of IFE-2 in neuronal P bodies counteracts age-related neuronal decline. The effects of mRNA decapping deficiency on stress resistance and longevity are orchestrated by a multimodal stress response involving the transcription factor SKN-1, which mediates lifespan extension upon reduced protein synthesis. Our findings elucidate a mechanism of proteostasis control during aging through P body-mediated regulation of protein synthesis in the soma.

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A salicylic acid derivative extends the lifespan of *Caenorhabditis elegans* by activating autophagy and the mitochondrial unfolded protein response

Plant extracts containing salicylates are probably the most ancient remedies to reduce fever and ease aches of all kind. Recently, it has been shown that salicylates activate adenosine monophosphate-activated kinase (AMPK), which is now considered as a promising target to slow down aging and prevent age-related diseases in humans. Beneficial effects of AMPK activation on lifespan have been discovered in the model organism *Caenorhabditis elegans* (*C. elegans*). Indeed, salicylic acid and acetylsalicylic acid extend lifespan in worms by activating AMPK and the forkhead transcription factor DAF-16/FOXO. Here, we investigated whether another salicylic acid derivative 5-octanoyl salicylic acid (C8-SA), developed as a controlled skin exfoliating ingredient, had similar properties using *C. elegans* as a model. We show that C8-SA increases lifespan of *C. elegans* and that a variety of pathways and genes are required for C8-SA-mediated lifespan extension. C8-SA activates AMPK and inhibits TOR both in nematodes and in primary human keratinocytes. We also show that C8-SA can induce both autophagy and the mitochondrial unfolded protein response (UPR^{mit}) in nematodes. This induction of both processes is fully required for lifespan extension in the worm. In addition, we found that the activation of autophagy by C8-SA fails to occur in worms with compromised UPR^{mit}, suggesting a mechanistic link between these two processes. Mutants that are defective in the mitochondrial unfolded protein response exhibit constitutive high autophagy levels. Taken together, these data therefore suggest that C8-SA positively impacts longevity in worms through induction of autophagy and the UPR^{mit}.

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Olfaction regulates organismal proteostasis and longevity via microRNA-dependent signaling

The maintenance of proteostasis is crucial for any organism to survive and reproduce in an ever-changing environment, but its efficiency declines with age[1,2]. Posttranscriptional regulators such as microRNAs control protein translation of target mRNAs with major consequences for development, physiology, and longevity[3,4]. However, the precise function of lifespan-determining microRNAs remains poorly understood. Here we show that the microRNA *mir-71* controls organismal proteostasis and aging in *Caenorhabditis elegans* by regulating its conserved target *tir-1* in AWC olfactory neurons. We screened a collection of microRNAs that control aging[4] to identify regulators of organismal proteostasis and discovered that the lifespan promoting *mir-71* affects ubiquitin-dependent protein turnover, particularly in the intestine. We show that *mir-71* directly inhibits the toll receptor domain protein TIR-1 in AWC olfactory neurons. Neuronal signaling is required for *mir-71*/*tir-1*-dependent and diet-dependent regulation of organismal proteostasis. Disruption of *mir-71*/*tir-1* or loss of AWC olfactory neurons eliminates the influence of food source on proteostasis. *Mir-71*-mediated regulation of TIR-1 controls chemotactic behavior and is regulated by odor. Our findings support a model whereby odor promotes *mir-71*-mediated inhibition of TIR-1 in AWC neurons to stimulate organismal protein turnover. Thus, odor perception influences cell-type specific miRNA-target interaction to regulate organismal proteostasis and longevity. We anticipate that the proposed mechanism of food perception will stimulate further research on neuroendocrine brain-to-gut communication and may open the possibility for therapeutic interventions to improve proteostasis and organismal health via the sense of smell, with potential implication for obesity, diabetes and aging.

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Fisetin is a senotherapeutic that extends health and lifespan

Background

Senescence is a tumor suppressor mechanism activated in stressed cells to prevent replication of damaged DNA. Senescent cells have been demonstrated to play a causal role in driving aging and age-related diseases using genetic and pharmacologic approaches. We previously demonstrated that the combination of dasatinib and the flavonoid quercetin is a potent senolytic improving numerous age-related conditions including frailty, osteoporosis and cardiovascular disease. The goal of this study was to identify flavonoids with more potent senolytic activity.

Methods

A panel of flavonoid polyphenols was screened for senolytic activity using senescent murine and human fibroblasts, driven by oxidative and genotoxic stress, respectively. The top senotherapeutic flavonoid was tested in mice modeling a progeroid syndrome carrying a $p16^{INK4a}$-luciferase reporter and aged wild-type mice to determine the effects of fisetin on senescence markers, age-related histopathology, disease markers, health span and lifespan. Human adipose tissue explants were used to determine if results translated.

Findings

Of the 10 flavonoids tested, fisetin was the most potent senolytic. Acute or intermittent treatment of progeroid and old mice with fisetin reduced senescence markers in multiple tissues, consistent with a hit-and-run senolytic mechanism. Fisetin reduced senescence in a subset of cells in murine and human adipose tissue, demonstrating cell-type specificity. Administration of fisetin to wild-type mice late in life restored tissue homeostasis, reduced age-related pathology, and extended median and maximum lifespan.

Interpretation

The natural product fisetin has senotherapeutic activity in mice and in human tissues. Late life intervention was sufficient to yield a potent health benefit. These characteristics suggest the feasibility to translation to human clinical studies.

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Persistent repair intermediates induce senescence

F. M. Feringa, J. A. Raaijmakers, M. A. Hadders, C. Vaarting, L. Macurek, L. Heitink, L. Krenning & R. H. Medema

Double-stranded DNA breaks activate a DNA damage checkpoint in G2 phase to trigger a cell cycle arrest, which can be reversed to allow for recovery. However, damaged G2 cells can also permanently exit the cell cycle, going into senescence or apoptosis, raising the question how an individual cell decides whether to recover or withdraw from the cell cycle. Here we find that the decision to withdraw from the cell cycle in G2 is critically dependent on the progression of DNA repair. We show that delayed processing of double strand breaks through HR-mediated repair results in high levels of resected DNA and enhanced ATR-dependent signalling, allowing p21 to rise to levels at which it drives cell cycle exit. These data imply that cells have the capacity to discriminate breaks that can be repaired from breaks that are difficult to repair at a time when repair is still ongoing.

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Clearance of senescent glial cells prevents tau-dependent pathology and cognitive decline

Cellular senescence, which is characterized by an irreversible cell-cycle arrest[1] accompanied by a distinctive secretory phenotype[2], can be induced through various intracellular and extracellular factors. Senescent cells that express the cell cycle inhibitory protein $p16^{INK4A}$ have been found to actively drive naturally occurring age-related tissue deterioration[3,4] and contribute to several diseases associated with ageing, including atherosclerosis[5] and osteoarthritis[6]. Various markers of senescence have been observed in patients with neurodegenerative diseases[7,8,9]; however, a role for senescent cells in the aetiology of these pathologies is unknown. Here we show a causal link between the accumulation of senescent cells and cognition-associated neuronal loss. We found that the $MAPT^{P301S}PS19$ mouse model of tau-dependent neurodegenerative disease[10] accumulates $p16^{INK4A}$-positive senescent astrocytes and microglia. Clearance of these cells as they arise using INK-ATTAC transgenic mice prevents gliosis, hyperphosphorylation of both soluble and insoluble tau leading to neurofibrillary tangle deposition, and degeneration of cortical and hippocampal neurons, thus preserving cognitive function. Pharmacological intervention with a first-generation senolytic modulates tau aggregation. Collectively, these results show that senescent cells have a role in the initiation and progression of tau-mediated disease, and suggest that targeting senescent cells may provide a therapeutic avenue for the treatment of these pathologies.

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Replacement of microglia in the aged brain reverses cognitive, synaptic, and neuronal deficits in mice

Microglia, the resident immune cell of the brain, can be eliminated via pharmacological inhibition of the colony-stimulating factor 1 receptor (CSF1R). Withdrawal of CSF1R inhibition then stimulates microglial repopulation, effectively replacing the microglial compartment. In the aged brain, microglia take on a "primed" phenotype and studies indicate that this coincides with age-related cognitive decline. Here, we investigated the effects of replacing the aged microglial compartment with new microglia using CSF1R inhibitor-induced microglial repopulation. With 28 days of repopulation, replacement of resident microglia in aged mice (24 months) improved spatial memory and restored physical microglial tissue characteristics (cell densities and morphologies) to those found in young adult animals (4 months). However, inflammation-related gene expression was not broadly altered with repopulation nor the response to immune challenges. Instead, microglial repopulation resulted in a reversal of age-related changes in neuronal gene expression, including expression of genes associated with actin cytoskeleton remodeling and synaptogenesis. Age-related changes in hippocampal neuronal complexity were reversed with both microglial elimination and repopulation, while microglial elimination increased both neurogenesis and dendritic spine densities. These changes were accompanied by a full rescue of age-induced deficits in long-term potentiation with microglial repopulation. Thus, several key aspects of the aged brain can be reversed by acute noninvasive replacement of microglia.

