Mitochondrial complex I as a therapeutic target for Alzheimer’s disease

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Abstract
Alzheimer’s disease (AD), the most prominent form of dementia in the elderly, has no cure. Strategies focused on the reduction of amyloid beta or hyperphosphorylated Tau protein have largely failed in clinical trials. Novel therapeutic targets and strategies are urgently needed. Emerging data suggest that in response to environmental stress, mitochondria initiate an integrated stress response (ISR) shown to be beneficial for healthy aging and neuroprotection. Here, we review data that implicate mitochondrial electron transport complexes involved in oxidative phosphorylation as a hub for small molecule-targeted therapeutics that could induce beneficial mitochondrial ISR. Specifically, partial inhibition of mitochondrial complex I has been exploited as a novel strategy for multiple human conditions, including AD, with several small molecules being tested in clinical trials. We discuss current understanding of the molecular mechanisms involved in this counterintuitive approach. Since this strategy has also shown to enhance health and life span, the development of safe and efficacious complex I inhibitors could promote healthy aging, delaying the onset of age-related neurodegenerative diseases.

Abbreviations: AD, Alzheimer’s disease; ADP, adenosine diphosphate; AIDS, acquired immunodeficiency syndrome; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; APP/PS1, amyloid precursor protein/presenilin 1; ATP, adenosine triphosphate; Aβ, amyloid beta; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; CP2, tricyclic pyrone compound two; ER, endoplasmic reticulum; ETC, electron transport chain; FADH2, flavin adenine dinucleotide; FDG-PET, fluorodeoxyglucose-positron emission tomography; GWAS, genome-wide association study; HD, Huntington’s disease; HIF-1α, hypoxia induced factor 1 alpha; ISR, integrated stress response; LTP, long term potentiation; MCI, mild cognitive impairment; MPTP, 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine; mtDNA, mitochondrial DNA; mtUPR, mitochondrial unfolded protein response; NAD+/NADH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NRF2, nuclear factor E2-related factor 2; OXPHOS, oxidative phosphorylation; PD, Parkinson’s disease; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; PMF, proton-motive force; pTau, hyper-phosphorylated Tau protein; RNAi, RNA interference; ROS, reactive oxygen species; T2DM, type II diabetes mellitus; TCA, the tricarboxylic acid cycle; ΔpH, proton gradient; Δψm, mitochondrial membrane potential.

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1. Introduction

Alzheimer’s disease (AD) is a common neurodegenerative disorder in the elderly without a cure. Common hallmarks of AD include extracellular plaques formed by amyloid beta (Aβ) peptides, neurofibrillary tangles comprised of hyper-phosphorylated Tau protein (pTau), reactive microgliosis, dystrophic neuritis, and loss of neurons and synapses. However, failure of recent clinical trials focused on the prevention of Aβ and pTau production or their clearance questions the amyloid cascade hypothesis and the role of Aβ and pTau in the underlying disease mechanism. The outcomes of the most recent studies conducted using advanced biochemistry and multi omics systems biology approaches in well characterized cohorts of AD patients, patient-derived cells and tissues suggest a multifactorial nature of AD where several mechanisms on a whole organismal level become individually or synergistically affected in a disease-stage- and sex-specific manner. These pathways include reduced glucose uptake and utilization, insulin resistance, altered autophagy and proteostasis, increased inflammation and oxidative stress, and mitochondrial dysfunction. Conclusive evidence demonstrating that brain hypometabolism precedes clinical presentation and the development of Aβ aggregates provided a justification for interventions focused on metabolism and mitochondrial function as potential disease-modifying strategies that could block the disease progression. However, most treatments aimed at boosting mitochondrial function or reducing the pathology associated with increased production of reactive oxygen species (ROS) have failed clinical trials. Unexpectedly, partial reduction of the activity of the complexes involved in the oxidative phosphorylation (OXPHOS) and electron transport chain (ETC) machinery using genetic or pharmacological down modulation approaches has been shown to provide significant health benefits, improving mitochondrial function and cellular energetics in multiple model systems in vitro and in vivo. In particular, partial inhibition of mitochondrial complex I using small molecules has emerged as a therapeutic strategy for multiple human conditions, including cancer and neurodegenerative diseases. This counterintuitive strategy has been shown to increase longevity and health span, which ultimately could delay the onset of neurodegenerative diseases of aging, such as AD. Indeed, the induction of mild energetic stress via partial complex I inhibition with subsequent mitochondria-mediated stress response may increase resilience to the greater stress associated with aging and ensure that mechanisms found instrumental for protection against AD, including inflammation, synaptic function, proteostasis, mitochondrial dynamics and function, and oxidative stress, remain in control. Below, we discuss the current understanding of the neuroprotective mechanisms behind complex I inhibition with respect to mitochondrial signaling via integrated stress response (ISR) and progress in the development of safe and efficacious partial complex I inhibitors.

