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Scientific News
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Sven Bulterijs

New paper from Ilia Stambler

Review article

The application of information theory for the research of aging and aging-related diseases

David Blokh[a], Ilia Stambler[b]

This article reviews the application of information-theoretical analysis, employing measures of entropy and mutual information, for the study of aging and aging-related diseases. The research of aging and aging-related diseases is particularly suitable for the application of information theory methods, as aging processes and related diseases are multi-parametric, with continuous parameters coexisting alongside discrete parameters, and with the relations between the parameters being as a rule non-linear. Information theory provides unique analytical capabilities for the solution of such problems, with unique advantages over common linear biostatistics. Among the age-related diseases, information theory has been used in the study of neurodegenerative diseases (particularly using EEG time series for diagnosis and prediction), cancer (particularly for establishing individual and combined cancer biomarkers), diabetes (mainly utilizing mutual information to characterize the diseased and aging states), and heart disease (mainly for the analysis of heart rate variability). Few works have employed information theory for the analysis of general aging processes and frailty, as underlying determinants and possible early preclinical diagnostic measures for aging-related diseases. Generally, the use of information-theoretical analysis permits not only establishing the (non-linear) correlations between diagnostic or therapeutic parameters of interest, but may also provide a theoretical insight into the nature of aging and related diseases by establishing the measures of variability, adaptation, regulation or homeostasis, within a system of interest. It may be hoped that the increased use of such measures in research may considerably increase diagnostic and therapeutic capabilities and the fundamental theoretical mathematical understanding of aging and disease.

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Developing criteria for evaluation of geroprotectors as a key stage toward translation to the clinic.

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Author information

Abstract

In the coming decades, a massive shift in the aging segment of the population will have major social and economic consequences around the world. One way to offset this increase is to expedite the development of geroprotectors, substances that slow aging, repair age-associated damage and extend healthy lifespan, or healthspan. While over 200 geroprotectors are now reported in model organisms and some are in human use for specific disease indications, the path toward determining whether they affect aging in humans remains obscure. Translation to the clinic is hampered by multiple issues including absence of a common set of criteria to define, select, and classify these substances, given the complexity of the aging process and their enormous diversity in mechanism of action. Translational research efforts would benefit from the formation of a scientific consensus on the following: the definition of 'geroprotector', the selection criteria for geroprotectors, a comprehensive classification system, and an analytical model. Here, we review current approaches to selection and put forth our own suggested selection criteria. Standardizing selection of geroprotectors will streamline discovery and analysis of new candidates, saving time and cost involved in translation to clinic.

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Repositioning tolcapone as a potent inhibitor of transthyretin amyloidogenesis and associated cellular toxicity

Transthyretin (TTR) is a plasma homotetrameric protein implicated in fatal systemic amyloidoses. TTR tetramer dissociation precedes pathological TTR aggregation. Native state stabilizers are promising drugs to treat TTR amyloidoses. Here we repurpose tolcapone, an FDA-approved molecule for Parkinson's disease, as a potent TTR aggregation inhibitor. Tolcapone binds specifically to TTR in human plasma, stabilizes the native tetramer *in vivo* in mice and humans and inhibits TTR cytotoxicity. Crystal structures of tolcapone bound to wild-type TTR and to the V122I cardiomyopathy-associated variant show that it docks better into the TTR T_4 pocket than tafamidis, so far the only drug on the market to treat TTR amyloidoses. These data indicate that tolcapone, already in clinical trials for familial amyloid polyneuropathy, is a strong candidate for therapeutic intervention in these diseases, including those affecting the central nervous system, for which no small-molecule therapy exists.

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Induction of histone deacetylases (HDACs) in human abdominal aortic aneurysm: therapeutic potential of HDAC inhibitors

María Galán, Saray Varona, Mar Orriols, José Antonio Rodríguez, Silvia Aguiló, Jaume Dilmé, Mercedes Camacho, José Martínez-González, Cristina Rodriguez

Clinical management of abdominal aortic aneurysms (AAA) is currently limited to elective surgical repair because an effective pharmacotherapy is still awaited. Inhibition of histone deacetylase (HDAC) activity could be a promising therapeutic option in cardiovascular diseases. We aimed to characterise HDACs expression in human AAA and to evaluate the therapeutic potential of class I and IIa HDAC inhibitors in the AAA model of angiotensin II (Ang II)–infused apolipoprotein E–deficient ($ApoE^{-/-}$) mice. Real-time PCR, western blot and immunohistochemistry evidenced an increased expression of HDACs 1, 2 (class I), 4 and 7 (class IIa) in abdominal aorta samples from patients undergoing AAA open repair (n=22) compared to those from donors (n=14). Aortic aneurysms from Ang II–infused $ApoE^{-/-}$ mice exhibited a similar HDACs expression profile. In these animals, treatment with a class I HDAC inhibitor (MS-275) or a class IIa inhibitor (MC-1568) improved survival, reduced the incidence and severity of AAA and limited aneurysmal expansion evaluated by Doppler ultrasonography. These beneficial effects were more patent in MC-1568-treated mice. The disorganization of elastin and collagen fibres and lymphocyte and macrophage infiltration were effectively reduced by both inhibitors. Additionally, HDAC inhibition attenuated the exacerbated expression of pro-inflammatory markers and the increase in metalloproteinase-2 and -9 activity induced by Ang II in this model. Therefore, our data evidence that HDAC expression is deregulated in human AAA and that class-selective HDAC inhibitors limit aneurysm expansion in an AAA mouse model. New generation HDAC inhibitors represent a promising therapeutic approach to overcome human aneurysm progression.

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FOXE3 mutations predispose to thoracic aortic aneurysms and dissections

Abstract

The ascending thoracic aorta is designed to withstand biomechanical forces from pulsatile blood. Thoracic aortic aneurysms and acute aortic dissections (TAADs) occur as a result of genetically triggered defects in aortic structure and a dysfunctional response to these forces. Here, we describe mutations in the forkhead transcription factor *FOXE3* that predispose mutation-bearing individuals to TAAD. We performed exome sequencing of a large family with multiple members with TAADs and identified a rare variant in *FOXE3* with an altered amino acid in the DNA-binding domain (p.Asp153His) that segregated with disease in this family. Additional pathogenic *FOXE3* variants were identified in unrelated TAAD families. In mice, *Foxe3* deficiency reduced smooth muscle cell (SMC) density and impaired SMC differentiation in the ascending aorta. *Foxe3* expression was induced in aortic SMCs after transverse aortic constriction, and *Foxe3* deficiency increased SMC apoptosis and ascending aortic rupture with increased aortic pressure. These phenotypes were rescued by inhibiting p53 activity, either by administration of a p53 inhibitor (pifithrin-α), or by crossing *Foxe3*$^{-/-}$ mice with *p53*$^{-/-}$ mice. Our data demonstrate that *FOXE3* mutations lead to a reduced number of aortic SMCs during development and increased SMC apoptosis in the ascending aorta in response to increased biomechanical forces, thus defining an additional molecular pathway that leads to familial thoracic aortic disease.

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Metformin Facilitates Amyloid-β Generation by β- and γ-Secretases via Autophagy Activation.

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Author information

Abstract

The evidence of strong pathological associations between type 2 diabetes and Alzheimer's disease (AD) has increased in recent years. Contrary to suggestions that anti-diabetes drugs may have potential for treating AD, we demonstrate here that the insulin sensitizing anti-diabetes drug metformin (Glucophage®) increased the generation of amyloid-β (Aβ), one of the major pathological hallmarks of AD, by promoting β- and γ-secretase-mediated cleavage of amyloid-β protein precursor (AβPP) in SH-SY5Y cells. In addition, we show that metformin caused autophagosome accumulation in Tg6799 AD model mice. Extremely high γ-secretase activity was also detected in autophagic vacuoles, apparently a novel site of Aβ peptide generation. Together, these data suggest that metformin-induced accumulation of autophagosomes resulted in increased γ-secretase activity and Aβ generation. Additional experiments indicated that metformin increased phosphorylation of AMP-activated protein kinase, which activates autophagy by suppressing mammalian target of rapamycin (mTOR). The suppression of mTOR then induces the abnormal accumulation of autophagosomes. We conclude that metformin, an anti-diabetes drug, may exacerbate AD pathogenesis by promoting amyloidogenic AβPP processing in autophagosomes.

Antidiabetic drug metformin (Glucophage^{R}) increases biogenesis of Alzheimer's amyloid peptides via up-regulating *BACE1* transcription

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Rise, stagnation, and rise of Danish women's life expectancy

Rune Lindahl-Jacobsen[a,b], Roland Rau[c,d], Bernard Jeune[a], Vladimir Canudas-Romo[a,b], Adam Lenart[a,b], Kaare Christensen[a,b,e,f], and James W. Vaupel[a,b,c,g,1]

Significance

Life expectancy is the most commonly used measure of health status in a population. Life expectancy has increased rapidly in most western populations over the past two centuries. There has been an ongoing debate about the relative contribution of cohort and period effects on a nation's life expectancy, but few concrete examples of strong cohort effects exist. In this study, we use demographic approaches to study cohort effects on the life expectancy of Danish women. We identify a clear-cut and strong cohort effect: the case of the interwar generations of Danish women.