Nilvadipine in mild to moderate Alzheimer disease: A randomised controlled trial

Methods and findings

NILVAD was an 18-month, randomised, placebo-controlled, double-blind trial that randomised participants between 15 May 2013 and 13 April 2015. The study was conducted at 23 academic centres in nine European countries. Of 577 participants screened, 511 were eligible and were randomised (258 to placebo, 253 to nilvadipine). Participants took a trial treatment capsule once a day after breakfast for 78 weeks. Participants were aged >50 years, meeting National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's disease Criteria (NINCDS-ADRDA) for diagnosis of probable Alzheimer disease, with a Standardised Mini-Mental State Examination (SMMSE) score of ≥12 and <27. Participants were randomly assigned to 8 mg sustained-release nilvadipine or matched placebo. The a priori defined primary outcome was progression on the Alzheimer's Disease Assessment Scale Cognitive Subscale-12 (ADAS-Cog 12) in the modified intention-to-treat (mITT) population (n = 498), with the Clinical Dementia Rating Scale sum of boxes (CDR-sb) as a gated co-primary outcome, eligible to be promoted to primary end point conditional on a significant effect on the ADAS-Cog 12. The analysis set had a mean age of 73 years and was 62% female. Baseline demographic and Alzheimer disease–specific characteristics were similar between treatment groups, with reported mean of 1.7 years since diagnosis and mean SMMSE of 20.4. The prespecified primary analyses failed to show any treatment benefit for nilvadipine on the co-primary outcome (p = 0.465). Decline from baseline in ADAS-Cog 12 on placebo was 0.79 (95% CI, −0.07–1.64) at 13 weeks, 6.41 (5.33–7.49) at 52 weeks, and 9.63 (8.33–10.93) at 78 weeks and on nilvadipine was 0.88 (0.02–1.74) at 13 weeks, 5.75 (4.66–6.85) at 52 weeks, and 9.41 (8.09–10.73) at 78 weeks. Exploratory analyses of the planned secondary outcomes showed no substantial effects, including on the CDR-sb or the Disability Assessment for Dementia. Nilvadipine appeared to be safe and well tolerated. Mortality was similar between groups (3 on nilvadipine, 4 on placebo); higher counts of adverse events (AEs) on nilvadipine (1,129 versus 1,030), and serious adverse events (SAEs; 146 versus 101), were observed. There were 14 withdrawals because of AEs. Major limitations of this study were that subjects had established dementia and the likelihood that non-Alzheimer subjects were included because of the lack of biomarker confirmation of the presence of brain amyloid.

Conclusions

The results do not suggest benefit of nilvadipine as a treatment in a population spanning mild to moderate Alzheimer disease.

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Cytomegalovirus Infection and Risk of Alzheimer Disease in Older Black and White Individuals FREE

Results

A solid-phase enzyme-linked immunosorbent assay was used for detecting type-specific immunoglobulin G antibody responses to CMV and herpes simplex virus type 1 (HSV-1) measured in archived serum samples. Of 849 participants, 73.4% had serologic evidence of exposure to CMV (89.0% black and 68.2% white; $P < .001$). During an average of 5.0 years of follow-up, 93 persons developed AD. CMV seropositivity was associated with an increased risk of AD (relative risk, 2.15; 95% confidence interval, 1.42–3.27) and a faster rate of decline in global cognition (estimate [±standard error], −0.02 ± 0.01; $P = .03$) in models that controlled for age, sex, education duration, race, vascular risk factors, vascular diseases, and apolipoprotein ε4 level. Results were similar in black and white individuals for both incident AD and change in cognitive function and were independent of HSV-1 status.

Conclusions

These results suggest that CMV infection is associated with an increased risk of AD and a faster rate of cognitive decline in older diverse populations.

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Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice

Results

Pg/gingipain was detected in the hippocampi of mice in the experimental group by immunohistochemistry, confocal microscopy, and qPCR confirming the translocation of orally applied Pg to the brain. Pg/gingipain was localized intra-nuclearly and peri-nuclearly in microglia (Iba1+), astrocytes (GFAP+), neurons (NeuN+) and was evident extracellularly. Significantly greater levels of expression of IL6, TNFα and IL1β were evident in experimental as compared to control group ($p<0.01$, $p<0.00001$, $p<0.00001$ respectively). In addition, microgliosis and astrogliosis were evident in the experimental but not in control group ($p<0.01$, $p<0.0001$ respectively). Neurodegeneration was evident in the experimental group based on a fewer number of intact neuronal cells assessed by NeuN positivity and rbFOX3 gene expression, and there was a greater number of degenerating neurons in the hippocampi of experimental mice assessed by Fluoro Jade C positivity. APP and BACE1 gene expression were increased in experimental group compared with control group ($p<0.05$, $p<0.001$ respectively). PSEN1 gene expression was higher in experimental than control group but the difference was not statistically significant ($p = 0.07$). ADAM10 gene expression was significantly decreased in experimental group compared with control group ($p<0.01$). Extracellular $A\beta_{42}$ was detected in the parenchyma in the experimental but not in the control group ($p< 0.00001$). Finally, phospho-Tau (Ser396) protein was detected and NFTs were evident in experimental but not in the control group ($p<0.00001$).

Conclusions

This study is the first to show neurodegeneration and the formation of extracellular $A\beta_{42}$ in young adult WT mice after repeated oral application of Pg. The neuropathological features observed in this study strongly suggest that low grade chronic periodontal pathogen infection can result in the development of neuropathology that is consistent with that of AD.

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Humanin Prevents Age-Related Cognitive Decline in Mice and is Associated with Improved Cognitive Age in Humans

Advanced age is associated with a decline in cognitive function, likely caused by a combination of modifiable and non-modifiable factors such as genetics and lifestyle choices. Mounting evidence suggests that humanin and other mitochondrial derived peptides play a role in several age-related conditions including neurodegenerative disease. Here we demonstrate that humanin administration has neuroprotective effects *in vitro* in human cell culture models and is sufficient to improve cognition *in vivo* in aged mice. Furthermore, in a human cohort, using mitochondrial GWAS, we identified a specific SNP (rs2854128) in the humanin-coding region of the mitochondrial genome that is associated with a decrease in circulating humanin levels. In a large, independent cohort, consisting of a nationally-representative sample of older adults, we find that this SNP is associated with accelerated cognitive aging, supporting the concept that humanin is an important factor in cognitive aging.

Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer's mouse model

RESULTS

Inducing AHN alone conferred minimal to no benefit for improving cognition in 5×FAD mice. Exercise-induced AHN improved cognition along with reduced Aβ load and increased levels of brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6), fibronectin type III domain–containing protein–5 (FNDC5), and synaptic markers. However, AHN activation was also required for exercise-induced improvement in memory. Inducing AHN genetically and pharmacologically in combination with elevating BDNF levels mimicked beneficial effects of exercise on AD mice. Conversely, suppressing AHN in early stages of AD exacerbated neuronal vulnerability in later stages of AD, leading to cognitive impairment and increased neuronal loss. However, no such effects from AHN ablation were observed in nontransgenic wild-type (WT) mice, suggesting that AHN has a specific role in AD.

CONCLUSION

Promoting AHN can only ameliorate AD pathology and cognitive deficits in the presence of a healthier, improved local brain environment, e.g., stimulated by exercise. Increasing AHN alone combined with overexpression of BDNF could mimic exercise-induced improvements in cognition, without reducing Aβ burden. Adult-born neurons generated very early in life are critical for maintaining hippocampal neuronal populations in the hostile brain environment created by AD later in life. Thus, AHN impairment may be a primary event that later mediates other aspects of AD pathogenesis. Future attempts to create pharmacological mimetics of the benefits of exercise on both increased AHN and BDNF may someday provide an effective means for improving cognition in AD. Moreover, increasing neurogenesis in the earliest stages of AD pathogenesis may protect against neuronal cell death later in the disease, providing a potentially powerful disease-modifying treatment strategy for AD.

Altered ER–mitochondria contact impacts mitochondria calcium homeostasis and contributes to neurodegeneration in vivo in disease models

Ca^{2+} regulates cellular metabolism, proliferation, and differentiation. Ca^{2+} homeostasis is critical for cellular function and health. Mitochondria help buffer transient Ca^{2+} elevations and prevent cell death induced by Ca^{2+} overload. Mito-Ca^{2+} is also required for optimal activity of certain key mitochondrial functions, such as oxidative phosphorylation and metabolism. Thus, mito-Ca^{2+} homeostasis assumes central roles in cellular health. Endoplasmic reticulum (ER) and mitochondria make intimate contacts and exchange molecules such as Ca^{2+} and lipids. We find that ER-to-mitochondria Ca^{2+} transfer is important for mito-Ca^{2+} homeostasis and that the conserved Miro protein is critically involved. We show that mito-Ca^{2+} homeostasis is disrupted in neurodegenerative disease models and its restoration is beneficial. Our findings have important implications for therapeutic intervention of neurodegenerative diseases.