2. Mitochondria function in energy production and as signaling organelles

Most of the energy required for cellular functions is produced by mitochondria (Fig. 1). These organelles are abundant, occupying up to 25% of the cytoplasmic volume. The mitochondrion is the only cellular organelle other than the nucleus that has its own DNA (mtDNA) and transcriptional and translational machinery (Fig. 1A). These features, together with the unusual dynamics of mitochondrial division and fusion (reminiscent of bacteria), have led to the theory of an ancient endosymbiosis of a nucleated cell and an aerobic prokaryote. Such cooperation provides the host with significantly increased energy supply, making mitochondria a “power plant” of the cell, while mitochondria enjoy the protection and resources of the host, including the outsourcing of the most of protein synthesis essential for their function. Successful symbiotic integration required the development of a robust communication system. The extensive arsenal of signaling molecules utilized by mitochondria for intracellular communication is discussed below.

2.1. OXPHOS machinery

Cell populations in the brain are diverse, and each cell type has distinctive energy demands and metabolic profiles. The conventional view is that neurons depend on energy produced by mitochondria via OXPHOS. In OXPHOS, a series of metabolic reactions leads to the oxidation of glucose or its metabolites, such as pyruvate and lactate, to produce energy in the form of adenosine triphosphate (ATP) (Fig. 1B). OXPHOS is the most efficient metabolic pathway, producing approximately 36 molecules of ATP per one molecule of glucose compared to 2 ATP molecules produced during glycolysis, a cytoplasmic process that also uses glucose but does not require mitochondria. Unexpectedly, mitochondrial morphology is essential to maintaining OXPHOS. The organelle has two membrane compartments (Fig. 1A). The outer membrane delimits the organelle and allows the passage of small molecules and ions to maintain mitochondrial homeostasis. An inner membrane consists of multiple folds called cristae and defines the mitochondrial matrix as a closed compartment. OXPHOS machinery is located at the inner mitochondrial membrane, while the tricarboxylic acid (TCA) cycle that produces essential components to power OXPHOS takes place in the matrix.

During OXPHOS, substrates such as nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide (FADH2) produced in the TCA cycle donate electrons that are transferred through the mitochondrial ETC via a series of redox reactions coupled to a final phosphorylation of adenosine diphosphate (ADP) to produce ATP, CO2 and water. This process requires oxygen, and oxygen consumption could be measured using oxygen electrodes or a Seahorse Extracellular Flux Analyzer to inform on the OXPHOS activity. The ETC (Fig. 1B) includes four protein complexes: NADH dehydrogenase (complex I), succinate dehydrogenase (complex II), cytochrome c oxidoreductase (complex III), and cytochrome c oxidase (complex IV). Electrons moving through the ETC promote the translocation of protons (H+) from the matrix to the intermembrane space, establishing an electrochemical gradient or proton-motive force (PMF). The energy generated from the PMF is used to
phosphorylate ADP to ATP via ATP synthase (complex V) (Fig. 1B). The PMF is regulated by the electrical potential difference established between the cytoplasm and the matrix, known as mitochondrial membrane potential ($\Delta \psi_m$), and the proton gradient ($\Delta p$H) across the inner mitochondrial membrane. Under normal physiological conditions, PMF is dominated by $\Delta \psi_m$, which accounts for over 70% of its potential. However, maintaining the PMF at high potential can lead to a breakdown of the membrane with subsequent formation of ROS, including hydrogen peroxide ($H_2O_2$) and superoxide ($O_2^-$). Hence, the PMF buildup during OXPHOS is counterbalanced by ATP synthase, during which protons re-enter the matrix diminishing the PMF. Under steady state conditions, the rate of electron transport equilibrates with proton translocation resulting in sufficient energy production and minimal generation of ROS. During OXPHOS, $H_2O_2$ and $O_2^-$ are produced as byproducts in mitochondria, primarily by complexes I and III, and are sequestered by the antioxidant enzymes, including superoxide dismutases, glutathione peroxidase, glutaredoxins, thioredoxins and catalases. Under disease conditions, this balance may be altered, leading to excessive ROS production and cellular damage. The balance amongst mitochondrial function, ROS production and the antioxidant defense is essential for normal function, since neuronal cells comprise most (80%–90%) of the energy demand of the brain and primarily depend on OXPHOS. Thus, levels of ROS, energy metabolites, metabolites of the TCA cycle, and $\Delta \psi_m$ are tightly linked to mitochondrial function, so changes in concentrations of all these metabolites could be used for intracellular signaling. In essence mitochondria serve as signaling organelles.