Abstract

Health conditions change from year to year, with a general tendency in many countries for improvement. These conditions also change from one birth cohort to another: some generations suffer more adverse events in childhood, smoke more heavily, eat poorer diets, etc., than generations born earlier or later. Because it is difficult to disentangle period effects from cohort effects, demographers, epidemiologists, actuaries, and other population scientists often disagree about cohort effects' relative importance. In particular, some advocate forecasts of life expectancy based on period trends; others favor forecasts that hinge on cohort differences. We use a combination of age decomposition and exchange of survival probabilities between countries to study the remarkable recent history of female life expectancy in Denmark, a saga of rising, stagnating, and now again rising lifespans. The gap between female life expectancy in Denmark vs. Sweden grew to 3.5 y in the period 1975–2000. When we assumed that Danish women born 1915–1945 had the same survival probabilities as Swedish women, the gap remained small and roughly constant. Hence, the lower Danish life expectancy is caused by these cohorts and is not attributable to period effects.

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Reevaluation of whether a soma–to–germ-line transformation extends lifespan in *Caenorhabditis elegans*

Significance

Understanding the genetic mechanisms that control lifespan is essential for the development of regenerative therapies that seek to reverse the aging process. In the nematode *Caenorhabditis elegans*, long-lived mutants that are defective in insulin signaling up-regulate a number of stress response genes to promote survival. A study published in 2009 reported that these long-lived mutants also express in their somatic cells factors that are normally restricted to germ cells and that these mutants rely on germ-line factors for some of their lifespan extension. Our studies call these findings into question and instead suggest that expression of certain germ-line factors in the somatic cells of worms is detrimental to the health of worms and reduces lifespan.

The germ lineage is considered to be immortal. In the quest to extend lifespan, a possible strategy is to drive germ-line traits in somatic cells, to try to confer some of the germ lineage's immortality on the somatic body. Notably, a study in *Caenorhabditis elegans* suggested that expression of germ-line genes in the somatic cells of long-lived *daf-2* mutants confers some of *daf-2*'s long lifespan. Specifically, mRNAs encoding components of *C. elegans* germ granules (P granules) were up-regulated in *daf-2* mutant worms, and knockdown of individual P-granule and other germ-line genes in *daf-2* young adults modestly reduced their lifespan. We investigated the contribution of a germ-line program to *daf-2*'s long lifespan and also tested whether other mutants known to express germ-line genes in their somatic cells are long-lived. Our key findings are as follows. (*i*) We could not detect P-granule proteins in the somatic cells of *daf-2* mutants by immunostaining or by expression of a P-granule transgene. (*ii*) Whole-genome transcript profiling of animals lacking a germ line revealed that germ-line transcripts are not up-regulated in the soma of *daf-2* worms compared with the soma of control worms. (*iii*) Simultaneous removal of multiple P-granule proteins or the entire germ-line program from *daf-2* worms did not reduce their lifespan. (*iv*) Several mutants that robustly express a broad spectrum of germ-line genes in their somatic cells are not long-lived. Together, our findings argue against the hypothesis that acquisition of a germ-cell program in somatic cells increases lifespan and contributes to *daf-2*'s long lifespan.

Amino Acids Rather than Glucose Account for the Majority of Cell Mass in Proliferating Mammalian Cells

Aaron M. Hosios, Vivian C. Hecht, Laura V. Danai, Marc O. Johnson, Jeffrey C. Rathmell, Matthew L. Steinhauser, Scott R. Manalis, Matthew G. Vander Heiden

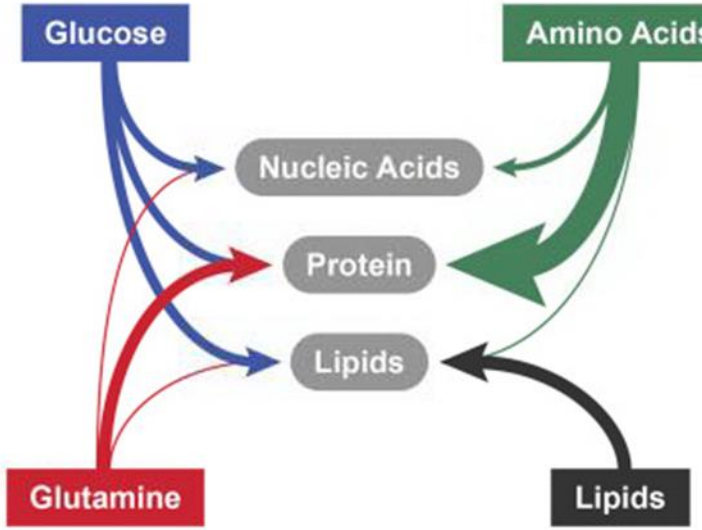
Highlights

- Glucose and glutamine are not the sources of the majority of mammalian cell mass
- Non-glutamine amino acids provide abundant carbon and nitrogen to proliferating cells
- Non-proliferating mammalian cells exhibit variable degrees of cell mass turnover
- Nutrient fates are determined, showing that glutamine contributes primarily to protein

Summary

Cells must duplicate their mass in order to proliferate. Glucose and glutamine are the major nutrients consumed by proliferating mammalian cells, but the extent to which these and other nutrients contribute to cell mass is unknown. We quantified the fraction of cell mass derived from different nutrients and found that the majority of carbon mass in cells is derived from other amino acids, which are consumed at much lower rates than glucose and glutamine. While glucose carbon has diverse fates, glutamine contributes most to protein, suggesting that glutamine's ability to replenish tricarboxylic acid cycle intermediates (anaplerosis) is primarily used for amino acid biosynthesis. These findings demonstrate that rates of nutrient consumption are indirectly associated with mass accumulation and suggest that high rates of glucose and glutamine consumption support rapid cell proliferation beyond providing carbon for biosynthesis.

Graphical Abstract



Vaccination with Necroptotic Cancer Cells Induces Efficient Anti-tumor Immunity

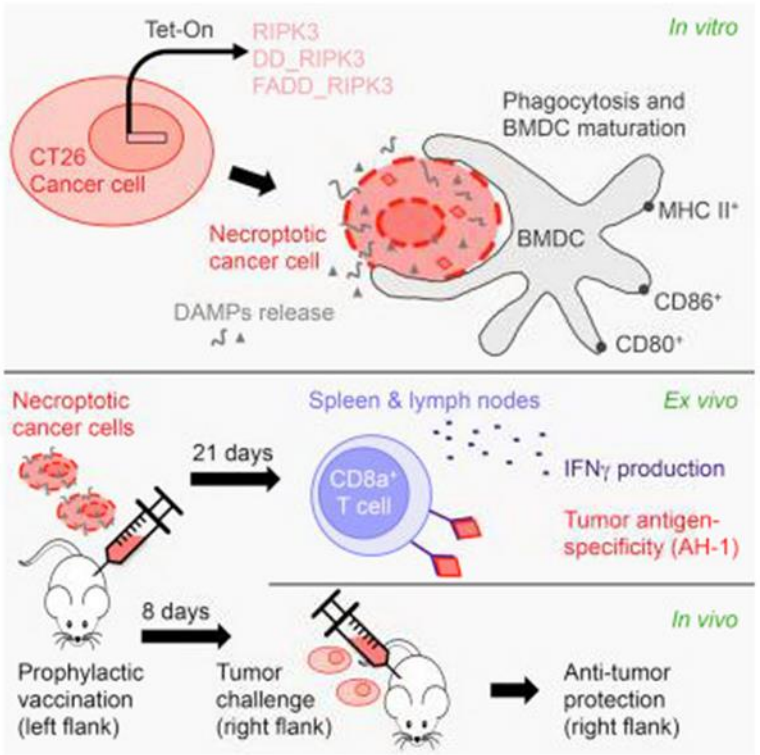
Highlights

- Necroptotic cancer cells release DAMPs and induce dendritic cell maturation in vitro
- Cross-priming of T cells was induced by necroptotic cancer cells in vivo
- Necroptotic cancer cells promote the tumor antigen-specific production of IFN-γ ex vivo
- Prophylactic injection of necroptotic cancer cells leads to an anti-tumor vaccination

Summary

Successful immunogenic apoptosis in experimental cancer therapy depends on the induction of strong host anti-tumor responses. Given that tumors are often resistant to apoptosis, it is important to identify alternative molecular mechanisms that elicit immunogenic cell death. We have developed a genetic model in which direct dimerization of FADD combined with inducible expression of RIPK3 promotes necroptosis. We report that necroptotic cancer cells release damage-associated molecular patterns and promote maturation of dendritic cells, the cross-priming of cytotoxic T cells, and the production of IFN-γ in response to tumor antigen stimulation. Using both FADD-dependent and FADD-independent RIPK3 induction systems, we demonstrate the efficient vaccination potential of immunogenic necroptotic cells. Our study broadens the current concept of immunogenic cell death and opens doors for the development of new strategies in cancer therapy.

