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The Ubiquitin Ligase CHIP Integrates Proteostasis and Aging by Regulation of Insulin Receptor Turnover

Summary

Aging is attended by a progressive decline in protein homeostasis (proteostasis), aggravating the risk for protein aggregation diseases. To understand the coordination between proteome imbalance and longevity, we addressed the mechanistic role of the quality-control ubiquitin ligase CHIP, which is a key regulator of proteostasis. We observed that CHIP deficiency leads to increased levels of the insulin receptor (INSR) and reduced lifespan of worms and flies. The membrane-bound INSR regulates the insulin and IGF1 signaling (IIS) pathway and thereby defines metabolism and aging. INSR is a direct target of CHIP, which triggers receptor monoubiquitylation and endocytic-lysosomal turnover to promote longevity. However, upon proteotoxic stress conditions and during aging, CHIP is recruited toward disposal of misfolded proteins, reducing its capacity to degrade the INSR. Our study indicates a competitive relationship between proteostasis and longevity regulation through CHIP-assisted proteolysis, providing a mechanistic concept for understanding the impact of proteome imbalance on aging.

cGAS is essential for cellular senescence

Cellular senescence is a natural barrier to tumorigenesis and it contributes to the antitumor effects of several therapies, including radiation and chemotherapeutic drugs. Senescence also plays an important role in aging, fibrosis, and tissue repair. The DNA damage response is a key event leading to senescence, which is characterized by the senescence-associated secretory phenotype (SASP) that includes expression of inflammatory cytokines. Here we show that cGMP-AMP (cGAMP) synthase (cGAS), a cytosolic DNA sensor that activates innate immunity, is essential for senescence. Deletion of cGAS accelerated the spontaneous immortalization of mouse embryonic fibroblasts. cGAS deletion also abrogated SASP induced by spontaneous immortalization or DNA damaging agents, including radiation and etoposide. cGAS is localized in the cytoplasm of nondividing cells but enters the nucleus and associates with chromatin DNA during mitosis in proliferating cells. DNA damage leads to accumulation of damaged DNA in cytoplasmic foci that contain cGAS. In human lung adenocarcinoma patients, low expression of cGAS is correlated with poor survival. These results indicate that cGAS mediates cellular senescence and retards immortalization. This is distinct from, and complementary to, the role of cGAS in activating antitumor immunity.

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Histone variant H2A.J accumulates in senescent cells and promotes inflammatory gene expression

The senescence of mammalian cells is characterized by a proliferative arrest in response to stress and the expression of an inflammatory phenotype. Here we show that histone H2A.J, a poorly studied H2A variant found only in mammals, accumulates in human fibroblasts in senescence with persistent DNA damage. H2A.J also accumulates in mice with aging in a tissue-specific manner and in human skin. Knock-down of H2A.J inhibits the expression of inflammatory genes that contribute to the senescent-associated secretory phenotype (SASP), and over expression of H2A.J increases the expression of some of these genes in proliferating cells. H2A.J accumulation may thus promote the signalling of senescent cells to the immune system, and it may contribute to chronic inflammation and the development of aging-associated diseases.

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Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug

Metformin is widely used in the treatment of type 2 diabetes (T2D), but its mechanism of action is poorly defined. Recent evidence implicates the gut microbiota as a site of metformin action. In a double-blind study, we randomized individuals with treatment-naive T2D to placebo or metformin for 4 months and showed that metformin had strong effects on the gut microbiome. These results were verified in a subset of the placebo group that switched to metformin 6 months after the start of the trial. Transfer of fecal samples (obtained before and 4 months after treatment) from metformin-treated donors to germ-free mice showed that glucose tolerance was improved in mice that received metformin-altered microbiota. By directly investigating metformin–microbiota interactions in a gut simulator, we showed that metformin affected pathways with common biological functions in species from two different phyla, and many of the metformin-regulated genes in these species encoded metalloproteins or metal transporters. Our findings provide support for the notion that altered gut microbiota mediates some of metformin's antidiabetic effects.