**Figure 1** Mitochondria structure and components of the OXPHOS machinery involved in mitochondrial intracellular signaling. (A) Electron micrograph (left) and cartoon (right) show a mitochondrion and its constituents. The organelle has an outer membrane and an inner membrane that folds into cristae that accommodate complexes of the OXPHOS machinery. The TCA cycle and mitochondrial DNA are located in the matrix. Scale bar, 500 nm. (B) The OXPHOS machinery. The series of protein complexes create a flow of electrons via redox reactions. The NADH and FADH$_2$ are converted to NAD$^+$ (complex I) and FAD (complex II), respectively, with $H_2O$ formed (complex IV) as a byproduct. This electron transfer causes protons to flow from mitochondrial matrix to intermembrane space, creating an outward gradient of positively charged protons. The inner mitochondrial membrane bound F$_0$ subunit of complex V (ATP synthase) uses this electrochemical gradient to rotate causing conformational changes to F$_1$ subunits that convert ADP to ATP. Changes in the concentrations of all these metabolites could be used for intracellular communication. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; FADH$_2$, flavin adenine dinucleotide; NAD$^+$, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid.
2.2. Mitochondria as signaling organelles

Mitochondria have long been recognized as central to ATP production, calcium buffering, and initiation of the apoptosis. In recent years, it became apparent that mitochondrial involvement in regulating cellular fate is more complex. Mitochondria communicate with the rest of the cell by releasing metabolites, mtDNA, and ROS, by changing their size and motility and by interacting with other subcellular organelles. For example, fluctuations in the TCA cycle metabolites (citrate, α-ketoglutarate, succinate and fumarate) induce epigenetic modifications, including nuclear DNA methylation, histone acetylation, and protein hydroxylation and acetylation. Changes in the levels of nicotinamide adenine dinucleotide (NAD⁺), a direct product of mitochondrial complex I function, affect the activity of sirtuins, the essential regulators of multiple cellular functions linked to improved mitochondrial function, increased health span and longevity. Alterations in ROS levels control hypoxic responses, immunity, and stem cell function. Recent findings demonstrate that mitochondrial ROS is an essential component of signaling that mediates antioxidant (redox) balance and stabilizes hypoxia-inducible factor 1α (HIF-1α), an important mediator of life-span extension linked to mitochondrial ISR. Recent studies also identified mitochondrial ROS signaling as a mitohormetic process where an increase in sublethal levels of ROS could predispose cells to a better response to increased oxidative stress in the future. Furthermore, mitochondria regulate immune response by releasing mtDNA and through the peptides (e.g., humanin and mitochondrial open-reading-frame of the twelve S rRNA-C, MOTS-c) encoded by mtDNA. Changes in mitochondrial dynamics (fission, fusion, axonal trafficking, biogenesis and mitophagy) are also important determinants of mitochondrial function and quality control. Multiple mechanisms are in place to respond to abnormal mitochondrial dynamics to ensure organelle preservation, including mitochondrial unfolded protein response (mtUPR, a process to maintain monomeric proteostasis), enhanced biogenesis (a mechanism to produce new mitochondria), and mitophagy (a process that removes damaged organelles). Changes in Δψm play a key role in mitochondrial homeostasis signaling for selective elimination of damaged organelles through mitophagy by recruiting PTEN-induced kinase 1 (PINK1) and Parkin proteins to the mitochondrial membrane. It is also a driving force for the translocation of ions and proteins essential for mitochondrial function. Mitochondria interact with other organelles, including the endoplasmic reticulum (ER), to modulate lipid homeostasis, immune response, and cell death.