Graphical Abstract



Longitudinal RNA-Seq Analysis of Vertebrate Aging Identifies Mitochondrial Complex I as a Small-Molecule-Sensitive Modifier of Lifespan

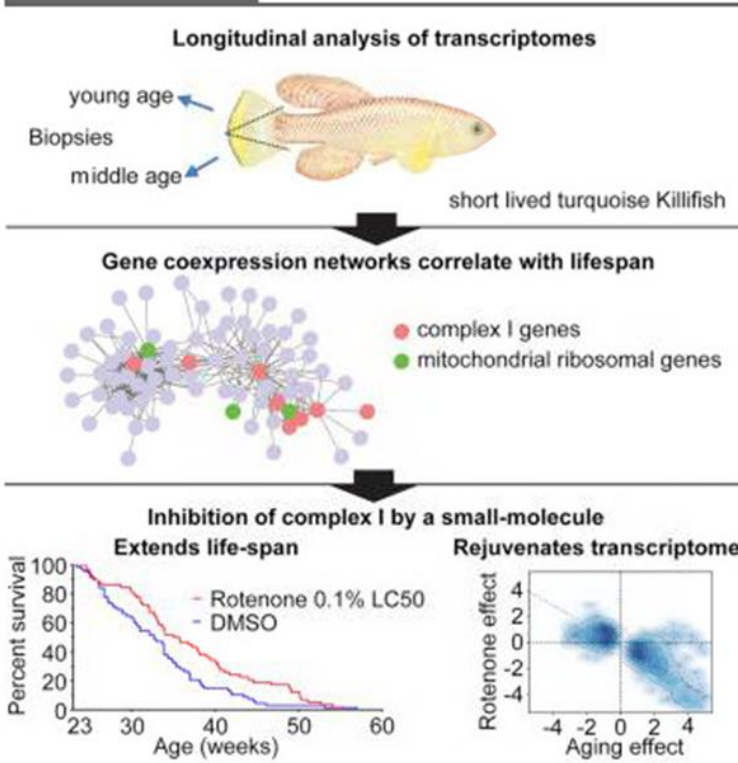
Highlights

- Longitudinal transcriptome correlation with lifespan in a short-lived vertebrate
- Shorter- and longer-lived individuals differ in transcriptome profiles at a young age
- Mitochondrial complex I identified as a hub for negative correlation with lifespan
- Inhibition of complex I prolongs lifespan and rejuvenates the transcriptome

Summary

Mutations and genetic variability affect gene expression and lifespan, but the impact of variations in gene expression within individuals on their aging-related mortality is poorly understood. We performed a longitudinal study in the short-lived killifish, *Nothobranchius furzeri*, and correlated quantitative variations in gene expression during early adult life with lifespan. Shorter- and longer-lived individuals differ in their gene expression before the onset of aging-related mortality; differences in gene expression are more pronounced early in life. We identified mitochondrial respiratory chain complex I as a hub in a module of genes whose expression is negatively correlated with lifespan. Accordingly, partial pharmacological inhibition of complex I by the small molecule rotenone reversed aging-related regulation of gene expression and extended lifespan in *N. furzeri* by 15%. These results support the use of *N. furzeri* as a vertebrate model for identifying the protein targets, pharmacological modulators, and individual-to-individual variability associated with aging.

Graphical Abstract



Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk

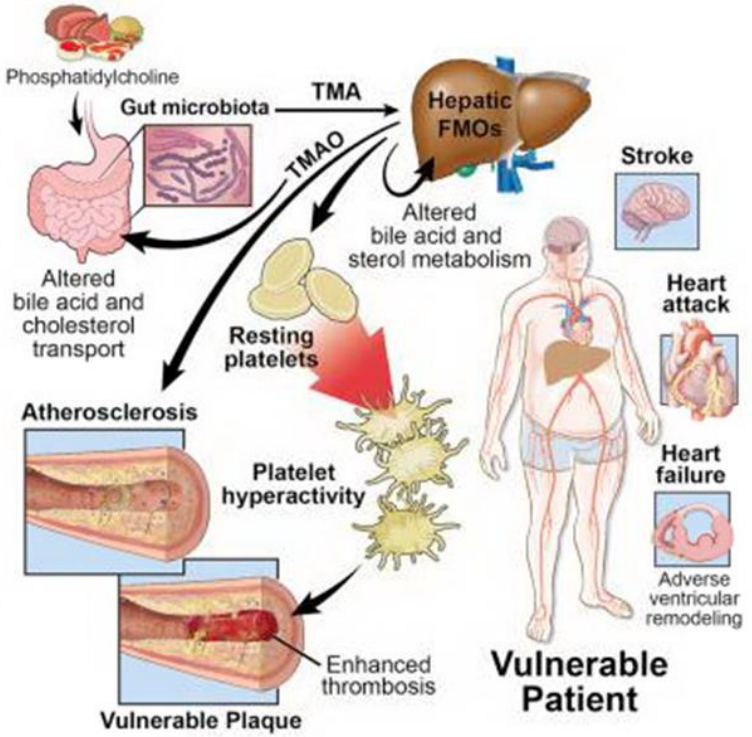
Highlights

- Elevated TMAO levels predict incident risk for thrombotic events in human subjects
- TMAO enhances sub-maximal stimulus-dependent platelet activation
- Dietary choline, gut microbes, and TMAO are linked to thrombotic potential in vivo
- Microbial transplantation shows that thrombosis potential is a transmissible trait

Summary

Normal platelet function is critical to blood hemostasis and maintenance of a closed circulatory system. Heightened platelet reactivity, however, is associated with cardiometabolic diseases and enhanced potential for thrombotic events. We now show gut microbes, through generation of trimethylamine N-oxide (TMAO), directly contribute to platelet hyperreactivity and enhanced thrombosis potential. Plasma TMAO levels in subjects (n > 4,000) independently predicted incident (3 years) thrombosis (heart attack, stroke) risk. Direct exposure of platelets to TMAO enhanced sub-maximal stimulus-dependent platelet activation from multiple agonists through augmented Ca^{2+} release from intracellular stores. Animal model studies employing dietary choline or TMAO, germ-free mice, and microbial transplantation collectively confirm a role for gut microbiota and TMAO in modulating platelet hyperresponsiveness and thrombosis potential and identify microbial taxa associated with plasma TMAO and thrombosis potential. Collectively, the present results reveal a previously unrecognized mechanistic link between specific dietary nutrients, gut microbes, platelet function, and thrombosis risk.

Graphical Abstract



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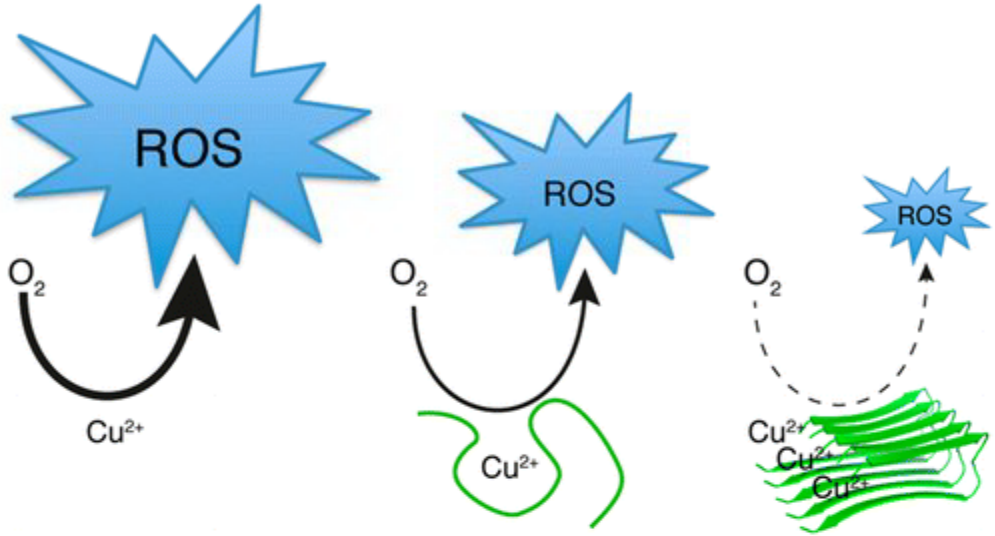
Trimethylamine N-Oxide Promotes Vascular Inflammation Through Signaling of Mitogen-Activated Protein Kinase and Nuclear Factor-κB

Background The choline-derived metabolite trimethylamine N-oxide (TMAO) has been demonstrated to contribute to atherosclerosis and is associated with coronary artery disease risk.

Methods and Results We explored the impact of TMAO on endothelial and smooth muscle cell function in vivo, focusing on disease-relevant outcomes for atherogenesis. Initially, we observed that aortas of $LDLR^{-/-}$ mice fed a choline diet showed elevated inflammatory gene expression compared with controls. Acute TMAO injection at physiological levels was sufficient to induce the same inflammatory markers and activate the well-known mitogen-activated protein kinase, extracellular signal–related kinase, and nuclear factor-κB signaling cascade. These observations were recapitulated in primary human aortic endothelial cells and vascular smooth muscle cells. We also found that TMAO promotes recruitment of activated leukocytes to endothelial cells. Through pharmacological inhibition, we further showed that activation of nuclear factor-κB signaling was necessary for TMAO to induce inflammatory gene expression in both of these relevant cell types as well as endothelial cell adhesion of leukocytes.

Conclusions Our results suggest a likely contributory mechanism for TMAO-dependent enhancement in atherosclerosis and cardiovascular risks.

Amyloid-β and α-Synuclein Decrease the Level of Metal-Catalyzed Reactive Oxygen Species by Radical Scavenging and Redox Silencing



The formation of reactive oxygen species (ROS) is linked to the pathogenesis of neurodegenerative diseases. Here we have investigated the effect of soluble and aggregated amyloid-β (Aβ) and α-synuclein (αS), associated with Alzheimer's and Parkinson's diseases, respectively, on the Cu^{2+}-catalyzed formation of ROS *in vitro* in the presence of a biological reductant. We find that the levels of ROS, and the rate by which ROS is generated, are significantly reduced when Cu^{2+} is bound to Aβ or αS, particularly when they are in their oligomeric or fibrillar forms. This effect is attributed to a combination of radical scavenging and redox silencing mechanisms. Our findings suggest that the increase in ROS associated with the accumulation of aggregated Aβ or αS does not result from a particularly ROS-active form of these peptides, but rather from either a local increase of Cu^{2+} and other ROS-active metal ions in the aggregates or as a downstream consequence of the formation of the pathological amyloid structures.