Paradoxical effects of mutant ubiquitin on Aβ plaque formation in an Alzheimer mouse model

Amyloid-β (Aβ) plaques are a prominent pathological hallmark of Alzheimer's disease (AD). They consist of aggregated Aβ peptides, which are generated through sequential proteolytic processing of the transmembrane protein amyloid precursor protein (APP) and several Aβ-associated factors. Efficient clearance of Aβ from the brain is thought to be important to prevent the development and progression of AD. The ubiquitin-proteasome system (UPS) is one of the major pathways for protein breakdown in cells and it has been suggested that impaired UPS-mediated removal of protein aggregates could play an important role in the pathogenesis of AD. To study the effects of an impaired UPS on Aβ pathology in vivo, transgenic APP_{Swe}/PS1ΔE9 mice (APPPS1) were crossed with transgenic mice expressing mutant ubiquitin (UBB^{+1}), a protein-based inhibitor of the UPS. Surprisingly, the APPPS1/UBB^{+1} crossbreed showed a remarkable decrease in Aβ plaque load during aging. Further analysis showed that UBB^{+1} expression transiently restored PS1-NTF expression and γ-secretase activity in APPPS1 mice. Concurrently, UBB^{+1} decreased levels of β-APP-CTF, which is a γ-secretase substrate. Although UBB^{+1} reduced Aβ pathology in APPPS1 mice, it did not improve the behavioral deficits in these animals.

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Carbamylation promotes amyloidogenesis and induces structural changes in Tau-core hexapeptide fibrils

V. Guru KrishnaKumar [a], Lokesh Baweja [a, b], Krittika Ralhan [a], Sharad Gupta [a]

Background

Carbamylation is a non-enzymatic post-translational modification (PTM), which involves the covalent modification of N-terminus of protein or ε-amino group of Lys. The role of carbamylation in several age-related disorders is well documented, however, the relationship between carbamylation and neurodegenerative disorders including Alzheimer's disease remains uncharted.

Methods

In the present study, using aggregation-prone tau-core hexapeptide fragments ^{306}VQIVYK^{311} (PHF6) and ^{275}VQIINK^{280} (PHF6*) as models, we have elucidated the effect of carbamylation on aggregation kinetics and the changes occurring in the 3-dimensional architecture of fibrils using biophysical assays and molecular dynamics simulations.

Results

We found that carbamylation aids in amyloid formation and can convert the unstructured off-pathway aggregates into robust amyloids, which were toxic to cells. Electron microscopy images and molecular dynamics simulations of PHF6 fibrils showed that carbamylated peptides can form excess hydrogen bonds and modulate the pitch length and twist of peptides fibrils. We have also compared N-terminal carbamylation to acetylation and further extended our finding to full length tau that exhibits aggregation upon carbamylation even in the absence of any external inducer.

Conclusion

Our *in vitro* and *in silico* results together suggest that carbamylation can modulate the aggregation pathway of the amyloidegenic sequences and cause structural changes in fibril assemblies.

General significance

Carbamylation acts as a switch, which triggers the aggregation in short amyloidogenic peptide fragments and modulate the structural changes in resulting amyloid fibrils.

Tau Protein Disrupts Nucleocytoplasmic Transport in Alzheimer's Disease

Tau is the major constituent of neurofibrillary tangles in Alzheimer's disease (AD), but the mechanism underlying tau-associated neural damage remains unclear. Here, we show that tau can directly interact with nucleoporins of the nuclear pore complex (NPC) and affect their structural and functional integrity. Pathological tau impairs nuclear import and export in tau-overexpressing transgenic mice and in human AD brain tissue. Furthermore, the nucleoporin Nup98 accumulates in the cell bodies of some tangle-bearing neurons and can facilitate tau aggregation *in vitro*. These data support the hypothesis that tau can directly interact with NPC components, leading to their mislocalization and consequent disruption of NPC function. This raises the possibility that NPC dysfunction contributes to tau-induced neurotoxicity in AD and tauopathies.

Studies on Degradation of 7-ketocholesterol by Environmental Bacterial Isolates

Medical bioremediation is a unique strategy of targeting pathogenic compounds with an exogenous enzyme of microbial origin. The objective of this study was to isolate and screen the microorganisms from diverse environmental samples for their ability to catabolize 7-ketocholesterol. Isolation of bacterial strains was performed and molecular identification was carried out by amplification and sequencing of 16S rDNA for 4 the best degrader isolates. Degradation was confirmed on the basis of UV spectrophotometric and HPLC analysis. Four bacterial isolates, showing high catabolic activity towards 7-ketocholesterol were isolated: *Alcanivorax jadensis* IP4 (accession number KP309836; sea water sediment), *Streptomyces auratus* IP2 (accession number KP309837; soil), *Serratia marcescens* IP3 (accession number KP309838; soil) and *Thermobifida fusca* IP1 (accession number KM677184; manure piles). All the isolates were capable of utilizing 7-ketocholesterol as the sole organic substrate, resulting in its mineralisation. The most rapid degradation was observed with *A. jadensis* IP4 followed by *T. fusca* IP1. The degradation was followed and analyzed by HPLC. *A. jadensis* IP4 removed 7-ketocholesterol below detection levels within 8 days.

Effect of Aspirin on Disability-free Survival in the Healthy Elderly

METHODS

From 2010 through 2014, we enrolled community-dwelling persons in Australia and the United States who were 70 years of age or older (or ≥65 years of age among blacks and Hispanics in the United States) and did not have cardiovascular disease, dementia, or physical disability. Participants were randomly assigned to receive 100 mg per day of enteric-coated aspirin or placebo orally. The primary end point was a composite of death, dementia, or persistent physical disability. Secondary end points reported in this article included the individual components of the primary end point and major hemorrhage.

RESULTS

A total of 19,114 persons with a median age of 74 years were enrolled, of whom 9525 were randomly assigned to receive aspirin and 9589 to receive placebo. A total of 56.4% of the participants were women, 8.7% were nonwhite, and 11.0% reported previous regular aspirin use. The trial was terminated at a median of 4.7 years of follow-up after a determination was made that there would be no benefit with continued aspirin use with regard to the primary end point. The rate of the composite of death, dementia, or persistent physical disability was 21.5 events per 1000 person-years in the aspirin group and 21.2 per 1000 person-years in the placebo group (hazard ratio, 1.01; 95% confidence interval [CI], 0.92 to 1.11; P=0.79). The rate of adherence to the assigned intervention was 62.1% in the aspirin group and 64.1% in the placebo group in the final year of trial participation. Differences between the aspirin group and the placebo group were not substantial with regard to the secondary individual end points of death from any cause (12.7 events per 1000 person-years in the aspirin group and 11.1 events per 1000 person-years in the placebo group), dementia, or persistent physical disability. The rate of major hemorrhage was higher in the aspirin group than in the placebo group (3.8% vs. 2.8%; hazard ratio, 1.38; 95% CI, 1.18 to 1.62; P<0.001).

CONCLUSIONS

Aspirin use in healthy elderly persons did not prolong disability-free survival over a period of 5 years but led to a higher rate of major hemorrhage than placebo. (Funded by the National Institute on Aging and others; ASPREE ClinicalTrials.gov number, NCT01038583.)

Efficacy and Safety of Further Lowering of Low-Density Lipoprotein Cholesterol in Patients Starting With Very Low Levels

A Meta-analysis

Main Outcomes and Measures The risk ratio (RR) of major vascular events (a composite of coronary heart death, myocardial infarction, ischemic stroke, or coronary revascularization) per 1-mmol/L (38.7-mg/dL) reduction in LDL-C level.

Results In the subgroup of patients from the CTTC meta-analysis of statins with a mean LDL-C in the control arm of 1.7 mmol/L (65.7 mg/dL), 1922 major vascular events occurred and the RR for major vascular events per 1-mmol/L (38.7-mg/dL) reduction in LDL-C was 0.78 (95% CI, 0.65-0.94). For 3 trials of nonstatin LDL-C–lowering therapies added to statins, there were 50 627 patients, the median LDL-C in the control arms ranged from 1.6 mmol/L to 1.8 mmol/L (63 mg/dL to 70 mg/dL), and 9570 major vascular events occurred. Nonstatin therapy lowered LDL-C by 0.3 to 1.2 mmol/L (11 mg/dL to 45 mg/dL), and the RR for major vascular events per 1-mmol/L (38.7-mg/dL) reduction in LDL-C was 0.79 (95% CI, 0.70-0.88). For statins and nonstatins combined, the RR was 0.79 (95% CI, 0.71-0.87; $P<.001$). Low-density lipoprotein cholesterol lowering was not associated with an increased risk of serious adverse events, myalgias and/or myositis, elevation in the level of aminotransferases, new-onset diabetes, hemorrhagic stroke, or cancer.