![Figure 2](image_url)  
**Figure 2** Mitochondrial arsenal for intracellular signaling. Δψm, mitochondrial membrane potential; NAD⁺, nicotinamide adenine dinucleotide; TCA, tricarboxylic acid cycle; ROS, reactive oxygen species; AMP, adenosine monophosphate; ATP, adenosine triphosphate; AMPK, AMP-activated protein kinase; mtDNA, mitochondrial DNA.

Finally, changes in ATP levels associated with either increased energy utilization or reduced mitochondrial capacity led to an increase in the cellular adenosine monophosphate (AMP)/ATP ratio, which activates AMP-activated protein kinase (AMPK), a master regulator of cellular energy homeostasis. AMPK initiates a robust signaling cascade to restore energy balance. This dynamic process involves changes in lipid and glucose metabolism, mitochondrial dynamics and biogenesis, autophagy, and protein synthesis. Directly relevant to aging and neurodegenerative diseases is the AMPK-dependent reduction of inflammation and increase in levels of sirtuins, signaling molecules that regulate vast networks of metabolic and non-metabolic enzymes essential for healthy aging. Numerous pathways affected by AMPK have been shown to be neuroprotective by triggering AMPK as a drug target for neurodegenerative diseases. However, the development of direct AMPK activators has been proven difficult given the delicate balance required for cellular energy homeostasis. Nevertheless, it is now broadly accepted that indirect AMPK activation via exercise or caloric restriction is associated with increased health span and slowing down the progression of age-related neurodegenerative diseases. The availability of such a robust signaling arsenal allows mitochondria to successfully adapt to environmental changes, ensuring sustained energy production and cell survival.

2.3. Beneficial consequences of mitochondrial stress response

The most common mitochondrial stressors that induce mtUPR and ISR include fluctuations in energy sources, mtDNA mutations, changes in Δψm, Ca²⁺ and other ions, increased ROS production, and inhibition of the OXPHOS complexes. The mechanisms of the mtUPR and the ISR studied in *Caenorhabditis elegans* and mammalian cells converged on changes in gene expression of chaperones, proteases, detoxification enzymes, and the engagement of mediators of metabolic and epigenetic reprogramming, including activation of AMPK (Fig. 2). In *C. elegans*, initiation of this signaling cascade extended life span with epigenetic modification transmitted over four generations through histone H3K4 methylation. Data in mammalian cells suggest that activation of ISR depends both on the nature of the mitochondrial stressor and the metabolic state of the cell. Multiple studies conducted to date indicate that mild mitochondrial stress associated with the inhibition of OXPHOS complexes could induce an adaptive stress response that promotes health and longevity and delays the development of neurodegenerative diseases. Early...
evidence from studies in model organisms have demonstrated that mutations that decrease the activity of the mitochondrial respiratory chain resulted in a 20%–30% increase in the mean adult life span in *C. elegans*. Similarly, effects on longevity were achieved with RNA interference (RNAi) reduction in expression of the ETC components. The complete ablation of major ETC subunits resulted in severe phenotype and shorter lifespan indicating that only mild decrease in ETC activity was beneficial. In flies, the RNAs of five genes encoding components of mitochondrial respiratory complexes I, III, IV, and V resulted in increased life span. This phenomenon was not associated with altered assembly of respiratory complexes or reduced ATP production. Targeted RNAi of two complex I genes in adult tissues or in neurons alone was sufficient to extend *Drosophila melanogaster* life span.