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Probes of ubiquitin E3 ligases enable systematic dissection of parkin activation

E3 ligases represent an important class of enzymes, yet there are currently no chemical probes for profiling their activity. We develop a new class of activity-based probe by re-engineering a ubiquitin-charged E2 conjugating enzyme and demonstrate the utility of these probes by profiling the transthiolation activity of the RING-in-between-RING (RBR) E3 ligase parkin *in vitro* and in cellular extracts. Our study provides valuable insight into the roles, and cellular hierarchy, of distinct phosphorylation events in parkin activation. We also profile parkin mutations associated with patients with Parkinson's disease and demonstrate that they mediate their effect largely by altering transthiolation activity. Furthermore, our probes enable direct and quantitative measurement of endogenous parkin activity, revealing that endogenous parkin is activated in neuronal cell lines (≥75%) in response to mitochondrial depolarization. This new technology also holds promise as a novel biomarker of PINK1-parkin signaling, as demonstrated by its compatibility with samples derived from individuals with Parkinson's disease.

Letter to the Editor, so hence no abstract

J Invest Dermatol. 2016 Mar;136(3):721-3. doi: 10.1016/j.jid.2015.12.005. Epub 2015 Dec 14.

Autoantibodies to Collagen XVII Are Present in Parkinson's Disease and Localize to Tyrosine-Hydroxylase Positive Neurons.

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Calcium is a key factor in α-synuclein induced neurotoxicity

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J Cell Sci 2016 : doi: 10.1242/jcs.180737

Article | Info & metrics | PDF

Abstract

Aggregation of α-synuclein leads to the formation of oligomeric intermediates that can interact with membranes to form pores. However it is unknown how this leads to cell toxicity in Parkinson's disease. We investigated the species-specific effects of α-synuclein on calcium signalling in primary neurons and astrocytes using live neuronal imaging and electrophysiology on artificial membranes. We demonstrate that α-synuclein induces an increase in basal intracellular calcium in its unfolded monomeric state as well as in its oligomeric state. Electrophysiology of artificial membranes demonstrated that α-synuclein monomers induce irregular ionic currents, while α-synuclein oligomers induce rare discrete channel formation. Despite the ability for monomeric α-synuclein to affect calcium signalling, it is only the oligomeric form of α-synuclein that induces cell death. Oligomer-induced cell death was abolished by the exclusion of extracellular calcium, which prevented the α-synuclein induced calcium dysregulation. The findings of this study confirm that α-synuclein interacts with membranes to affect calcium signalling in a structure-specific manner and the oligomeric beta sheet rich α-synuclein species ultimately leads to calcium dysregulation and calcium dependent cell death.

Nanoscopic insights into seeding mechanisms and toxicity of α-synuclein species in neurons

Significance

The self-assembly of normally soluble proteins into fibrillar amyloid structures is associated with a range of neurodegenerative disorders. Here, we monitor the fate of different forms of α-synuclein (AS), a protein implicated in Parkinson's disease, via optical nanoscopy directly in neuronal cells. We show that exogenously added preformed AS fibrils elongate by the addition of endogenous AS, naturally present in neurons. In contrast, exogenously added monomeric AS induces aggregate formation within the cells and leads to apoptosis. The latter is significantly reduced by the addition of preformed fibrils, suggesting a neuroprotective role of fibrillar species. The visualization of these effects at the nanoscale shown here opens up new avenues for understanding the links between AS aggregation and neuronal toxicity.

Abstract

New strategies for visualizing self-assembly processes at the nanoscale give deep insights into the molecular origins of disease. An example is the self-assembly of misfolded proteins into amyloid fibrils, which is related to a range of neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases. Here, we probe the links between the mechanism of α-synuclein (AS) aggregation and its associated toxicity by using optical nanoscopy directly in a neuronal cell culture model of Parkinson's disease. Using superresolution microscopy, we show that protein fibrils are taken up by neuronal cells and act as prion-like seeds for elongation reactions that both consume endogenous AS and suppress its de novo aggregation. When AS is internalized in its monomeric form, however, it nucleates and triggers the aggregation of endogenous AS, leading to apoptosis, although there are no detectable cross-reactions between externally added and endogenous protein species. Monomer-induced apoptosis can be reduced by pretreatment with seed fibrils, suggesting that partial consumption of the externally added or excess soluble AS can be significantly neuroprotective.

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Disrupted iron homeostasis causes dopaminergic neurodegeneration in mice

Significance

The brain requires iron for mitochondrial respiration and synthesis of myelin, neurotransmitters, and monoamine oxidases. Iron accumulates in distinct parts of the brain in patients with neurodegenerative diseases, and some have proposed that neurons die because they contain too much iron. Neuronal iron handling is not well understood. We focused on dopaminergic neurons, affected in Parkinson's disease, and manipulated molecules involve in iron uptake and release. We showed that loss of ferroportin, which exports cellular iron, had no apparent effect. In contrast, loss of transferrin receptor, involved in iron uptake, caused neuronal iron deficiency and neurodegeneration with features similar to Parkinson's disease. We propose that neuronal iron deficiency may contribute to neurodegeneration in human disease.

Abstract

Disrupted brain iron homeostasis is a common feature of neurodegenerative disease. To begin to understand how neuronal iron handling might be involved, we focused on dopaminergic neurons and asked how inactivation of transport proteins affected iron homeostasis in vivo in mice. Loss of the cellular iron exporter, ferroportin, had no apparent consequences. However, loss of transferrin receptor 1, involved in iron uptake, caused neuronal iron deficiency, age-progressive degeneration of a subset of dopaminergic neurons, and motor deficits. There was gradual depletion of dopaminergic projections in the striatum followed by death of dopaminergic neurons in the substantia nigra. Damaged mitochondria accumulated, and gene expression signatures indicated attempted axonal regeneration, a metabolic switch to glycolysis, oxidative stress, and the unfolded protein response. We demonstrate that loss of transferrin receptor 1, but not loss of ferroportin, can cause neurodegeneration in a subset of dopaminergic neurons in mice.

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Response

A role for iron deficiency in dopaminergic neurodegeneration

Yvette Y. Yien[a,b] and Barry H. Paw[a,b,c,d,1]

Author Affiliations

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Disorders of iron metabolism, manifesting in iron overload or iron deficiency, are implicated in neurodegeneration (1⇓⇓⇓–5). In PNAS, Matak et al. (6) report the specific inactivation of ferroportin (*Fpn*, *Slc40a1*) and transferrin receptor 1 (*TfR1*) in dopaminergic (DA) neurons. The animal models generated in this study demonstrated that *Fpn* did not play a major role in DA neurophysiology, whereas a defect in *Tfr1*-dependent iron uptake caused severe iron deficiency that resulted in neurodegeneration, manifesting in behaviors that are similar to those in Parkinson's disease (6).

Iron transporters play a key role in iron homeostasis and tightly couple intracellular iron levels with cellular requirements (7). This Matak et al. (6) study examines the requirement for FPN, the only known cellular iron exporter (8⇓–10), and TFR1, a key component of the iron uptake machinery, to dissect the effects of iron overload and iron deficiency in the biology of DA neurons. Loss of *Fpn* function in several cell types causes iron overload (11, 12) and has been implicated in myelination defects (13). *TfR1*-mediated iron uptake is the main source of iron for actively proliferating cells, and is essential for iron transport in erythroid cells and neural tissue (14), epithelial enterocytes (15), skeletal muscle (16), and cardiac muscle (17), in addition to DA neurons, as described in Matak et al. (6). *TfR1* deficiency in erythroid …

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Complement and microglia mediate early synapse loss in Alzheimer mouse models

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Synapse loss in Alzheimer's disease (AD) correlates with cognitive decline. Involvement of microglia and complement in AD has been attributed to neuroinflammation, prominent late in disease. Here we show in mouse models that complement and microglia mediate synaptic loss early in AD. C1q, the initiating protein of the classical complement cascade, is increased and associated with synapses before overt plaque deposition. Inhibition of C1q, C3 or the microglial complement receptor CR3, reduces the number of phagocytic microglia as well as the extent of early synapse loss. C1q is necessary for the toxic effects of soluble β-amyloid (Aβ) oligomers on synapses and hippocampal long-term potentiation (LTP). Finally, microglia in adult brains engulf synaptic material in a CR3-dependent process when exposed to soluble Aβ oligomers. Together, these findings suggest that the complement-dependent pathway and microglia that prune excess synapses in development are inappropriately activated and mediate synapse loss in AD.

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A new structural model of Alzheimer's Aβ42 fibrils based on electron paramagnetic resonance data and Rosetta modeling

Lei Gu, Joyce Tran, Lin Jiang, Zhefeng Guo

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Abstract

Brain deposition of Aβ in the form of amyloid plaques is a pathological hallmark of Alzheimer's disease. There are two major species of Aβ in the brain: Aβ42 and Aβ40. Although Aβ40 is several-fold more abundant than Aβ42 in soluble form, Aβ42 is the major component of amyloid plaques. Structural knowledge of Aβ42 fibrils is important both for understanding the process of Aβ aggregation and for designing fibril-targeting drugs. Here we report site-specific structural information of Aβ42 fibrils at 22 residue positions based on electron paramagnetic resonance data. In combination with structure prediction program Rosetta, we modeled Aβ42 fibril structure at atomic resolution. Our Aβ42 fibril model consists of four parallel in-register β-sheets: β_N (residues ~7–13), β_1 (residues ~17–20), β_2 (residues ~32–36), and β_C (residues 39–41). The region of β_1-loop-β_2 in Aβ42 fibrils adopts similar structure as that in Aβ40 fibrils. This is consistent with our cross seeding data that Aβ42 fibril seeds shortened the lag phase of Aβ40 fibrillization. On the other hand, Aβ42 fibrils contain a C-terminal β-arc-β motif with a special turn, termed "arc", at residues 37–38, which is absent in Aβ40 fibrils. Our results can explain both the higher aggregation propensity of Aβ42 and the importance of Aβ42 to Aβ40 ratio in the pathogenesis of Alzheimer's disease.