Conclusions and Relevance There is a consistent relative risk reduction in major vascular events per change in LDL-C in patient populations starting as low as a median of 1.6 mmol/L (63 mg/dL) and achieving levels as low as a median of 0.5 mmol/L (21 mg/dL), with no observed offsetting adverse effects. These data suggest further lowering of LDL-C beyond the lowest current targets would further reduce cardiovascular risk.

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Calorie Restriction Curbs Proinflammation That Accompanies Arterial Aging, Preserving a Youthful Phenotype

Background

Aging exponentially increases the incidence of morbidity and mortality of quintessential cardiovascular disease mainly due to arterial proinflammatory shifts at the molecular, cellular, and tissue levels within the arterial wall. Calorie restriction (CR) in rats improves arterial function and extends both health span and life span. How CR affects the proinflammatory landscape of molecular, cellular, and tissue phenotypic shifts within the arterial wall in rats, however, remains to be elucidated.

Methods and Results

Aortae were harvested from young (6-month-old) and old (24-month-old) Fischer 344 rats, fed ad libitum and a second group maintained on a 40% CR beginning at 1 month of age. Histopathologic and morphometric analysis of the arterial wall demonstrated that CR markedly reduced age-associated intimal medial thickening, collagen deposition, and elastin fractionation/degradation within the arterial walls. Immunostaining/blotting showed that CR effectively prevented an age-associated increase in the density of platelet-derived growth factor, matrix metalloproteinase type II activity, and transforming growth factor beta 1 and its downstream signaling molecules, phospho-mothers against decapentaplegic homolog-2/3 (p-SMAD-2/3) in the arterial wall. In early passage cultured vascular smooth muscle cells isolated from AL and CR rat aortae, CR alleviated the age-associated vascular smooth muscle cell phenotypic shifts, profibrogenic signaling, and migration/proliferation in response to platelet-derived growth factor.

Conclusions

CR reduces matrix and cellular proinflammation associated with aging that occurs within the aortic wall and that are attributable to platelet-derived growth factor signaling. Thus, CR reduces the platelet-derived growth factor–associated signaling cascade, contributing to the postponement of biological aging and preservation of a more youthful aortic wall phenotype.

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Daily Fasting Improves Health and Survival in Male Mice Independent of Diet Composition and Calories

The importance of dietary composition and feeding patterns in aging remains largely unexplored, but was implicated recently in two prominent nonhuman primate studies. Here, we directly compare in mice the two diets used in the primate studies focusing on three paradigms: *ad libitum* (AL), 30% calorie restriction (CR), and single-meal feeding (MF), which accounts for differences in energy density and caloric intake consumed by the AL mice. MF and CR regimes enhanced longevity regardless of diet composition, which alone had no significant impact within feeding regimens. Like CR animals, MF mice ate quickly, imposing periods of extended daily fasting on themselves that produced significant improvements in morbidity and mortality compared with AL. These health and survival benefits conferred by periods of extended daily fasting, independent of dietary composition, have major implications for human health and clinical applicability.

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Enhanced longevity and metabolism by brown adipose tissue with disruption of the regulator of G protein signaling 14

Dorothy E. Vatner, Jie Zhang, Marko Oydanich, John Guers, Elena Katsyuba, Lin Yan, David Sinclair, Johan Auwerx, Stephen F. Vatner

Disruption of the regulator for G protein signaling 14 (RGS14) knockout (KO) in mice extends their lifespan and has multiple beneficial effects related to healthful aging, that is, protection from obesity, as reflected by reduced white adipose tissue, protection against cold exposure, and improved metabolism. The observed beneficial effects were mediated by improved mitochondrial function. But most importantly, the main mechanism responsible for the salutary properties of the RGS14 KO involved an increase in brown adipose tissue (BAT), which was confirmed by surgical BAT removal and transplantation to wild-type (WT) mice, a surgical simulation of a molecular knockout. This technique reversed the phenotype of the RGS14 KO and WT, resulting in loss of the improved metabolism and protection against cold exposure in RGS14 KO and conferring this protection to the WT BAT recipients. Another mechanism mediating the salutary features in the RGS14 KO was increased SIRT3. This mechanism was confirmed in the RGS14 X SIRT3 double KO, which no longer demonstrated improved metabolism and protection against cold exposure. Loss of function of the *Caenorhabditis elegans* RGS-14 homolog confirmed the evolutionary conservation of this mechanism. Thus, disruption of RGS14 is a model of healthful aging, as it not only enhances lifespan, but also protects against obesity and cold exposure and improves metabolism with a key mechanism of increased BAT, which, when removed, eliminates the features of healthful aging.

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Population level mitogenomics of long-lived bats reveals dynamic heteroplasmy and challenges the Free Radical Theory of Ageing

Bats are the only mammals capable of true, powered flight, which drives an extremely high metabolic rate. The "Free Radical Theory of Ageing" (FTRA) posits that a high metabolic rate causes mitochondrial heteroplasmy and the progressive ageing phenotype. Contrary to this, bats are the longest-lived order of mammals given their small size and high metabolic rate. To investigate if bats exhibit increased mitochondrial heteroplasmy with age, we performed targeted, deep sequencing of mitogenomes and measured point heteroplasmy in wild, long lived *Myotis myotis*. Blood was sampled from 195 individuals, aged between <1 and at 6+ years old, and whole mitochondria deep-sequenced, with a subset sampled over multiple years. The majority of heteroplasmies were at a low frequency and were transitions. Oxidative mutations were present in only a small number of individuals, suggesting local oxidative stress events. Cohort data showed no significant increase in heteroplasmy with age, while longitudinal data from recaptured individuals showed heteroplasmy is dynamic, and does not increase uniformly over time. We show that bats do not suffer from the predicted, inevitable increase in heteroplasmy as posited by the FRTA, instead heteroplasmy was found to be dynamic, questioning its presumed role as a primary driver of ageing.

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Multidimensional comparison of countries' adaptation to societal aging

As long-term changes in life expectancy and fertility drive the emergence of aging societies across the globe, individual countries vary widely in the development of age-relevant policies and programs. While failure to adapt to the demographic transformation carries not only important financial risks but also social risks, most efforts to gauge countries' preparedness focus on economic indicators. Using data from the Organization for Economic Cooperation and Development (OECD) and other sources, we developed a multidimensional Aging Society Index that assesses the status of older populations across five specific domains, including productivity and engagement, well-being, equity, economic and physical security, and intergenerational cohesion. For 18 OECD countries, the results demonstrate substantial diversity in countries' progress in adapting to aging. For any given domain, there are wide differences across countries, and within most countries, there is substantial variation across domains. Overall, Norway and Sweden rank first in adaptation to aging, followed by the United States, The Netherlands, and Japan. Central and eastern European countries rank at the bottom, with huge untapped potential for successful aging. The United States ranks best in productivity and engagement, in the top half for cohesion, and in the middle in well-being, but it ranks third from the bottom in equity. Only well-being and security showed significant between-domain correlation ($r = 0.59$, $P = 0.011$), strengthening the case for a multidimensional index. Examination of heterogeneity within and across domains of the index can be used to assess the need for, and effectiveness of, various programs and policies and facilitate successful adaptation to the demographic transition.

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Biological Processes Modulating Longevity across Primates: A Phylogenetic Genome-Phenome Analysis

Aging is a complex process affecting different species and individuals in different ways. Comparing genetic variation across species with their aging phenotypes will help understanding the molecular basis of aging and longevity. Although most studies on aging have so far focused on short-lived model organisms, recent comparisons of genomic, transcriptomic, and metabolomic data across lineages with different lifespans are unveiling molecular signatures associated with longevity. Here, we examine the relationship between genomic variation and maximum lifespan across primate species. We used two different approaches. First, we searched for parallel amino-acid mutations that co-occur with increases in longevity across the primate linage. Twenty-five such amino-acid variants were identified, several of which have been previously reported by studies with different experimental setups and in different model organisms. The genes harboring these mutations are mainly enriched in functional categories such as wound healing, blood coagulation, and cardiovascular disorders. We demonstrate that these pathways are highly enriched for pleiotropic effects, as predicted by the antagonistic pleiotropy theory of aging. A second approach was focused on changes in rates of protein evolution across the primate phylogeny. Using the phylogenetic generalized least squares, we show that some genes exhibit strong correlations between their evolutionary rates and longevity-associated traits. These include genes in the Sphingosine 1-phosphate pathway, PI3K signaling, and the Thrombin/protease-activated receptor pathway, among other cardiovascular processes. Together, these results shed light into human senescence patterns and underscore the power of comparative genomics to identify pathways related to aging and longevity.