In mice, decreased expression of proteins involved in the ETC, especially the matrix arm subunits of complex I, increased longevity by 30% and was associated with improved complex I assembly, higher complex I-linked state 3 respiration and decreased ROS production. Partial inhibition of complex IV and cytochrome *c* oxidase activity not only increased longevity in mice but also protected from neurodegeneration. The severe deficiency in complex IV or mild deficiency in complex III expression in neurons resulted in a reduction of ROS and Aβ deficiency in complex IV or mild deficiency in complex III or in complexes I and V appeared to be detrimental. Thus, the depletion of mtDNA also led to a decrease in plaque accumulation in the same AD mouse model. Inhibition of complex V has been linked to mitohormetic signaling, which increased neuronal survival in response to toxic agents in vitro and in vivo where mechanistic pathways converged on AMPK and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Furthermore, the uncoupling of OXPHOS using molecules that depolarize mitochondria causing an ATP decrease and activation of AMPK has been considered as a therapeutic strategy for aging, obesity, neurodegeneration, and cancer. Despite showing an improvement of mitochondrial function and oxidative metabolism via adaptive stress response, the OXPHOS uncouplers had multiple off-target effects and toxicity, which limited their clinical use.

Longitudinal RNA sequencing analysis identified mitochondrial complex I as a hub in a module of genes whose expression was negatively correlated with lifespan in *Nothobranchius furzeri*, an African turquoise killifish. Partial pharmacological inhibition of complex I with picomolar concentrations of the small molecule rotenone reversed aging-related regulation of gene expression rejuvenating the transcriptome and increasing life span in *N. furzeri* by 15%. This is particularly interesting since high concentrations of rotenone are devastating for the organism due to a high ROS production. In humans, data generated in a cohort of 2200 ultranonagenarians (and an equal number of controls) have shown that mutations in subunits of complex I that resulted in partial loss of its activity had a beneficial effect on longevity, while the simultaneous presence of mutations in complexes I and III or in complexes I and V appeared to be detrimental. Thus, the beneficial adaptive stress response could be induced by multiple mitochondria-targeted stressors, including inhibition of OXPHOS complexes, mtDNA depletion, and mitochondrial uncoupling, among others. However, clinical translation in most cases is impeded by the lack of selectivity, specificity, and deleterious side effects.

Molecular mechanisms linked to life-extending interventions associated with mild inhibition of OXPHOS across species included adaptive response to energetic stress via activation of AMPK. Additional important outcomes involved protection against oxidative stress that was attributed to the decreased rate of OXPHOS leading to overall lower production of ROS, AMPK-induced activation of the nuclear factor E2-related factor 2 (NRF2) signaling pathway, and mitohormetic response where sublethal ROS production associated with the ETC inhibition increased antioxidant defense via retrograde ROS signaling. Furthermore, AMPK activation enhanced autophagy mediating the removal of damaged organelles and misfolded proteins to improve cellular proteostasis, while increasing the production of “young” mitochondria via biogenesis. Importantly, these mechanisms overlap with the outcomes of non-pharmacological interventions, such as exercise and caloric restriction, known to reduce oxidative damage and inflammation and improve health, life span, and cognitive function.

### 3. Mitochondria-targeted therapeutics

While inhibition of mitochondrial ETC to achieve healthy aging and prevent neurodegeneration appears counterintuitive, broad application of metformin, an inexpensive U.S. Food and Drug Administration (FDA)-approved drug to treat type II diabetes mellitus (T2DM), supports the feasibility of such an approach in humans. Metformin is a natural product derived from the medicinal herb ‘goat’s-rue’, *Galega officinalis*. It has a robust safety record having been used in herbal medicine since medieval times. Metformin became the most prescribed antidiabetic drug after the results of a prospective study conducted in overweight patients with T2DM with a median follow up of over 10 years where blood glucose control with metformin reduced the incidence of diabetes-related endpoints and all-cause mortality. Metformin exerts its glucose-lowering effect by inhibiting hepatic gluconeogenesis and opposing the action of glucagon. Among other multiple targets, metformin could inhibit mitochondrial complex I to result in defective cyclic AMP and protein kinase A signaling in response to glucagon and the stimulation of AMPK. Metformin can cross the blood–brain barrier (BBB) and have specific effects on the central nervous system. Biological, clinical, and epidemiological data suggest that T2DM increases risk of mild cognitive impairment (MCI), vascular dementia and AD. Clinical trials have found that application of antidiabetic drugs including metformin protected against cognitive decline in patients with MCI and AD, improving executive functioning, learning, memory, and attention. These antidiabetic drugs positively affected mitochondrial and synaptic function, reduced neuroinflammation, and improved brain metabolism.