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Therapeutic correction of ApoER2 splicing in Alzheimer's disease mice using antisense oligonucleotides

Apolipoprotein E receptor 2 (*ApoER2*) is an apolipoprotein E receptor involved in long-term potentiation, learning, and memory. Given its role in cognition and its association with the Alzheimer's disease (AD) risk gene, apoE, *ApoER2* has been proposed to be involved in AD, though a role for the receptor in the disease is not clear. *ApoER2* signaling requires amino acids encoded by alternatively spliced exon 19. Here, we report that the balance of *ApoER2* exon 19 splicing is deregulated in postmortem brain tissue from AD patients and in a transgenic mouse model of AD. To test the role of deregulated *ApoER2* splicing in AD, we designed an antisense oligonucleotide (ASO) that increases exon 19 splicing. Treatment of AD mice with a single dose of ASO corrected ApoER2 splicing for up to 6 months and improved synaptic function and learning and memory. These results reveal an association between *ApoER2* isoform expression and AD, and provide preclinical evidence for the utility of ASOs as a therapeutic approach to mitigate Alzheimer's disease symptoms by improving *ApoER2* exon 19 splicing.

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Generation and deposition of Aβ43 by the virtually inactive presenilin-1 L435F mutant contradicts the presenilin loss-of-function hypothesis of Alzheimer's disease

As stated by the prevailing amyloid cascade hypothesis, Alzheimer's disease (AD) is caused by the aggregation and cerebral deposition of long amyloid-β peptide (Aβ) species, which are released from a C-terminal amyloid precursor protein fragment by γ-secretase. Mutations in its catalytic subunit presenilin-1 (PS1) increase the Aβ42 to Aβ40 ratio and are the major cause of familial AD (FAD). An opposing hypothesis states that loss of essential presenilin functions underlies the disease. A major argument for this hypothesis is the observation that the nearly inactive PS1 L435F mutant, paradoxically, causes FAD. We now show that the very little Aβ generated by PS1 L435F consists primarily of Aβ43, a highly amyloidogenic species which was overlooked in previous studies of this mutant. We further demonstrate that the generation of Aβ43 is not due to a trans-dominant effect of this mutant on WT presenilin. Furthermore, we found Aβ43-containing plaques in brains of patients with this mutation. The aberrant generation of Aβ43 by this particular mutant provides a direct objection against the presenilin hypothesis.

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Expression of A152T human tau causes age-dependent neuronal dysfunction and loss in transgenic mice

A152T-variant human tau (hTau-A152T) increases risk for tauopathies, including Alzheimer's disease. Comparing mice with regulatable expression of hTau-A152T or wild-type hTau (hTau-WT), we find age-dependent neuronal loss, cognitive impairments, and spontaneous nonconvulsive epileptiform activity primarily in hTau-A152T mice. However, overexpression of either hTau species enhances neuronal responses to electrical stimulation of synaptic inputs and to an epileptogenic chemical. hTau-A152T mice have higher hTau protein/mRNA ratios in brain, suggesting that A152T increases production or decreases clearance of hTau protein. Despite their functional abnormalities, aging hTau-A152T mice show no evidence for accumulation of insoluble tau aggregates, suggesting that their dysfunctions are caused by soluble tau. In human amyloid precursor protein (hAPP) transgenic mice, co-expression of hTau-A152T enhances risk of early death and epileptic activity, suggesting copathogenic interactions between hTau-A152T and amyloid-β peptides or other hAPP metabolites. Thus, the A152T substitution may augment risk for neurodegenerative diseases by increasing hTau protein levels, promoting network hyperexcitability, and synergizing with the adverse effects of other pathogenic factors.

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Glycation alter the process of Tau phosphorylation to change Tau isoforms aggregation property

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Abstract

The risk of tauopathies depends in part on the levels and modified composition of six Tau isoforms in the human brain. Abnormal phosphorylation of the Tau protein and the shift of the ratio of 3R Tau to 4R Tau are presumed to result in neurofibrillary pathology and neurodegeneration. Glycation has recently been linked to dementia and metabolic syndrome. To determine the contribution of Tau protein glycation and phosphorylation on Tau aggregation propensity, the assembled kinetics were examined in vitro using Thioflavin T fluorescence assays. We found that glycation and phosphorylation have different effects on aggregation propensity in different Tau isoforms. Different Tau proteins play important parts in each tauopathies, but 3R0N, fetal Tau protein, has no effect on tauopathies. Conversely, 4R2N has more modified sites and a higher tendency to aggregate, playing the most important role in 4R tauopathies. Finally, Glycation, which could modulate Tau phosphorylation, may occur before any other modification. It also regulates the 3R to 4R ratio and promotes 4R2N Tau protein aggregation. Decreasing the sites of glycation, as well as shifting other Tau proteins to 3R0N Tau proteins has potential therapeutic implications for tauopathies.

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Cdk5-FOXO3a axis: initially neuroprotective, eventually neurodegenerative in Alzheimer's disease models

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Article | Info & metrics | PDF

Abstract

Deregulated Cdk5 causes neurotoxic Aβ processing and cell death, two hallmarks of Alzheimer's disease (AD) via FOXO3a transcriptional factor in hippocampal cells, primary neurons and an AD mouse model. Using an innovative chemical-genetic screen, we identified Foxo3a as a direct substrate of Cdk5 in brain lysates. Cdk5 directly phosphorylates FOXO3a, which increased its levels and nuclear translocation. Nuclear FOXO3a initially rescued cells from ensuing oxidative stress by upregulating MnSOD. However, following prolonged exposure, FOXO3a upregulated Bim and FasL causing cell death. Active FOXO3a also increased Aβ(1-42) levels in a phosphorylation-dependent manner. These events were completely inhibited either by expressing phosphorylation-resistant FOXO3a or by depleting Cdk5 or Foxo3, highlighting a key role of Cdk5 in regulating FOXO3a. These results were confirmed in an AD mouse model, which exhibited increased levels and nuclear localization of FOXO3a in hippocampal neurons, which preceded neurodegeneration and Aβ plaque formation, suggesting it is an early event in AD pathogenesis. These results show that Cdk5-mediated phospho-regulation of FOXO3a can activate several genes that promote neuronal death and aberrant Aβ processing, thereby contributing to the progression of neurodegenerative pathologies.

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S-nitrosation of proteins relevant to Alzheimer's disease during early stages of neurodegeneration

Protein *S*-nitrosation (*SNO*-protein), the nitric oxide-mediated posttranslational modification of cysteine thiols, is an important regulatory mechanism of protein function in both physiological and pathological pathways. A key first step toward elucidating the mechanism by which *S*-nitrosation modulates a protein's function is identification of the targeted cysteine residues. Here, we present a strategy for the simultaneous identification of *SNO*-cysteine sites and their cognate proteins to profile the brain of the CK-p25–inducible mouse model of Alzheimer's disease-like neurodegeneration. The approach—*SNO*TRAP (*SNO* trapping by triaryl phosphine)—is a direct tagging strategy that uses phosphine-based chemical probes, allowing enrichment of *SNO*-peptides and their identification by liquid chromatography tandem mass spectrometry. *SNO*TRAP identified 313 endogenous *SNO*-sites in 251 proteins in the mouse brain, of which 135 *SNO*-proteins were detected only during neurodegeneration. *S*-nitrosation in the brain shows regional differences and becomes elevated during early stages of neurodegeneration in the CK-p25 mouse. The *SNO*-proteome during early neurodegeneration identified increased *S*-nitrosation of proteins important for synapse function, metabolism, and Alzheimer's disease pathology. In the latter case, proteins related to amyloid precursor protein processing and secretion are *S*-nitrosated, correlating with increased amyloid formation. Sequence analysis of *SNO*-cysteine sites identified potential linear motifs that are altered under pathological conditions. Collectively, *SNO*TRAP is a direct tagging tool for global elucidation of the *SNO*-proteome, providing functional insights of endogenous *SNO* proteins in the brain and its dysregulation during neurodegeneration.

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Synaptosomal Mitochondrial Dysfunction in 5xFAD Mouse Model of Alzheimer's Disease

Brain mitochondrial dysfunction is hallmark pathology of Alzheimer's disease (AD). Recently, the role of synaptosomal mitochondrial dysfunction in the development of synaptic injury in AD has received increasing attention. Synaptosomal mitochondria are a subgroup of neuronal mitochondria specifically locating at synapses. They play an essential role in fueling synaptic functions by providing energy on the site; and their defects may lead to synaptic failure, which is an early and pronounced pathology in AD. In our previous studies we have determined early synaptosomal mitochondrial dysfunction in an AD animal model (J20 line) overexpressing human Amyloid beta (Aβ), the key mediator of AD. In view of the limitations of J20 line mice in representing the full aspects of amyloidopathy in AD cases, we employed 5xFAD mice which are thought to be a desirable paradigm of amyloidopathy as seen in AD subjects. In addition, we have also examined the status of synaptosomal mitochondrial dynamics as well as Parkin-mediated mitophagy which have not been previously investigated in this mouse model. In comparison to nontransgenic (nonTg mice), 5xFAD mice demonstrated prominent synaptosomal mitochondrial dysfunction. Moreover, synaptosomal mitochondria from the AD mouse model displayed imbalanced mitochondrial dynamics towards fission along with activated Parkin and LC3BII recruitment correlating to spatial learning & memory impairments in 5xFAD mice in an age-dependent manner. These results suggest that synaptosomal mitochondrial deficits are primary pathology in Aβ-rich environments and further confirm the relevance of synaptosomal mitochondrial deficits to the development of AD.