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Two forms of death in ageing *Caenorhabditis elegans*

Ageing generates senescent pathologies, some of which cause death. Interventions that delay or prevent lethal pathologies will extend lifespan. Here we identify life-limiting pathologies in *Caenorhabditis elegans* with a necropsy analysis of worms that have died of old age. Our results imply the presence of multiple causes of death. Specifically, we identify two classes of corpse: early deaths with a swollen pharynx (which we call 'P deaths'), and later deaths with an atrophied pharynx (termed 'p deaths'). The effects of interventions on lifespan can be broken down into changes in the frequency and/or timing of either form of death. For example, *glp-1* mutation only delays p death, while *eat-2* mutation reduces P death. Combining pathology and mortality analysis allows mortality profiles to be deconvolved, providing biological meaning to complex survival and mortality profiles.

Impairment of insulin signalling in peripheral tissue fails to extend murine lifespan

Impaired insulin/IGF1 signalling has been shown to extend lifespan in model organisms ranging from yeast to mammals. Here we sought to determine the effect of targeted disruption of the insulin receptor (IR) in non-neuronal tissues of adult mice on the lifespan. We induced hemizygous (PerIRKO$^{+/-}$) or homozygous (PerIRKO$^{-/-}$) disruption of the IR in peripheral tissue of 15-weeks-old mice using a tamoxifen-inducible Cre transgenic mouse with only peripheral tissue expression, and subsequently monitored glucose metabolism, insulin signalling and spontaneous death rates over 4 years. Complete peripheral IR disruption resulted in a diabetic phenotype with increased blood glucose and plasma insulin levels in young mice. Although blood glucose levels returned to normal, and fat mass was reduced in aged PerIRKO$^{-/-}$ mice, their lifespan was reduced. By contrast, heterozygous disruption had no effect on lifespan. This was despite young male PerIRKO$^{+/-}$ mice showing reduced fat mass and mild increase in hepatic insulin sensitivity. In conflict with findings in metazoans like *Caenorhabditis elegans* and *Drosophila melanogaster*, our results suggest that heterozygous impairment of the insulin signalling limited to peripheral tissues of adult mice fails to extend lifespan despite increased systemic insulin sensitivity, while homozygous impairment shortens lifespan.

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The *In Vitro* Influence of a Genetic Superoxide-Hydrogen Peroxide Imbalance on Immunosenescence

As superoxide is a key molecule of inflammatory activation, superoxide-hydrogen peroxide (S-HP) imbalance genetically caused could alter immunosenescence patterns. To test this hypothesis, we collected and cultured peripheral blood mononuclear cells (PBMCs) carrier's different genotypes of a genetic polymorphism located in the superoxide dismutase manganese-dependent gene (Val16Ala-SOD2). We used an *in vitro* genetic model based on previous studies, which suggested an association between homozygous genotypes (AA and VV) and alterations in oxidative–inflammatory mediators. PBMCs collected from young healthy volunteers were cultured in the presence of phytohemagglutinin, as well as the following cell culture passages obtained from the 72-hour initial culture. Each follow passage started with the same cell concentration (1×10^5 cells). The general immunosenescence pattern was observed independent of SOD2 genotypes: cellular proliferation until the 15th passage, when cellular arrestment occurred in the G0/G1 phase. From the 10th passage, a higher proliferative state was observed, indicating inflammatory hyperactivation, with an increase in the levels of inflammatory cytokines (IL-1, IL-6, and TNFα), nitric oxide, superoxide, lipoperoxidation, protein carbonylation, reactive oxygen species, and DNA damage. The S-HP imbalance affected the intensity of some immunosenescence parameters. AA cells, which present basal high HP levels, were associated with higher DNA damage and lipoperoxidation levels, whereas VV, which present basal high S levels, was associated with higher proinflammatory cytokine levels. In summary, the results suggested that a basal S-HP imbalance could affect the intensity of some immunosenescence markers, and this influence could explain the potential association between an imbalance of genotypes (AA and VV) and the risk of developing some chronic diseases.