Interestingly, a recent systematic review reported that metformin reduced mortality and diseases of aging (cardiovascular disease and cancer) in patients who did not have diabetes, demonstrating that the effect of metformin on health span is independent of its antidiabetic properties. Thus, metformin appears to mimic mechanisms involved in caloric restriction and exercise shown to slow the aging process, improve memory, and reduce oxidative stress. However, a few reports based on data generated in experimental animal models and collected in studies in diabetic patients suggest that metformin could increase amyloid accumulation and risk of developing AD. These effects have been linked to overactivation of AMPK and vitamin B12 deficiency potentiated by metformin, which contribute to cognitive impairment. Furthermore, it remains uncertain to what extent
Table 1  Mitochondrial complex I inhibitors in clinical trials.

| Complex I inhibitor | Structure | Condition or disease | Clinical trial ID a |
|---------------------|-----------|----------------------|---------------------|
| Metformin           |           | AD, MCI, T2DM, obesity, cancer, inflammation, infectious diseases | NCT01965756, NCT00620191, NCT02432287, NCT02502253, NCT01219244, NCT01504854, NCT02095873, 1681 trials completed and 2587 trials in total |
| Resveratrol (also inhibits complexes III and V) |           | AD, MCI, AD, Aging, inflammation, T2DM, metabolic syndrome, mitochondrial myopathies, COVID-19 | NCT02502253, NCT01504854, NCT02095873, NCT03221894, 124 trials completed and 185 trials in total |
| Berberine           |           | AD, MCI, Inflammation, T2DM, obesity, metabolic disorder, hypertension, COVID-19 | NCT03221894, 39 trials completed and 73 trials in total |
| Epigallocatechin-3-gallate (also inhibits complexes II and V) |           | AD, AD, Huntington’s disease, Multiple sclerosis, Down syndrome, T2DM, metabolic syndrome, hypertension, inflammation, cancer | NCT00951834, NCT03978052, NCT01357681, NCT03740295, NCT01699711, NCT00951834, NCT03978052, NCT01357681, NCT03740295, NCT01699711, 60 trials completed and 95 trials in total |
| Droquinone and tricyclic ortho-carbonyl analogs |           | Melasma, HI/AIDS, COVID-19 | NCT03221894, 22 trials completed and 38 trials in total |
| Elesclomol          |           | Cancer | NCT00888615, 6 trials completed and 9 trials in total |
| IACS-10759          |           | Acute myeloid leukemia, Cancer | NCT02882321, NCT03291938 |
| BAY 87-2243         |           | Cancer | NCT01297530 terminated |
| Benzophenone        |           | Breast cancer, Melasma, Infertility | NCT03885648, 4 trials completed |
| Capsaicin (also inhibits complex III) |           | Pain, neuropathy | NCT01401868, 193 trials completed and 286 trials in total |
| ME-143              |           | Solid tumors | NCT01401868 |
complex I inhibition contributes to the beneficial effect of metformin. Analysis of the literature indicates that plasma protein binding of metformin is negligible, and after oral administration at the recommended doses and dosing schedules, steady-state plasma concentrations are reached within 24–48 h and are generally less than 1 μg/mL (6.04 μmol/L). In controlled clinical trials, maximum metformin plasma levels did not exceed 5 μg/mL even at maximum doses. The experimental data, however, indicate that metformin does not inhibit complex I at concentrations below 25 μmol/L. Nevertheless, it was reported that metformin accumulates in mitochondria where it could reach concentrations sufficient for complex I inhibition. Thus, while increasing evidence supports strong therapeutic potential for metformin as a neuroprotective therapy for neurodegenerative diseases of aging, additional safety and feasibility studies and mechanistic studies aimed at evaluating the contribution of complex I inhibition in different tissues to the drug efficacy are needed to identify potential risk factors, windows of therapeutic opportunity, and regimens.