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Individual variability in human blood metabolites identifies age-related differences

Metabolites present in human blood document individual physiological states influenced by genetic, epigenetic, and lifestyle factors. Using high-resolution liquid chromatography-mass spectrometry (LC-MS), we performed nontargeted, quantitative metabolomics analysis in blood of 15 young (29 ± 4 y of age) and 15 elderly (81 ± 7 y of age) individuals. Coefficients of variation (CV = SD/mean) were obtained for 126 blood metabolites of all 30 donors. Fifty-five RBC-enriched metabolites, for which metabolomics studies have been scarce, are highlighted here. We found 14 blood compounds that show remarkable age-related increases or decreases; they include 1,5-anhydroglucitol, dimethyl-guanosine, acetyl-carnosine, carnosine, ophthalmic acid, UDP-acetyl-glucosamine, *N*-acetyl-arginine, N_6-acetyl-lysine, pantothenate, citrulline, leucine, isoleucine, NAD^+, and $NADP^+$. Six of them are RBC-enriched, suggesting that RBC metabolomics is highly valuable for human aging research. Age differences are partly explained by a decrease in antioxidant production or increasing inefficiency of urea metabolism among the elderly. Pearson's coefficients demonstrated that some age-related compounds are correlated, suggesting that aging affects them concomitantly. Although our CV values are mostly consistent with those CVs previously published, we here report previously unidentified CVs of 51 blood compounds. Compounds having moderate to high CV values (0.4–2.5) are often modified. Compounds having low CV values, such as ATP and glutathione, may be related to various diseases because their concentrations are strictly controlled, and changes in them would compromise health. Thus, human blood is a rich source of information about individual metabolic differences.

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Mitochondrial stress induces cellular senescence in an mTORC1-dependent manner

Highlights

- Low level ROS leads to mTORC1 activation, high levels of ROS inhibit mTORC1 through AMPK.
- mTORC1/S6 kinase activity can target HDM2 following mitochondrial dysfunction to activate p53.
- Changes in mTORC1 activity alter oxygen consumption rates and are tied to energy status.
- Elevated mTORC1 activity may represent a chronic stress response leading to senescence.

Although mitochondrial stress is a key determinant of cellular homeostasis, the intracellular mechanisms by which this stress is communicated to the nucleus and its impact on cell fate decisions are not well defined. In this study, we report that activation of mTORC1 signaling triggered by mitochondrial-generated reactive oxygen species (ROS) results in activation of the senescence program. We show that exposure of human fibroblasts to nucleoside analogs commonly used in antiretroviral therapies, and known to induce mitochondrial dysfunction, increases mitochondrial ROS and leads to a rise in intracellular ROS concomitant with activation of mTORC1. In this setting, it appears that mTORC1 activates senescence through HDM2 phosphorylation, facilitating a p53-mediated response. Inhibition of mTORC1 by rapamycin decreases HDM2 phosphorylation and blocks activation of the senescence program in human cells. In addition, decreasing mitochondrial ROS directly blocks mTORC1 signaling and prevents the onset of senescence. Consistent with these results, both total and mitochondrial-specific ROS increased in cells undergoing replicative senescence along with ribosomal p70 phosphorylation. The results reveal a novel link between mitochondrial dysfunction, mTORC1 signaling, and the senescence program.

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Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice

Abstract

Rationale

While reports suggest a single dose of senolytics may improve vasomotor function, the structural and functional impact of long-term senolytic treatment is unknown.

Objective

To determine whether long-term senolytic treatment improves vasomotor function, vascular stiffness, and intimal plaque size and composition in aged or hypercholesterolemic mice with established disease.

Methods and Results

Senolytic treatment (intermittent treatment with Dasatinib + Quercetin via oral gavage) resulted in significant reductions in senescent cell markers (TAF^+ cells) in the medial layer of aorta from aged and hypercholesterolemic mice, but not in intimal atherosclerotic plaques. While senolytic treatment significantly improved vasomotor function (isolated organ chamber baths) in both groups of mice, this was due to increases in nitric oxide bioavailability in aged mice and increases in sensitivity to NO donors in hypercholesterolemic mice. Genetic clearance of senescent cells in aged normocholesterolemic *INK-ATTAC* mice phenocopied changes elicited by D+Q. Senolytics tended to reduce aortic calcification (alizarin red) and osteogenic signaling (qRT-PCR, immunohistochemistry) in aged mice, but both were significantly reduced by senolytic treatment in hypercholesterolemic mice. Intimal plaque fibrosis (picrosirius red) was not changed appreciably by chronic senolytic treatment.

Conclusions

This is the first study to demonstrate that chronic clearance of senescent cells improves established vascular phenotypes associated with aging and chronic hypercholesterolemia, and may be a viable therapeutic intervention to reduce morbidity and mortality from cardiovascular diseases.

$p16^{Ink4a}$-induced senescence of pancreatic beta cells enhances insulin secretion

Cellular senescence is thought to contribute to age-associated deterioration of tissue physiology. The senescence effector $p16^{Ink4a}$ is expressed in pancreatic beta cells during aging and limits their proliferative potential; however, its effects on beta cell function are poorly characterized. We found that beta cell–specific activation of $p16^{Ink4a}$ in transgenic mice enhances glucose-stimulated insulin secretion (GSIS). In mice with diabetes, this leads to improved glucose homeostasis, providing an unexpected functional benefit. Expression of $p16^{Ink4a}$ in beta cells induces hallmarks of senescence—including cell enlargement, and greater glucose uptake and mitochondrial activity—which promote increased insulin secretion. GSIS increases during the normal aging of mice and is driven by elevated $p16^{Ink4a}$ activity. We found that islets from human adults contain $p16^{Ink4a}$-expressing senescent beta cells and that senescence induced by $p16^{Ink4a}$ in a human beta cell line increases insulin secretion in a manner dependent, in part, on the activity of the mechanistic target of rapamycin (mTOR) and the peroxisome proliferator-activated receptor (PPAR)-γ proteins. Our findings reveal a novel role for $p16^{Ink4a}$ and cellular senescence in promoting insulin secretion by beta cells and in regulating normal functional tissue maturation with age.

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Ageing-induced changes in the redox status of peripheral motor nerves imply an effect on redox signalling rather than oxidative damage

Ageing is associated with loss of skeletal muscle fibres, atrophy of the remaining fibres and weakness. These changes in muscle are accompanied by disruption of motor neurons and neuromuscular junctions although the direct relationship between the nerve and muscle degeneration is not understood. Oxidative changes have been implicated in the mechanisms leading to age-related loss of muscle mass and in degeneration of the central nervous system, but little is known about age-related changes in oxidation in specific peripheral nerves that supply muscles that are affected by ageing. We have therefore examined the sciatic nerve of old mice at an age when loss of tibialis anterior muscle mass and function is apparent. Sciatic nerve from old mice did not show a gross increase in oxidative damage, but electron paramagnetic resonance (EPR) studies indicated an increase in the activity of superoxide and/or peroxynitrite in the nerves of old mice at rest that was further exacerbated by electrical stimulation of the nerve to activate muscle contractions. Proteomic analyses indicated that specific redox-sensitive proteins are increased in content in the nerves of old mice that may reflect an adaptation to regulate the increased superoxide/peroxynitrite and maintain redox homoeostasis. Analysis of redox active cysteines showed some increase in reversible oxidation in specific proteins in nerves of old mice, but this was not universally seen across all redox-active cysteines. Detailed analysis of the redox-active cysteine in one protein in the nerve of old mice that is key to redox signalling (Peroxiredoxin 6, Cys 47) showed a minor increase in reversible oxidation that would be compatible with a change in its redox signalling function. In conclusion, the data presented indicate that sciatic nerve from old mice does not show a gross increase in oxidative damage similar to that seen in the TA and other muscles that it innervates. Our results indicate an adaptation to increased oxidation with minor changes in the oxidation of key cysteines that may contribute to defective redox signalling in the nerve.

A Systems Approach to Reverse Engineer Lifespan Extension by Dietary Restriction

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Highlights

- We obtain temporally resolved effects of diet restriction on aging transcriptomes
- Early responses involve metabolism; late involve cell cycle and DNA damage
- We find three regulator groups with novel regulators separated by target specificity
- Regulator feedbacks are leveraged to fully recapitulate diet restriction effects

Summary

Dietary restriction (DR) is the most powerful natural means to extend lifespan. Although several genes can mediate responses to alternate DR regimens, no single genetic intervention has recapitulated the full effects of DR, and no unified system is known for different DR regimens. Here we obtain temporally resolved transcriptomes during calorie restriction and intermittent fasting in *Caenorhabditis elegans* and find that early and late responses involve metabolism and cell cycle/DNA damage, respectively. We uncover three network modules of DR regulators by their target specificity. By genetic manipulations of nodes representing discrete modules, we induce transcriptomes that progressively resemble DR as multiple nodes are perturbed. Targeting all three nodes simultaneously results in extremely long-lived animals that are refractory to DR. These results and dynamic simulations demonstrate that extensive feedback controls among regulators may be leveraged to drive the regulatory circuitry to a younger steady state, recapitulating the full effect of DR.

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Calories or Protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone.