Epigenetic age-predictor for mice based on three CpG sites

Epigenetic clocks for mice were generated based on deep-sequencing analysis of the methylome. Here, we demonstrate that site-specific analysis of DNA methylation levels by pyrosequencing at only three CG dinucleotides (CpGs) in the genes *Prima1*, *Hsf4*, and *Kcns1* facilitates precise estimation of chronological age in murine blood samples, too. DBA/2 mice revealed accelerated epigenetic aging as compared to C57BL6 mice, which is in line with their shorter life-expectancy. The three-CpG-predictor provides a simple and cost-effective biomarker to determine biological age in large intervention studies with mice.

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The Role of Advanced Glycation End Products in Aging and Metabolic Diseases: Bridging Association and Causality

Accumulation of advanced glycation end products (AGEs) on nucleotides, lipids, and peptides/proteins are an inevitable component of the aging process in all eukaryotic organisms, including humans. To date, a substantial body of evidence shows that AGEs and their functionally compromised adducts are linked to and perhaps responsible for changes seen during aging and for the development of many age-related morbidities. However, much remains to be learned about the biology of AGE formation, causal nature of these associations, and whether new interventions might be developed that will prevent or reduce the negative impact of AGEs-related damage. To facilitate achieving these latter ends, we show how invertebrate models, notably *Drosophila melanogaster* and *Caenorhabditis elegans*, can be used to explore AGE-related pathways in depth and to identify and assess drugs that will mitigate against the detrimental effects of AGE-adduct development.

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Genome organization and chromatin analysis identify transcriptional downregulation of insulin-like growth factor signaling as a hallmark of aging in developing B cells

Results

Our analysis reveals that the expression levels of most genes are generally preserved in B cell precursors isolated from aged compared with young mice. Nonetheless, age-specific expression changes are observed at numerous genes, including microRNA encoding genes. Importantly, these changes are underpinned by multi-layered alterations in chromatin structure, including chromatin accessibility, histone modifications, long-range promoter interactions, and nuclear compartmentalization. Previous work has shown that differentiation is linked to changes in promoter-regulatory element interactions. We find that aging in B cell precursors is accompanied by rewiring of such interactions. We identify transcriptional downregulation of components of the insulin-like growth factor signaling pathway, in particular downregulation of Irs1 and upregulation of Let-7 microRNA expression, as a signature of the aged phenotype. These changes in expression are associated with specific alterations in H3K27me3 occupancy, suggesting that Polycomb-mediated repression plays a role in precursor B cell aging.

Conclusions

Changes in chromatin and 3D genome organization play an important role in shaping the altered gene expression profile of aged precursor B cells. Components of the insulin-like growth factor signaling pathways are key targets of epigenetic regulation in aging in bone marrow B cell precursors.

REVIEWS/COMMENTS/ METHODS/EDITORIALS

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Facing up to the global challenges of ageing

Linda Partridge ✉, Joris Deelen & P. Eline Slagboom ✉

Longer human lives have led to a global burden of late-life disease. However, some older people experience little ill health, a trait that should be extended to the general population. Interventions into lifestyle, including increased exercise and reduction in food intake and obesity, can help to maintain healthspan. Altered gut microbiota, removal of senescent cells, blood factors obtained from young individuals and drugs can all improve late-life health in animals. Application to humans will require better biomarkers of disease risk and responses to interventions, closer alignment of work in animals and humans, and increased use of electronic health records, biobank resources and cohort studies.

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Viewpoint | Scientific Discovery and the Future of Medicine

October 2, 2018

Aging as a Biological Target for Prevention and Therapy

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JAMA. 2018;320(13):1321-1322. doi:10.1001/jama.2018.9562

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Chronic health problems related to the unprecedented aging of the human population in the 21st century threaten to disrupt economies and degrade the quality of later life throughout the developed world. Fortunately, research has shown that fundamental aging processes can be targeted by nutritional, genetic, and pharmacologic interventions to enhance and extend both health and longevity in experimental animal models. These findings clearly demonstrate that the biological rate of aging can be slowed.

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Viewpoint | Scientific Discovery and the Future of Medicine

October 2, 2018

Aging, Cell Senescence, and Chronic Disease
Emerging Therapeutic Strategies

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JAMA. 2018;320(13):1319-1320. doi:10.1001/jama.2018.12440

Related Articles

Age is the leading predictive factor for most of the chronic diseases that account for the majority of morbidity, hospitalizations, health costs, and mortality worldwide. These diseases include Alzheimer disease and other neurodegenerative diseases, cardiovascular disease, and most cancers. Chronological age is also the main risk factor for the geriatric syndromes, including frailty and immobility as well as decreased physical resilience, which is manifested by delayed or incomplete recovery from stressors, such as surgery, hip fracture, and pneumonia. The prevalence of these problems not only increases with age, but these conditions tend to cluster within older individuals, leading to multimorbidity. Therefore, if any single major age-related disease were cured, it would only be supplanted by others, adding little to quality or length of life and limiting the effectiveness of treating age-related chronic diseases one at a time.

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How healthy is the healthspan concept?

Authors | Authors and affiliations

Matt Kaeberlein

The concept of healthspan is relatively new in geroscience research, which seeks to understand the biological mechanisms of aging (Burch et al. 2014; Sierra and Kohanski 2017). Prior to the year 2000, only 14 papers were indexed on PubMed with "healthspan" or "health span" in the title or abstract. By mid-2018, that number has grown to more than 900 (Fig. 1). Notably, several of these use the exact phrases "increases healthspan," "improves healthspan," or "extends healthspan," implying that healthspan is a quantifiable phenotype. In contrast, a recent discussion session at the 2018 Nathan Shock Center Summit and American Aging Association Annual Meeting entitled "How healthy is the healthspan concept?" indicates that there are no accepted or validated metrics for measuring healthspan. How then are claims of increased healthspan so routinely making it into the peer-reviewed literature? A widespread lack of clarity and precision in the use and meaning of this term among both authors and reviewers is evident, and this author will somewhat shamefacedly admit to being among the offenders (Bitto et al. 2016; Leiser et al. 2011; Sutphin et al. 2012; Urfer et al. 2017).

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Rebuking the concept of ageing as a disease

Cathal McCrory • Rose Anne Kenny

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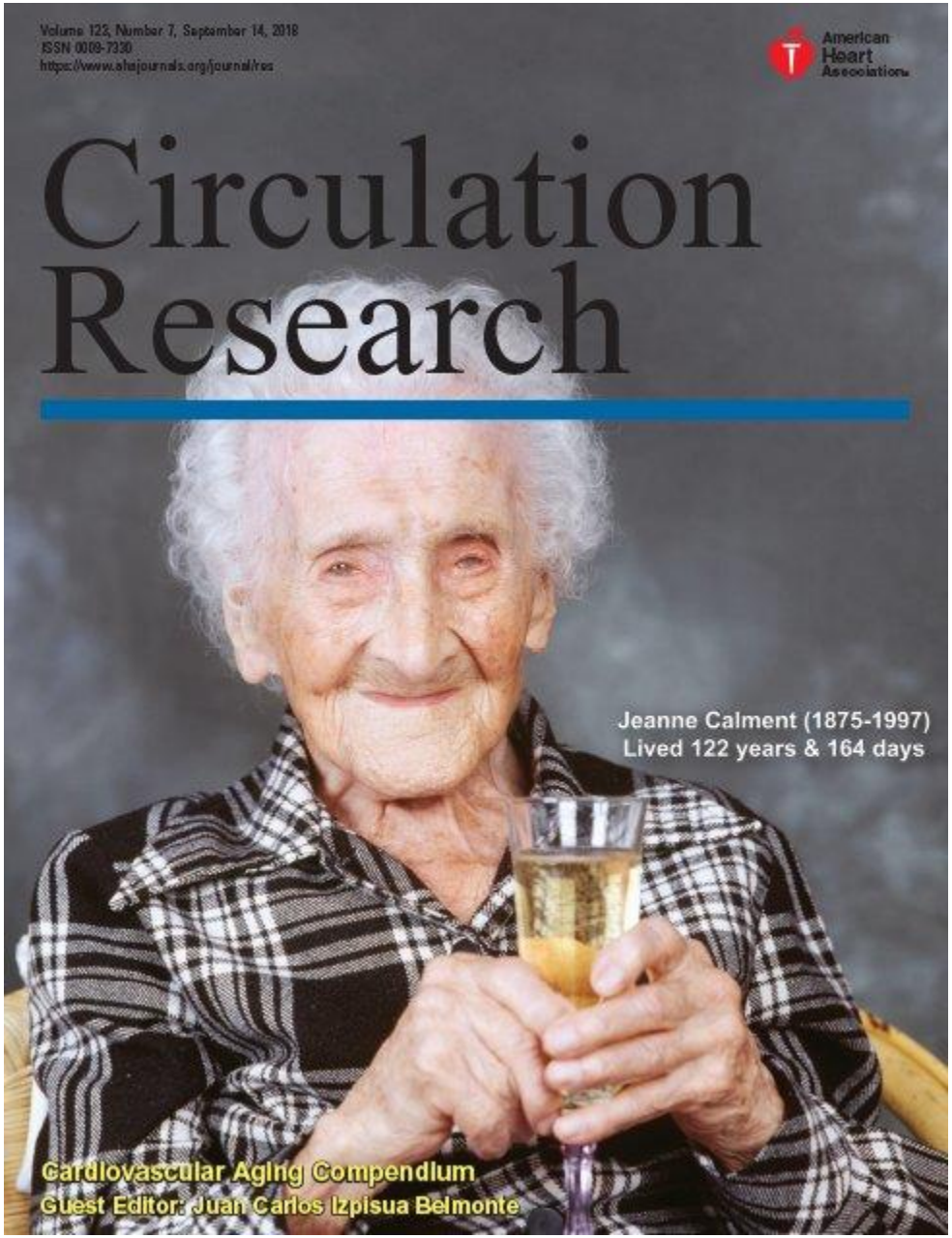
It was with concern that we read the title of a recent Editorial[1] in *The Lancet Diabetes & Endocrinology* entitled "Opening the door to treating ageing as a disease". Although the purpose of the Editorial may have been to stimulate debate, we believe that any attempt to rebrand ageing as a disease is fundamentally misguided and retrograde.