Recent studies have identified other ETC inhibitors with a wide range of biological properties, including antioxidant, anticancer, anti-inflammatory, and neuroprotective effects. Resveratrol, a promising therapeutic compound that activates sirtuins, has been shown to reduce the activity of mitochondrial complexes I, III, and V. Similar to metformin, resveratrol stimulates key signaling pathways, including antioxidant defenses, reduction of inflammation via inhibiting NF-κB signaling, and AMPK activation, leading to improved mitochondrial function and biogenesis through sirtuin 1/AMPK/peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) pathway and vitagenes, which prevent the deleterious effects triggered by oxidative stress. Results of studies conducted in in vitro and in vivo models of AD provided evidence that resveratrol normalizes cholinergic neurotransmission and brain-derived neurotrophic factor (BDNF) expression, reduces oxidative stress, promotes Aβ peptide clearance and anti-amyloidogenic cleavage of APP, and reduces neuronal apoptosis. Application of resveratrol was also beneficial in models of metabolic disorders, Huntington’s disease (HD), and Parkinson’s disease (PD), amyotrophic lateral sclerosis, stroke, and alcohol-induced neurodegenerative disorders. However, the use of resveratrol in humans has been challenging, with limited bioavailability, pronounced adverse side effects, and inconsistent results were reported in healthy and unhealthy participants of clinical trials. While data generated to date strongly support the importance of resveratrol for human health, the design of better analogs with greater potency, solubility, and bioavailability are needed. Taken together, these data demonstrate that mild inhibition of the OXPHOS complexes engages a multifaceted mitochondria-mediated signaling cascade that improves multiple mechanisms of AD pathogenesis, including inflammation, mitochondrial dysfunction, abnormal energy and lipid homeostasis, the ER and oxidative stress making this therapeutic approach appealing.

### 4. Partial mitochondrial complex I inhibition as a therapeutic strategy for AD and other diseases

Partial inhibition of complex I with small molecules emerged as a promising strategy to induce beneficial ISR. Table I lists complex I inhibitors that are in clinical trials for various human conditions, including T2DM, cancers, metabolic disorder, obesity, inflammatory and infectious diseases. Only metformin, resveratrol, berberine, and epigallocatechin-3-gallate were trialed in a limited number of studies for neurodegenerative diseases, including AD, HD, MCI, multiple sclerosis, and Down syndrome. Metformin improved cognitive function in patients with amnestic MCI, while resveratrol, berberine and epigallocatechin-3-gallate did not show statistically significant improvements in cognitive performance in patients with AD, HD, or MCI. While all four complex I inhibitors penetrate the BBB, the therapeutic effect of resveratrol, berberine and epigallocatechin-3-gallate was limited, probably due to a poor stability, short half-life, and a very low bioavailability (<1%) in contrast to metformin, which is stable and has better bioavailability. Therefore, modifications of current complex I inhibitors or the development of new small molecules with improved drug-like properties and bioavailability are needed to increase therapeutic efficacy for neurodegenerative diseases.

**Complex I is the largest (970 kDa) multisubunit complex of the ETC with 14 central subunits involved in the oxidation of NADH to NAD⁺ at the flavin mononucleotide domain (FMN), transfer of the electrons along eight canonical iron-sulfur clusters to ubiquinone and its reduction, and proton pumping (Fig. 3).** There are an additional 31 accessory subunits that are not directly associated with energy production. Structures of bacterial and mammalian complex I have been determined by X-ray crystallography and cryogenic electron microscopy (cryo-EM) at high resolution, providing new insights into its assembly, proton-pumping machinery, the enzyme’s catalytic mechanism, and dysfunctions associated with disease-causing mutations. Complex I contributes significantly to the formation of ROS. Interestingly, there are more than 60 complex I inhibitors that have a differential effect on the enzyme kinetics or ROS production, where molecules including rotenone, piericidin A, and rolliniastatin 1 and 2 increase ROS, while inhibitors such as stigmatellin, mucidin, capsacin, and coenzyme Q2 prevent ROS formation. Similarly, some mutations in complex I could preserve the conversion of

| Complex I inhibitor | Structure | Condition or disease | Clinical trial ID* |
|---------------------|-----------|---------------------|-------------------|
| ME-344              | ![Structure](image) | Solid tumors, Breast cancers | NCT01544322, NCT02806817 |