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Author information

Abstract

Almost exactly 100 years ago Osborne and colleagues demonstrated that restricting the food intake of a small number of female rats extended their lifespan. In the 1930s experiments on the impact of diet on lifespan were extended by Slonaker, and subsequently McCay. Slonaker concluded that there was a strong impact of protein intake on lifespan, while McCay concluded that calories are the main factor causing differences in lifespan when animals are restricted (Calorie restriction or CR). Hence from the very beginning the question of whether food restriction acts on lifespan via reduced calorie intake or reduced protein intake was disputed. Subsequent work supported the idea that calories were the dominant factor. More recently, however, this role has again been questioned, particularly in studies of insects. Here we review the data regarding previous studies of protein and calorie restriction in rodents. We show that increasing CR (with simultaneous protein restriction: PR) increases lifespan, and that CR with no PR generates an identical effect. None of the residual variation in the impact of CR (with PR) on lifespan could be traced to variation in macronutrient content of the diet. Other studies show that low protein content in the diet does increase median lifespan, but the effect is smaller than the CR effect. We conclude that CR is a valid phenomenon in rodents that cannot be explained by changes in protein intake, but that there is a separate phenomenon linking protein intake to lifespan, which acts over a different range of protein intakes than is typical in CR studies. This suggests there may be a fundamental difference in the responses of insects and rodents to CR. This may be traced to differences in the physiology of these groups, or reflect a major methodological difference between 'restriction' studies performed on rodents and insects. We suggest that studies where the diet is supplied ad libitum, but diluted with inert components, should perhaps be called dietary or caloric dilution, rather than dietary or caloric restriction, to distinguish these potentially important methodological differences.

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Safety of two-year caloric restriction in non-obese healthy individuals.

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⊕ Author information

Abstract

BACKGROUND: The extent to which sustained caloric restriction (CR) in healthy non-obese adults is safe has not been previously investigated.

OBJECTIVE: Assess the safety and tolerability of sustained two-year CR intervention in healthy, non-obese adults.

DESIGN: A multi-center, randomized controlled trial. Participants were randomized using a 2:1 allocation in favor of 25% CR vs. Ad-Libitum intake (AL). Adverse and serious adverse events (AE, SAE), safety laboratory tests, and other safety parameters were closely monitored.

RESULTS: Three participants were withdrawn from the CR intervention because of the safety concerns. No deaths and one SAE was reported by participants in the CR group. Although the difference in AE between AL and CR groups was not significant, within the CR group, the incidence of nervous system ($p = 0.02$), musculoskeletal ($p = 0.02$) and reproductive system ($p = 0.002$) disorders was significantly higher in the normal-weight than in the overweight participants. At months 12 and 24, bone mineral densities at the lumbar spine, total hip, and femoral neck of participants in the CR group were significantly lower than in those in the AL group.

CONCLUSIONS: Two-years of CR at levels achieved in CALERIE was safe and well tolerated. Close monitoring for excessive bone loss and anemia is important.

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Oxidative stress at low levels can induce clustered DNA lesions leading to NHEJ mediated mutations.

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Author information

Abstract

DNA damage and mutations induced by oxidative stress are associated with various different human pathologies including cancer. The facts that most human tumors are characterized by large genome rearrangements and glutathione depletion in mice results in deletions in DNA suggest that reactive oxygen species (ROS) may cause gene and chromosome mutations through DNA double strand breaks (DSBs). However, the generation of DSBs at low levels of ROS is still controversial. In the present study, we show that H2O2 at biologically-relevant levels causes a marked increase in oxidative clustered DNA lesions (OCDLs) with a significant elevation of replication-independent DSBs. Although it is frequently reported that OCDLs are fingerprint of high-energy IR, our results indicate for the first time that H2O2, even at low levels, can also cause OCDLs leading to DSBs specifically in G1 cells. Furthermore, a reverse genetic approach revealed a significant contribution of the non-homologous end joining (NHEJ) pathway in H2O2-induced DNA repair & mutagenesis. This genomic instability induced by low levels of ROS may be involved in spontaneous mutagenesis and the etiology of a wide variety of human diseases like chronic inflammation-related disorders, carcinogenesis, neuro-degeneration and aging.

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Structural, biological and biophysical properties of glycated and glycoxidized phosphatidylethanolamines.

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Author information

Abstract

Glycation and glycoxidation of proteins and peptides have been intensively studied and are considered as reliable diagnostic biomarkers of hyperglycemia and early stages of type II diabetes. However, glucose can also react with primary amino groups present in other cellular components, such as aminophospholipids (aminoPLs). Although it is proposed that glycated aminoPLs can induce many cellular responses and contribute to the development and progression of diabetes, the routes of their formation and their biological roles are only partially revealed. The same is true for the influence of glucose-derived modifications on the biophysical properties of PLs. Here we studied structural, signaling, and biophysical properties of glycated and glycoxidized phosphatidylethanolamines (PEs). By combining high resolution mass spectrometry and nuclear magnetic resonance spectroscopy it was possible to deduce the structures of several intermediates indicating an oxidative cleavage of the Amadori product yielding glycoxidized PEs including advanced glycation end products, such as carboxyethyl- and carboxymethyl-ethanolamines. The pro-oxidative role of glycated PEs was demonstrated and further associated with several cellular responses including activation of NFκB signaling pathways. Label free proteomics indicated significant alterations in proteins regulating cellular metabolisms. Finally, the biophysical properties of PL membranes changed significantly upon PE glycation, such as melting temperature (T_m), membrane surface charge, and ion transport across the phospholipid bilayer.

Int J Cardiol. 2016 May 1;210:100-8. doi: 10.1016/j.ijcard.2016.02.095. Epub 2016 Feb 18.

Cross-linking versus RAGE: How do high molecular weight advanced glycation products induce cardiac dysfunction?

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Author information

Abstract

BACKGROUND: Several clinical and experimental studies have demonstrated that advanced glycation end products (AGEs) are associated with adverse cardiac outcome. Growing evidence shows that high molecular weight AGEs (HMW-AGEs) might be as important as the characterized low molecular weight AGEs. To date, the role of HMW-AGEs in the pathogenesis of cardiac remodeling remains unknown. In this study, we investigated whether HMW-AGEs are involved in cardiac dysfunction.

METHODS: Healthy rats were daily ip injected with 20mg/kg BSA-derived HMW-AGEs or, as a control, unmodified BSA, during 6weeks. Cardiac function was assessed with echocardiography. Plasma levels of glucose, AGEs and soluble RAGE (sRAGE) were measured. AGEs, RAGE and lysyl oxidase (LOX) expression were determined by western blot.

RESULTS: After 6weeks, animals displayed a sustained increase in circulating total AGEs without hyperglycemia. HMW-AGEs injections induced cardiac dysfunction characterized by wall hypertrophy, increased heart sphericity, reduced strain and strain rate with preserved ejection fraction. Plasma sRAGE levels were significantly higher compared to control and correlated significantly with decreased strain. RAGE expression, TNF-α and IL-6 remained unchanged. Finally, HMW-AGEs induced prominent cardiac fibrosis associated with an increased LOX expression.

CONCLUSION: Our data demonstrate that rather than via a specific activation of RAGE, the deleterious effects of HMW-AGEs are likely mediated via an increased collagen cross-linking responsible for the observed cardiac stiffness. Additionally, we show that in the setting of elevated HMW-AGEs, increased sRAGE levels are markers of altered cardiac function.

KEYWORDS: Echocardiography; Fibrosis; High molecular weight advanced glycation end products; RAGE

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Does transcription-associated DNA damage limit lifespan?

Callegari AJ[1].

⊕ Author information

Abstract

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

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Age-Related and Heteroplasmy-Related Variation in Human mtDNA Copy Number.

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Author information

Abstract

The mitochondrial (mt) genome is present in many copies in human cells, and intra-individual variation in mtDNA sequences is known as heteroplasmy. Recent studies found that heteroplasmies are highly tissue-specific, site-specific, and allele-specific, however the functional implications have not been explored. This study investigates variation in mtDNA copy numbers (mtCN) in 12 different tissues obtained at autopsy from 152 individuals (ranging in age from 3 days to 96 years). Three different methods to estimate mtCN were compared: shotgun sequencing (in 4 tissues), capture-enriched sequencing (in 12 tissues) and droplet digital PCR (ddPCR, in 2 tissues). The highest precision in mtCN estimation was achieved using shotgun sequencing data. However, capture-enrichment data provide reliable estimates of relative (albeit not absolute) mtCNs. Comparisons of mtCN from different tissues of the same individual revealed that mtCNs in different tissues are, with few exceptions, uncorrelated. Hence, each tissue of an individual seems to regulate mtCN in a tissue-related rather than an individual-dependent manner. Skeletal muscle (SM) samples showed an age-related decrease in mtCN that was especially pronounced in males, while there was an age-related increase in mtCN for liver (LIV) samples. MtCN in SM samples was significantly negatively correlated with both the total number of heteroplasmic sites and with minor allele frequency (MAF) at two heteroplasmic sites, 408 and 16327. Heteroplasmies at both sites are highly specific for SM, accumulate with aging and are part of functional elements that regulate mtDNA replication. These data support the hypothesis that selection acting on these heteroplasmic sites is reducing mtCN in SM of older individuals.

REVIEWS/COMMENTS/EDITORIALS

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The amyloid hypothesis of Alzheimer's disease at 25 years

Dennis J Selkoe, John Hardy

Despite continuing debate about the amyloid β-protein (or Aβ hypothesis, new lines of evidence from laboratories and clinics worldwide support the concept that an imbalance between production and clearance of Aβ42 and related Aβ peptides is a very early, often initiating factor in Alzheimer's disease (AD). Confirmation that presenilin is the catalytic site of γ-secretase has provided a linchpin: all dominant mutations causing early-onset AD occur either in the substrate (amyloid precursor protein, APP) or the protease (presenilin) of the reaction that generates Aβ. Duplication of the wild-type APP gene in Down's syndrome leads to Aβ deposits in the teens, followed by microgliosis, astrocytosis, and neurofibrillary tangles typical of AD. Apolipoprotein E4, which predisposes to AD in > 40% of cases, has been found to impair Aβ clearance from the brain. Soluble oligomers of Aβ42 isolated from AD patients' brains can decrease synapse number, inhibit long-term potentiation, and enhance long-term synaptic depression in rodent hippocampus, and injecting them into healthy rats impairs memory. The human oligomers also induce hyperphosphorylation of tau at AD-relevant epitopes and cause neuritic dystrophy in cultured neurons. Crossing human APP with human tau transgenic mice enhances tau-positive neurotoxicity. In humans, new studies show that low cerebrospinal fluid (CSF) Aβ42 and amyloid-PET positivity precede other AD manifestations by many years. Most importantly, recent trials of three different Aβ antibodies (solanezumab, crenezumab, and aducanumab) have suggested a slowing of cognitive decline in *post hoc* analyses of mild AD subjects. Although many factors contribute to AD pathogenesis, Aβ dyshomeostasis has emerged as the most extensively validated and compelling therapeutic target.