First, age is not a stable category but rather humans are part of different age cohorts throughout life. Ageing is a normative biological process that varies within and between species with marked biological heterogeneity. Whereas chronological age represents a major risk factor for disease, the ageing process is characterised by the presence of high inter-individual variation between individuals of the same chronological age.[2] If we accept that there is large heterogeneity in the rate of ageing, on what basis are we justified in treating ageing as a disease?

Second, rebranding ageing as a disease does nothing to further our understanding of the ageing process—the life-course social, behavioural, biological, and genetic influences that contribute to it—nor indeed the underlying cellular and molecular processes that accelerate it. To this end, much research effort is currently being directed in the field of geroscience towards identifying ageing biomarkers that predict lifespan better than chronological age.[3, 4]

Special issue “Cardiovascular Aging Compendium”



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Mouse Models to Disentangle the Hallmarks of Human Aging

Alicia R. Folgueras, Sandra Freitas-Rodríguez, Gloria Velasco, and Carlos López-Otín

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Abstract

Model organisms have provided fundamental evidence that aging can be delayed and longevity extended. These findings gave rise to a new era in aging research aimed at elucidating the pathways and networks controlling this complex biological process. The identification of 9 hallmarks of aging has established a framework to evaluate the relative contribution of each hallmark and the interconnections among them. In this review, we revisit these hallmarks with the information obtained exclusively through the generation of genetically modified mouse models that have a significant impact on the aging process. We discuss within each hallmark those interventions that accelerate aging or that have been successful at increasing lifespan, with the final goal of identifying the most promising antiaging avenues based on the current knowledge provided by in vivo models.

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DNA Methylation Clocks in Aging: Categories, Causes, and Consequences

Adam E. Field • Neil A. Robertson • Tina Wang • ... Aaron Havas • Trey Ideker • Peter D. Adams

Age-associated changes to the mammalian DNA methylome are well documented and thought to promote diseases of aging, such as cancer. Recent studies have identified collections of individual methylation sites whose aggregate methylation status measures chronological age, referred to as the DNA methylation clock. DNA methylation may also have value as a biomarker of healthy versus unhealthy aging and disease risk; in other words, a biological clock. Here we consider the relationship between the chronological and biological clocks, their underlying mechanisms, potential consequences, and their utility as biomarkers and as targets for intervention to promote healthy aging and longevity.

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iScience. 2018 Sep 28;7:154-169. doi: 10.1016/j.isci.2018.08.023. Epub 2018 Sep 3.

The Systems Biology of Single-Cell Aging.

Song R[1], Sarnoski EA[2], Acar M[3].

Author information

Abstract

Aging is a leading cause of human morbidity and mortality, but efforts to slow or reverse its effects are hampered by an incomplete understanding of its multi-faceted origins. Systems biology, the use of quantitative and computational methods to understand complex biological systems, offers a toolkit well suited to elucidating the root cause of aging. We describe the known components of the aging network and outline innovative techniques that open new avenues of investigation to the aging research community. We propose integration of the systems biology and aging fields, identifying areas of complementarity based on existing and impending technological capabilities.

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In vivo somatic cell reprogramming for tissue regeneration: the emerging role of the local microenvironment

Martina Pesaresi [1], Sergi A Bonilla-Pons [1, 2], Maria Pia Cosma [1, 3, 4] ✉

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https://doi.org/10.1016/j.ceb.2018.07.002

The past few years have witnessed an exponential increase of interest in the reprogramming process. This has been motivated by the enthusiasm of unravelling key aspects not only of cell identity and dedifferentiation, but also of the endogenous regenerative capacities of mammalian organs.

Here, we present the most recent advances in the field of reprogramming, stressing how they are re-defining the rules of cell fate and plasticity *in vivo*. Specifically, we focus on the emerging role of the tissue microenvironment, with particular emphasis on tissue damage, inflammation and senescence that can facilitate *in vivo* reprogramming and regeneration through cell-extrinsic mechanisms.

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Therapeutic implications of how TNF links apolipoprotein E, phosphorylated tau, α-synuclein, amyloid-β and insulin resistance in neurodegenerative diseases

While cytokines such as TNF have long been recognized as essential to normal cerebral physiology, the implications of their chronic excessive production within the brain are now also increasingly appreciated. Syndromes as diverse as malaria and lead poisoning, as well as non-infectious neurodegenerative diseases, illustrate this. These cytokines also orchestrate changes in tau, α-synuclein, amyloid-β levels and degree of insulin resistance in most neurodegenerative states. New data on the effects of salbutamol, an indirect anti-TNF agent, on α-synuclein and Parkinson's disease, APOE4 and tau add considerably to the rationale of the anti-TNF approach to understanding, and treating, these diseases. Therapeutic advances being tested, and arguably useful for a number of the neurodegenerative diseases, include a reduction of excess cerebral TNF, whether directly, with a specific anti-TNF biological agent such as etanercept via Batson's plexus, or indirectly via surgically implanting stem cells. Inhaled salbutamol also warrants investigating further across the neurodegenerative disease spectrum. It is now timely to integrate this range of new information across the neurodegenerative disease spectrum, rather than keep seeing it through the lens of individual disease states.

G Protein-Coupled Receptor Systems as Crucial Regulators of DNA Damage Response Processes

Hanne Leysen [1], Jaana van Gastel [1,2], Jhana O. Hendrickx [1,2], Paula Santos-Otte [3], Bronwen Martin [1] and Stuart Maudsley [1,2,*]

G protein-coupled receptors (GPCRs) and their associated proteins represent one of the most diverse cellular signaling systems involved in both physiological and pathophysiological processes. Aging represents perhaps the most complex biological process in humans and involves a progressive degradation of systemic integrity and physiological resilience. This is in part mediated by age-related aberrations in energy metabolism, mitochondrial function, protein folding and sorting, inflammatory activity and genomic stability. Indeed, an increased rate of unrepaired DNA damage is considered to be one of the 'hallmarks' of aging. Over the last two decades our appreciation of the complexity of GPCR signaling systems has expanded their functional signaling repertoire. One such example of this is the incipient role of GPCRs and GPCR-interacting proteins in DNA damage and repair mechanisms. Emerging data now suggest that GPCRs could function as stress sensors for intracellular damage, e.g., oxidative stress. Given this role of GPCRs in the DNA damage response process, coupled to the effective history of drug targeting of these receptors, this suggests that one important future activity of GPCR therapeutics is the rational control of DNA damage repair systems. View Full-Text

Mechanisms of mitophagy in cellular homeostasis, physiology and pathology

Konstantinos Palikaras, Eirini Lionaki & Nektarios Tavernarakis

Mitophagy is an evolutionarily conserved cellular process to remove dysfunctional or superfluous mitochondria, thus fine-tuning mitochondrial number and preserving energy metabolism. In this Review, we survey recent advances towards elucidating the molecular mechanisms that mediate mitochondrial elimination and the signalling pathways that govern mitophagy. We consider the contributions of mitophagy in physiological and pathological contexts and discuss emerging findings, highlighting the potential value of mitophagy modulation in therapeutic intervention.