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Short-term caloric restriction exerts neuroprotective effects following mild traumatic brain injury by promoting autophagy and inhibiting astrocyte activation.

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Author information

Abstract

Cognitive deficits may occur after mild traumatic brain injury (mTBI), but effective treatment modalities are presently unavailable. Caloric restriction (CR) has beneficial effects on neurodegenerative diseases and brain injury. However, the underlying mechanisms have not yet been clearly defined. Therefore, the aim of the present study was to investigate the short-term effects of CR treatment on cognitive function in mice after mTBI. Forty-five 12-week-old C57/BL6 mice were subjected to closed-head mTBI using a weight drop device. The mice were then randomly divided into three groups according to their diet for 30 days: the normal calorie group (mTBI+NC group, n=15), the caloric restriction group (mTBI+CR group, n=15), and the high energy group (mTBI+HE group, n=15). After 30 days, the Morris water maze test was performed to evaluate learning abilities. Nissl staining, immunohistochemistry, and western blotting were used to monitor pathological changes and changes in autophagy-associated proteins in the hippocampus. The average escape latency was significantly shorter in the mTBI+CR group than in the mTBI+NC and mTBI+HE groups, and the number of target platform crossings in the mTBI+CR group was significantly higher than in the other two groups. In the hippocampus, the expression of GFAP and mTOR was increased in the mTBI+HE group and decreased in the mTBI+CR group. Conversely, the expression of LC3B was decreased in the mTBI+HE group and increased in the mTBI+CR group. Our findings suggest that short-term CR after mTBI may ameliorate cognitive dysfunction induced by mTBI by increasing the level of autophagy and suppressing astrocyte activation.

Attenuation of β-Amyloid Toxicity *In Vitro* and *In Vivo* by Accelerated Aggregation

Accumulation and aggregation of β-amyloid (Aβ) peptides result in neuronal death, leading to cognitive dysfunction in Alzheimer's disease. The self-assembled Aβ molecules form various intermediate aggregates including oligomers that are more toxic to neurons than the mature aggregates, including fibrils. Thus, one strategy to alleviate Aβ toxicity is to facilitate the conversion of Aβ intermediates to larger aggregates such as fibrils. In this study, we designed a peptide named A3 that significantly enhanced the formation of amorphous aggregates of Aβ by accelerating the aggregation kinetics. Thioflavin T fluorescence experiments revealed an accelerated aggregation of Aβ monomers, accompanying reduced Aβ cytotoxicity. Transgenic *Caenorhabditis elegans* over-expressing amyloid precursor protein exhibited paralysis due to the accumulation of Aβ oligomers, and this phenotype was attenuated by feeding the animals with A3 peptide. These findings suggest that the Aβ aggregation-promotion effect can potentially be useful for developing strategies to reduce Aβ toxicity.

REVIEWS/COMMENTS/EDITORIALS

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Aging and the Inevitable Limit to Human Life Span.

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Author information

Abstract

There is a long-lasting debate about a natural limit to human life span, and it has been argued that the maximum reported age at death, which has not increased for ca 25 years, fluctuates around 115 years, even if some persons live beyond this age. We argue that the close connection of species-specific longevity with life history strategies explains why human life span is limited and cannot reach the considerably longer life spans of several other species.