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Periodic acid-Schiff granules in the brain of aged mice: From amyloid aggregates to degenerative structures containing neo-epitopes

Highlights

- PAS granules are degenerative structures formed in mice brain with age.
- Oxidative stress and genetic background play a significant role in PAS granules formation.
- PAS granules have been erroneously identified as amyloid deposits, among others.
- PAS granules contain neo-epitopes that are a target of natural IgM auto-antibodies.
- Autoimmunity could be a new focus in the study of age-related degenerative processes.

Brain ageing in mice leads to the progressive appearance and expansion of degenerative granular structures frequently referred as "PAS granules" because of their positive staining with periodic acid-Schiff (PAS). PAS granules are present mainly in the hippocampus, although they have also been described in other brain areas such as piriform and entorhinal cortices, and have been observed in other mammals than mice, like rats and monkeys. PAS granules have been identified as a wide range of brain deposits related to numerous neurodegenerative diseases, such as amyloid deposits, neurofibrillary tangles, Lafora bodies, corpora amylacea and polyglucosan bodies, and these identifications have generated controversy and particular theories about them. We have recently reported the presence of a neo-epitope in mice hippocampal PAS granules and the existence of natural IgM auto-antibodies directed against the neo-epitope in the plasma of the animals. The significance of the neo-epitope and the autoantibodies is discussed in this review. Moreover, we observed that the IgM anti-neo-epitope is frequently present as a contaminant in numerous commercial antibodies and is responsible of a considerable amount of false positive immunostainings, which may produce misinterpretations in the identification of the granules. Now that this point has been clarified, this article reviews and reconsiders the nature and physiopathological significance of these degenerative granules. Moreover, we suggest that neo-epitopes may turn into a useful brain-ageing biomarker and that autoimmunity could become a new focus in the study of age-related degenerative processes.

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Lysosomal cell death mechanisms in aging

Highlights

- Lysosomal cell death participates in physiological processes, aging, and disease.
- Lysosomal membrane permeabilization plays a key role in lysosomal cell death.
- Inducers and inhibitors of lysosomal cell death may be of therapeutic value.

Abstract

Lysosomes are degradative organelles essential for cell homeostasis that regulate a variety of processes, from calcium signaling and nutrient responses to autophagic degradation of intracellular components. Lysosomal cell death is mediated by the lethal effects of cathepsins, which are released into the cytoplasm following lysosomal damage. This process of lysosomal membrane permeabilization and cathepsin release is observed in several physiopathological conditions and plays a role in tissue remodeling, the immune response to intracellular pathogens and neurodegenerative diseases. Many evidences indicate that aging strongly influences lysosomal activity by altering the physical and chemical properties of these organelles, rendering them more sensitive to stress. In this review we focus on how aging alters lysosomal function and increases cell sensitivity to lysosomal membrane permeabilization and lysosomal cell death, both in physiological conditions and age-related pathologies.

Nuclear DNA damage signalling to mitochondria in ageing

Mitochondrial dysfunction is a hallmark of ageing, and mitochondrial maintenance may lead to increased healthspan. Emerging evidence suggests a crucial role for signalling from the nucleus to mitochondria (NM signalling) in regulating mitochondrial function and ageing. An important initiator of NM signalling is nuclear DNA damage, which accumulates with age and may contribute to the development of age-associated diseases. DNA damage-dependent NM signalling constitutes a network that includes nuclear sirtuins and controls genomic stability and mitochondrial integrity. Pharmacological modulation of NM signalling is a promising novel approach for the prevention and treatment of age-associated diseases.

OTHER RESEARCH

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Design and synthesis of a minimal bacterial genome

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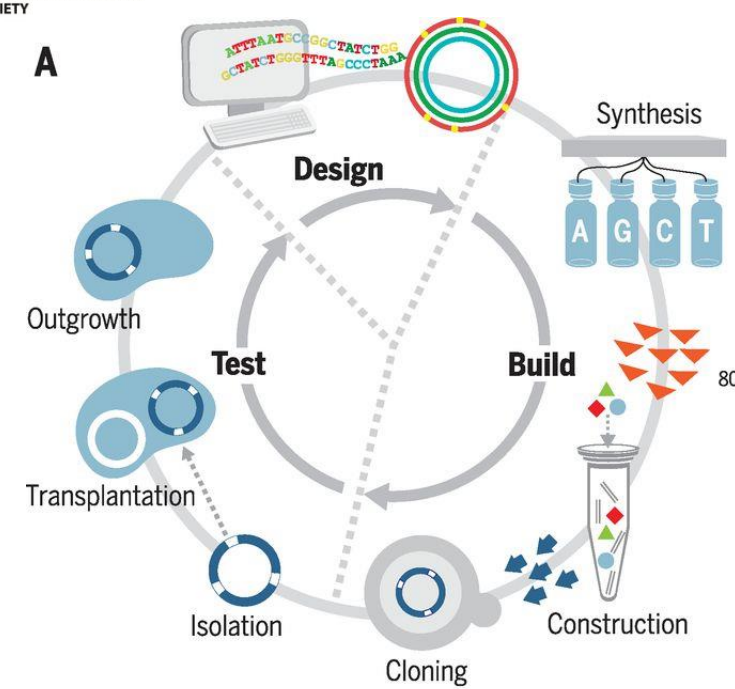
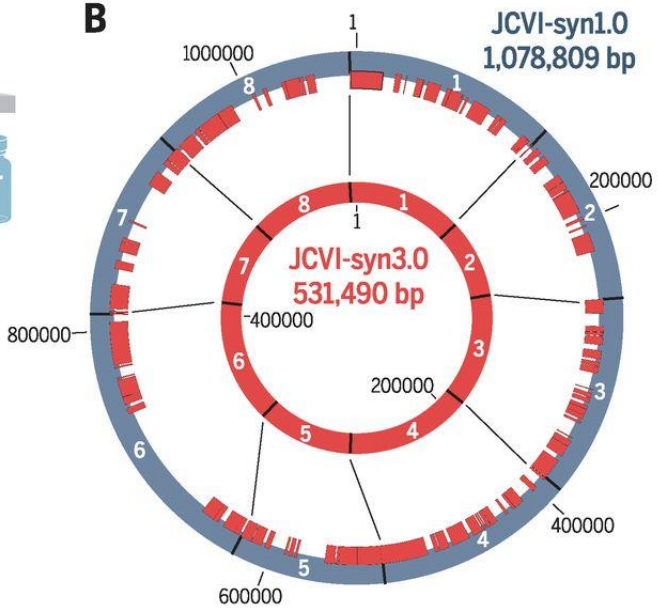
Designing and building a minimal genome

A goal in biology is to understand the molecular and biological function of every gene in a cell. One way to approach this is to build a minimal genome that includes only the genes essential for life. In 2010, a 1079-kb genome based on the genome of *Mycoplasma mycoides* (JCV-syn1.0) was chemically synthesized and supported cell growth when transplanted into cytoplasm. Hutchison III *et al.* used a design, build, and test cycle to reduce this genome to 531 kb (473 genes). The resulting JCV-syn3.0 retains genes involved in key processes such as transcription and translation, but also contains 149 genes of unknown function.





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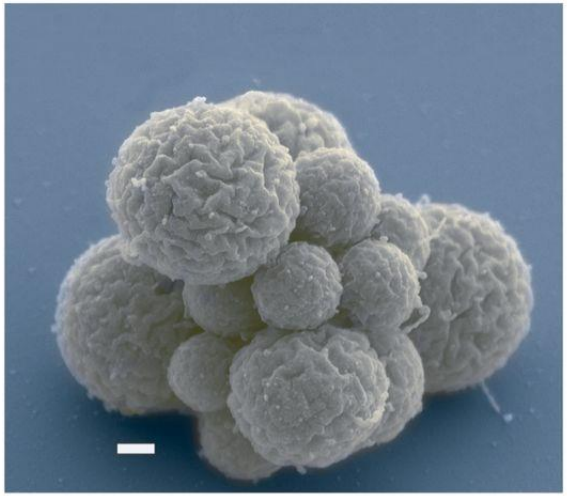

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Adaptation in CRISPR-Cas Systems

Samuel H. Sternberg[6], Hagen Richter[6], Emmanuelle Charpentier, Udi Qimron

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins constitute an adaptive immune system in prokaryotes. The system preserves memories of prior infections by integrating short segments of foreign DNA, termed spacers, into the CRISPR array in a process termed adaptation. During the past 3 years, significant progress has been made on the genetic requirements and molecular mechanisms of adaptation. Here we review these recent advances, with a focus on the experimental approaches that have been developed, the insights they generated, and a proposed mechanism for self- versus non-self-discrimination during the process of spacer selection. We further describe the regulation of adaptation and the protein players involved in this fascinating process that allows bacteria and archaea to harbor adaptive immunity.