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Bench to bedside: Current advances in regenerative medicine

Gabriella Clarke [1], Peter Harley [1], Ella-Louise Hubber [1], Teodora Manea [1], Luigi Manuelli [1], Emily Read [1], Fiona M Watt ✉

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Regenerative medicine is a diverse and rapidly evolving field, employing core expertise from biologists, engineers, and clinicians. Recently the field has made significant progress towards regenerating or replacing tissues lost to age, disease or injury. Current strategies include transplantation of adult or pluripotent stem cells to replace tissue or support tissue healing. Promising approaches for the future of regenerative medicine include stimulating endogenous stem cells for *in situ* repair, transplantation of organoids to repair minor tissue injury, and the use of interspecies chimerism to produce functional metabolic organs for transplantation. In our review we focus on these emerging strategies, paying particular attention to their current and prospective translational impacts and challenges.

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Vascular smooth muscle cell senescence and age-related diseases: State of the art ☆

Aging is a worldwide challenge, and it is accompanied by the accumulation of senescent cells. Cellular senescence is traditionally defined as permanent cell growth arrest and currently includes the senescence-associated secretory phenotype (SASP). There are two main types of cellular senescence, including telomere-dependent replicative senescence and stress-induced premature senescence. The process of cellular senescence is mainly controlled by two effector pathways, namely, the p53-p21 and p16-retinoblastoma protein (pRB) pathways. Vascular smooth muscle cells (VSMCs) are integral parts of arteries and play an important role in vascular structure and function. VSMC senescence may be triggered by many factors, such as angiotensin II, oxidative stress, inflammation, DNA damage, and small molecule compounds. These inducers are able to genetically and epigenetically regulate VSMC senescence. The senescence of VSMCs together with the SASP contributes to chronic vascular inflammation, the loss of arterial function, and the development of age-related diseases. Current evidence suggests that the senescence of VSMCs might be harmful to individual health, whereas its influence on the lifespan is not clear. The purpose of this paper was to review the current knowledge regarding VSMC senescence and its relevance to hypertension, atherosclerosis, and diabetes, as well as the potential mechanisms responsible for VSMC senescence in these age-related diseases.

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Deep learning in biomedicine

Michael Wainberg, Daniele Merico, Andrew Delong & Brendan J Frey

Deep learning is beginning to impact biological research and biomedical applications as a result of its ability to integrate vast datasets, learn arbitrarily complex relationships and incorporate existing knowledge. Already, deep learning models can predict, with varying degrees of success, how genetic variation alters cellular processes involved in pathogenesis, which small molecules will modulate the activity of therapeutically relevant proteins, and whether radiographic images are indicative of disease. However, the flexibility of deep learning creates new challenges in guaranteeing the performance of deployed systems and in establishing trust with stakeholders, clinicians and regulators, who require a rationale for decision making. We argue that these challenges will be overcome using the same flexibility that created them; for example, by training deep models so that they can output a rationale for their predictions. Significant research in this direction will be needed to realize the full potential of deep learning in biomedicine.

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Amyloids of multiple species: are they helpful in survival?

Arun Upadhyay, Amit Mishra

Amyloids are primarily known for their roles in neurodegenerative disorders, as well as in systemic diseases like diabetes. Evolutionary forces tend to maintain a healthy set of heritable characteristics, while eliminating toxic or unfavourable elements; but amyloids seem to represent an exception to this fundamental concept. In addition to their presence in mammals, amyloids also persist in the proteome of many lower organisms that may be linked with possible roles in survival, which are still unexplored. Herein, we address some unanswered questions regarding amyloids: are these well-structured proteinaceous aggregates a by-product of inefficient folding events, or have they been retained in our protein repertoire for as yet unknown functional roles; and how do protein misfolding and associated disorders originate, despite the presence of protein quality-control systems inside the cells? This review aims to extend our current understanding about the multifaceted useful properties of amyloids and their functional interactions with other molecular pathways in various species; this may provide new insights to identify novel therapeutic strategies for ageing and neurodegenerative diseases.

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Genetic and epigenetic regulation of human aging and longevity ☆

Here we summarize the latest data on genetic and epigenetic contributions to human aging and longevity. Whereas environmental and lifestyle factors are important at younger ages, the contribution of genetics appears more important in reaching extreme old age. Genome-wide studies have implicated ~57 gene loci in lifespan. Epigenomic changes during aging profoundly affect cellular function and stress resistance. Dysregulation of transcriptional and chromatin networks is likely a crucial component of aging. Large-scale bioinformatic analyses have revealed involvement of numerous interaction networks. As the young well-differentiated cell replicates into eventual senescence there is drift in the highly regulated chromatin marks towards an entropic middle-ground between repressed and active, such that genes that were previously inactive "leak". There is a breakdown in chromatin connectivity such that topologically associated domains and their insulators weaken, and well-defined blocks of constitutive heterochromatin give way to generalized, senescence-associated heterochromatin, foci. Together, these phenomena contribute to aging.

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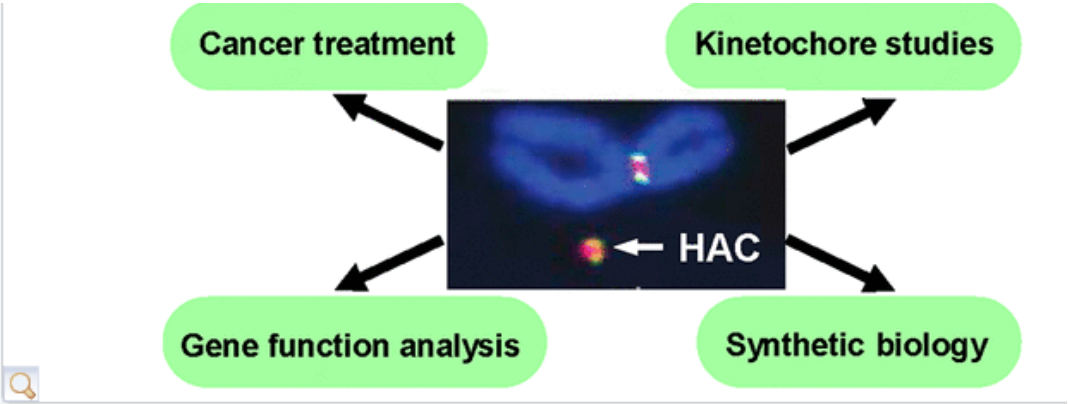
Telomere Length and All-Cause Mortality: A Meta-analysis

Qi Wang [1, 2], Yiqiang Zhan [2], Nancy L. Pedersen [2], Fang Fang [2], Sara Hägg [2]

Telomere attrition is associated with increased morbidity and mortality of various age-related diseases. Reports of association between telomere length (TL) and all-cause mortality remain inconsistent. In the present study, a meta-analysis was performed using published cohort studies and un-published data from the Swedish Twin Registry (STR). Twenty-five studies were included: four STR cohorts (12,083 individuals with 2517 deaths) and 21 published studies. In the STR studies, one standard deviation (SD) decrement of leukocyte TL corresponded to 13% increased all-cause mortality risk (95% confidence interval [CI]: 7%–19%); individuals in the shortest TL quarter had 44% higher hazard (95% CI: 27%–63%) than those in the longest quarter. Meta-analysis of all eligible studies (121,749 individuals with 21,763 deaths) revealed one SD TL decrement-associated hazard ratio of 1.09 (95% CI: 1.06–1.13); those in the shortest TL quarter had 26% higher hazard (95% CI: 15%–38%) compared to the longest quarter, although between-study heterogeneity was observed. Analyses stratified by age indicated that the hazard ratio was smaller in individuals over 80 years old. In summary, short telomeres are associated with increased all-cause mortality risk in the general population. However, TL measurement techniques and age at measurement contribute to the heterogeneity of effect estimation.

Human Artificial Chromosome with Regulated Centromere: A Tool for Genome and Cancer Studies

Natalay Kouprina*†, Nikolai Petrov†, Oscar Molina‡, Mikhail Liskovykh†, Elisa Pesenti§, Jun-ichirou Ohzeki‖, Hiroshi Masumoto*‖, William C. Earnshaw*§, and Vladimir Larionov*†



Since their description in the late 1990s, Human Artificial Chromosomes (HACs) bearing functional kinetochores have been considered as promising systems for gene delivery and expression. More recently a HAC assembled from a synthetic alphoid DNA array has been exploited in studies of centromeric chromatin and in assessing the impact of different epigenetic modifications on kinetochore structure and function in human cells. This HAC was termed the alphoid^{tetO}-HAC, as the synthetic monomers each contained a tetO sequence in place of the CENP-B box that can be targeted specifically with tetR-fusion proteins. Studies in which the kinetochore chromatin of the alphoid^{tetO}-HAC was specifically modified, revealed that heterochromatin is incompatible with centromere function and that centromeric transcription is important for centromere assembly and maintenance. In addition, the alphoid^{tetO}-HAC was modified to carry large gene inserts that are expressed in target cells under conditions that recapitulate the physiological regulation of endogenous loci. Importantly, the phenotypes arising from stable gene expression can be reversed when cells are “cured” of the HAC by inactivating its kinetochore in proliferating cell populations, a feature that provides a control for phenotypic changes attributed to expression of HAC-encoded genes. Alphoid^{tetO}-HAC-based technology has also been used to develop new drug screening and assessment strategies to manipulate the CIN phenotype in cancer cells. In summary, the alphoid^{tetO}-HAC is proving to be a versatile tool for studying human chromosome transactions and structure as well as for genome and cancer studies.