KEYWORDS: Centenarians; Dietary restriction; Life expectancy; Life-history strategy; Limits to life span; Maximum life span; Maximum reported age at death; Negligible senescence

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Molecular and physiological manifestations and measurement of aging in humans

Biological aging is associated with a reduction in the reparative and regenerative potential in tissues and organs. This reduction manifests as a decreased physiological reserve in response to stress (termed homeostenosis) and a time-dependent failure of complex molecular mechanisms that cumulatively create disorder. Aging inevitably occurs with time in all organisms and emerges on a molecular, cellular, organ, and organismal level with genetic, epigenetic, and environmental modulators. Individuals with the same chronological age exhibit differential trajectories of age-related decline, and it follows that we should assess biological age distinctly from chronological age. In this review, we outline mechanisms of aging with attention to well-described molecular and cellular hallmarks and discuss physiological changes of aging at the organ-system level. We suggest methods to measure aging with attention to both molecular biology (e.g., telomere length and epigenetic marks) and physiological function (e.g., lung function and echocardiographic measurements). Finally, we propose a framework to integrate these molecular and physiological data into a composite score that measures biological aging in humans. Understanding the molecular and physiological phenomena that drive the complex and multifactorial processes underlying the variable pace of biological aging in humans will inform how researchers assess and investigate health and disease over the life course. This composite biological age score could be of use to researchers seeking to characterize normal, accelerated, and exceptionally successful aging as well as to assess the effect of interventions aimed at modulating human aging.

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Kidney, heart and brain: three organs targeted by ageing and glycation

Marie Frimat, Maité Daroux, Rachel Litke, Rémi Nevière, Frédéric J. Tessier, Eric Boulanger

Advanced glycation end-product (AGE) is the generic term for a heterogeneous group of derivatives arising from a non-enzymatic reaction between reducing sugars and proteins. In recent years, evidence has accumulated that incriminates AGEs in pathogenic processes associated with both chronic hyperglycaemia and age-related diseases. Regardless of their exogenous or endogenous origin, the accumulation of AGEs and their derivatives could promote accelerated ageing by leading to protein modifications and activating several inflammatory signalling pathways via AGE-specific receptors. However, it remains to be demonstrated whether preventing the accumulation of AGEs and their effects is an important therapeutic option for successful ageing. The present review gives an overview of the current knowledge on the pathogenic role of AGEs by focusing on three AGE target organs: kidney, heart and brain. For each of these organs we concentrate on an age-related disease, each of which is a major public health issue: chronic kidney disease, heart dysfunction and neurodegenerative diseases. Even though strong connections have been highlighted between glycation and age-related pathogenesis, causal links still need to be validated. In each case, we report evidence and uncertainties suggested by animal or epidemiological studies on the possible link between pathogenesis and glycation in a chronic hyperglycaemic state, in the absence of diabetes, and with exogenous AGEs alone. Finally, we present some promising anti-AGE strategies that are currently being studied.

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Aging-associated mitochondrial DNA mutations alter oxidative phosphorylation machinery and cause mitochondrial dysfunctions

Our previous study generated a series of cybrids containing mitochondria of synaptosomes from mice at different ages. The following functional analysis on these cybrids revealed an age-dependent decline of mitochondrial function. To understand the underlying mechanisms that contribute to the age-related mitochondrial dysfunction, we focused on three cybrids carrying mitochondria derived from synaptosomes of the old mice that exhibited severe respiratory deficiencies. In particular, we started with a comprehensive analysis of mitochondrial genome by high resolution, high sensitive deep sequencing method. Compared with young control, we detected a significant accumulation of heteroplasmic mtDNA mutations. These mutations included six alterations in main control region that has been shown to regulate overall gene-expression, and four alterations in protein coding region, two of which led to significant changes in complex I subunit ND5 and complex III subunit CytB. Interestingly, a reduced mtDNA-encoded protein synthesis was associated with the changes in the main control region. Likewise, mutations in ND5 and CytB were associated with defects in assembly of respiratory complexes. All together, the identified age-dependent accumulation of mtDNA mutations in mouse brain likely contributes to the decline in mitochondrial function.