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Synthetic materials at the forefront of gene delivery

Irene Lostalé-Seijo & Javier Montenegro

Nature Reviews Chemistry (2018) | Download Citation

Abstract

The delivery of nucleic acids with transient activity for genetic engineering is a promising methodology with potential applications in the treatment of diseases ranging from cancer and infectious diseases to heritable disorders. Restoring the expression of a missing protein, correcting defective splicing of transcripts and silencing or modulating the expression of genes are powerful approaches that could have substantial benefits in biological research and medicine. Impressive progress in improving gene delivery has been made in the past decade, and several products have reached the market. However, translating the results of in vitro and preclinical studies into functional therapies is hindered by the suboptimal performance of gene delivery vehicles in capturing, protecting and delivering nucleic acid cargoes safely and efficaciously. Chemistry has a key role in the development of innovative synthetic materials to overcome the challenges of producing next-generation gene delivery therapies and protocols. In this Review, we discuss the latest chemical advances in the production of materials for the delivery of nucleic acids to cells and for gene therapy.

OTHER RESEARCH

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Large-scale investigation of the reasons why potentially important genes are ignored

Thomas Stoeger, Martin Gerlach, Richard I. Morimoto, Luís A. Nunes Amaral

Biomedical research has been previously reported to primarily focus on a minority of all known genes. Here, we demonstrate that these differences in attention can be explained, to a large extent, exclusively from a small set of identifiable chemical, physical, and biological properties of genes. Together with knowledge about homologous genes from model organisms, these features allow us to accurately predict the number of publications on individual human genes, the year of their first report, the levels of funding awarded by the National Institutes of Health (NIH), and the development of drugs against disease-associated genes. By explicitly identifying the reasons for gene-specific bias and performing a meta-analysis of existing computational and experimental knowledge bases, we describe gene-specific strategies for the identification of important but hitherto ignored genes that can open novel directions for future investigation.

existence of related medical drugs. We find that biomedical research is primarily guided by a handful of generic chemical and biological characteristics of genes, which facilitated experimentation during the 1980s and 1990s, rather than the physiological importance of individual genes or their relevance to human disease.

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Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*

The Tabula Muris Consortium*

Here we present a compendium of single-cell transcriptomic data from the model organism *Mus musculus* that comprises more than 100,000 cells from 20 organs and tissues. These data represent a new resource for cell biology, reveal gene expression in poorly characterized cell populations and enable the direct and controlled comparison of gene expression in cell types that are shared between tissues, such as T lymphocytes and endothelial cells from different anatomical locations. Two distinct technical approaches were used for most organs: one approach, microfluidic droplet-based 3′-end counting, enabled the survey of thousands of cells at relatively low coverage, whereas the other, full-length transcript analysis based on fluorescence-activated cell sorting, enabled the characterization of cell types with high sensitivity and coverage. The cumulative data provide the foundation for an atlas of transcriptomic cell biology.

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Transcriptional recording by CRISPR spacer acquisition from RNA

Florian Schmidt, Mariia Y. Cherepkova & Randall J. Platt

Nature (2018) | Download Citation

Abstract

The ability to record transcriptional events within a cell over time would help to elucidate how molecular events give rise to complex cellular behaviours and states. However, current molecular recording technologies capture only a small set of defined stimuli. Here we use CRISPR spacer acquisition to capture and convert intracellular RNAs into DNA, enabling DNA-based storage of transcriptional information. In *Escherichia coli*, we show that defined stimuli, such as an RNA virus or arbitrary sequences, as well as complex stimuli, such as oxidative stress, result in quantifiable transcriptional records that are stored within a population of cells. We demonstrate that the transcriptional records enable us to classify and describe complex cellular behaviours and to identify the precise genes that orchestrate differential cellular responses. In the future, CRISPR spacer acquisition-mediated recording of RNA followed by deep sequencing (Record–seq) could be used to reconstruct transcriptional histories that describe complex cell behaviours or pathological states.

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Re-Evaluating One-step Generation of Mice Carrying Conditional Alleles by CRISPR-Cas9-Mediated Genome Editing Technology

CRISPR–Cas9 gene editing technology has considerably facilitated the generation of mouse knockout alleles, relieving many of the cumbersome and time-consuming steps of traditional mouse embryonic stem cell technology. However, the generation of conditional knockout alleles remains an important challenge. An earlier study reported up to 16% efficiency in generating conditional knockout alleles in mice using 2 single guide RNAs (sgRNA) and 2 single-stranded oligonucleotides (ssODN) (2sgRNA–2ssODN). We re-evaluated this method from a large data set generated from a consortium consisting of 17 transgenic core facilities or laboratories or programs across the world. The dataset constituted 17,887 microinjected or electroporated zygotes and 1,718 live born mice, of which only 15 (0.87%) mice harbored 2 correct LoxP insertions in cis configuration indicating a very low efficiency of the method. To determine the factors required to successfully generate conditional alleles using the 2sgRNA–2ssODN approach, we performed a generalized linear regression model. We show that factors such as the concentration of the sgRNA, Cas9 protein or the distance between the placement of LoxP insertions were not predictive for the success of this technique. The major predictor affecting the method's success was the probability of simultaneously inserting intact proximal and distal LoxP sequences, without the loss of the DNA segment between the two sgRNA cleavage sites. Our analysis of a large data set indicates that the 2sgRNA–2ssODN method generates a large number of undesired alleles (>99%), and a very small number of desired alleles (<1%) requiring, on average 1,192 zygotes.

United States Patent Application *20180282722*
Kind Code **A1**
Jakimo; Noah ; et al. **October 4, 2018**

Chimeric DNA:RNA Guide for High Accuracy Cas9 Genome Editing

Abstract

A chimeric DNA:RNA guide for very high accuracy Cas9 genome editing employs nucleotide-type substitutions in nucleic acid-guided endonucleases for enhanced specificity. The CRISPR-Cas9 gene editing system is manipulated to generate chimeric DNA:RNA guide strands to minimize the off-target cleavage events of the S. pyogenes Cas9 endonuclease. A DNA:RNA chimeric guide strand is sufficient to guide Cas9 to a specified target sequence for indel formation and minimize off-target cleavage events due to the specificity conferred by DNA-DNA interactions. Use of chimeric mismatch-evading lowered-thermostability guides ("melt-guides") demonstrate that nucleotide-type substitutions in the spacer can reduce cleavage of sequences mismatched by as few as a single base pair. The chimeric mismatch-evading lowered-thermostability guides replace most gRNA spacer positions with DNA bases to suppress mismatched targets under Cas9's catalytic threshold.

Inventors: **Jakimo; Noah**; *(Boston, MA)* **; Chatterjee; Pranam**; *(Cambridge, MA)* **; Jacobson; Joseph M.**; *(Newton, MA)*

Applicant:	**Name**	**City**	**State**	**Country**	**Type**
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Assignee: **Massachusetts Institute of Technology**
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In vivo reprogramming of wound-resident cells generates skin epithelial tissue

Large cutaneous ulcers are, in severe cases, life threatening[1,2]. As the global population ages, non-healing ulcers are becoming increasingly common[1,2]. Treatment currently requires the transplantation of pre-existing epithelial components, such as skin grafts, or therapy using cultured cells[2]. Here we develop alternative supplies of epidermal coverage for the treatment of these kinds of wounds. We generated expandable epithelial tissues using in vivo reprogramming of wound-resident mesenchymal cells. Transduction of four transcription factors that specify the skin-cell lineage enabled efficient and rapid de novo epithelialization from the surface of cutaneous ulcers in mice. Our findings may provide a new therapeutic avenue for treating skin wounds and could be extended to other disease situations in which tissue homeostasis and repair are impaired.

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A naked mole rat iPSC line expressing drug-inducible mouse pluripotency factors developed from embryonic fibroblasts

Naked mole rats (NMRs, *Heterocephalus glaber*) are long-lived, cancer-resistant rodents. Here, we report the development of an induced pluripotent stem cell (iPSC) line generated from immortalized NMR embryonic fibroblasts transduced with a doxycycline-inducible mouse OSKM polycistronic vector. This iPSC line was shown to express pluripotency-associated markers, form embryoid bodies, differentiate *in vitro* to the derivatives of three germ layers, and exhibit normal karyotype. The ability of iPSCs to differentiate *in vivo* was supported by the contribution to interspecific chimera upon injection into mouse blastocysts. This NMR iPSC line may be a useful tool in cancer and aging research.