OTHER RESEARCH

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A subcellular map of the human proteome

RESULTS

We report a high-resolution characterization of the spatial subcellular distribution of the human proteome based on more than 80,000 confocal IF images. A total of 12,003 proteins targeted by 13,993 antibodies were classified into one or several of 30 cellular compartments and substructures, altogether defining the proteomes of 13 major organelles. The organelles with the largest proteomes were the nucleus and its substructures (6245 proteins), such as bodies and speckles, and the cytosol (4279 proteins). However, smaller organelles such as the midbody, rods and rings, and nucleoli also showed a larger diversity than previously recognized. Intriguingly, about half of all proteins were localized to multiple compartments, showing that there is a shared pool of proteins even among functionally unrelated organelles. Single-cell analysis revealed 1855 proteins with variation in their expression pattern, either in terms of expression levels or spatial distribution. Last, the spatial information was used to refine biological networks. Our location-pruned network that restricts protein interaction to the same organelle improved the accuracy of the human interactome model. The analysis also included transcriptomics data for all putative protein-coding genes (19,628) in 56 human cell lines of various origins. On average, cell lines expressed 11,490 genes, with half of them (6295) being expressed across all samples, suggesting a "housekeeping" role.

In vivo mapping of tissue- and subcellular-specific proteomes in *Caenorhabditis elegans*

Abstract

Multicellular organisms are composed of tissues that have distinct functions requiring specialized proteomes. To define the proteome of a live animal with tissue and subcellular resolution, we adapted a localized proteomics technology for use in the multicellular model organism *Caenorhabditis elegans*. This approach couples tissue- and location-specific expression of the enzyme ascorbate peroxidase (APX), which enables proximity-based protein labeling in vivo, and quantitative proteomics to identify tissue- and subcellular-restricted proteomes. We identified and localized more than 3000 proteins from strains of *C. elegans* expressing APX in either the nucleus or cytoplasm of the intestine, epidermis, body wall muscle, or pharyngeal muscle. We also identified several hundred proteins that were specifically localized to one of the four tissues analyzed or specifically localized to the cytoplasm or the nucleus. This approach resulted in the identification both of proteins with previously characterized localizations and of those not known to localize to the nucleus or cytoplasm. Further, we confirmed the tissue- and subcellular-specific localization of a subset of identified proteins using green fluorescent protein tagging and fluorescence microscopy, validating our in vivo proximity-based proteomics technique. Together, these results demonstrate a new approach that enables the tissue- and subcellular-specific identification and quantification of proteins within a live animal.

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An Atomic Structure of the Human Spliceosome

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Summary

Mechanistic understanding of pre-mRNA splicing requires detailed structural information on various states of the spliceosome. Here we report the cryo electron microscopy (cryo-EM) structure of the human spliceosome just before exon ligation (the C* complex) at an average resolution of 3.76 Å. The splicing factor Prp17 stabilizes the active site conformation. The step II factor Slu7 adopts an extended conformation, binds Prp8 and Cwc22, and is poised for selection of the 3′-splice site. Remarkably, the intron lariat traverses through a positively charged central channel of RBM22; this unusual organization suggests mechanisms of intron recruitment, confinement, and release. The protein PRKRIP1 forms a 100-Å α helix linking the distant U2 snRNP to the catalytic center. A 35-residue fragment of the ATPase/helicase Prp22 latches onto Prp8, and the quaternary exon junction complex (EJC) recognizes upstream 5′-exon sequences and associates with Cwc22 and the GTPase Snu114. These structural features reveal important mechanistic insights into exon ligation.

ATP as a biological hydrotrope

Hydrotropes are small molecules that solubilize hydrophobic molecules in aqueous solutions. Typically, hydrotropes are amphiphilic molecules and differ from classical surfactants in that they have low cooperativity of aggregation and work at molar concentrations. Here, we show that adenosine triphosphate (ATP) has properties of a biological hydrotrope. It can both prevent the formation of and dissolve previously formed protein aggregates. This chemical property is manifested at physiological concentrations between 5 and 10 millimolar. Therefore, in addition to being an energy source for biological reactions, for which micromolar concentrations are sufficient, we propose that millimolar concentrations of ATP may act to keep proteins soluble. This may in part explain why ATP is maintained in such high concentrations in cells.