AD, Alzheimer’s disease; MCI, mild cognitive impairment; T2DM, type 2 diabetes; HI, human immunodeficiency; AIDS, acquired immunodeficiency syndrome.

*Listed are the most recent representative clinical trials as of September, 2021. Additional trials could be found on [https://clinicaltrials.gov/](https://clinicaltrials.gov/).
NADH to NAD$^+$ and, therefore, complex I activity while completely blocking pathological ROS production$^{35}$. These data suggest that it is possible to develop safe and efficacious complex I inhibitors that are selective to the target and do not induce mitochondrial dysfunction associated with increased ROS production. These observations help to address concerns associated with the development of complex I inhibitors for chronic use in the elderly population to treat/delay the development of AD. For example, it is well established that mitochondrial complex I inhibitors such as 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 3-nitropropionic acid could be used to mimic PD by reducing mitochondrial complex I activity while

**Figure 3** Redox-linked proton translocation by complex I. Electrons are transferred from the nicotinamide adenine dinucleotide (NADH) oxidation site (the flavin mononucleotide domain, FMN) to the ubiquinone reduction site via a chain of iron–sulfur clusters (in gold); selected critical residues of the ubiquinone reduction site are shown in green (Tyr144, His95, His91). The FMN and ubiquinone are the main sites of reactive oxygen species (ROS) production. The membrane arm comprises three antipporter type subunits with discontinuous helices (ND5, marine; ND4, cyan; ND2, pink) corresponding to three potential proton translocation sites (black arrows). In the proximal part of the membrane arm (PP module) the $\pi$-bulge helix of ND6 (orange) and the discontinuous helix of ND1 (red) are highlighted. Residues constituting a fourth putative proton pathway (dashed arrow) are found in subunits ND2 and ND4L. In the center of the membrane arm a series of protonable residues (basic, blue; acidic, red) extends from subunit ND5 to subunit ND1 and terminates below the ubiquinone reduction site with a loop comprising a cluster of highly conserved acidic residues. Conformational changes linked to the redox chemistry of ubiquinone are proposed to induce an electric pulse that ultimately triggers proton translocation events in the membrane arm. Reprinted from Ref. 131 with the permission from the Elsevier.

disease pathogenesis$^{39,40}$. Our data generated in primary mouse neurons from HD mice$^{118}$ and in a bacterial artificial chromosome (BAC)-mediated transgenic mouse model of HD (unpublished observations) demonstrate that partial reduction of complex I activity improves multiple mechanisms affected by the expression of mutant huntingtin protein. Thus, a mounting body of evidence supports the feasibility of targeting complex I as a therapeutic strategy for neurodegenerative diseases. However, to develop safe and efficacious complex I inhibitors, it is imperative to determine the binding site, extent of inhibition of complex I activity, selectivity, and levels of ROS production.

We recently identified a small molecule tricyclic pyrrole compound (CP2) that penetrates the BBB and accumulates in mitochondria where it mildly inhibits the activity of complex I$^{117,141,142}$. CP2 is bioavailable, has low toxicity in vitro and in vivo, and has good drug-like properties and safety profile, demonstrating the lack of off-target activity against human receptors and ion channels$^{141-144}$. The genome-wide associations study using 196 human lymphoblastoid cell lines from healthy individuals with diverse age, sex and racial background demonstrated the safety of CP2 application at therapeutic doses$^{142}$. The effect of CP2 on the activity of each of the respiratory complexes examined using enzymatic assays and mitochondria isolated from the mouse or postmortem human brain confirmed selective and specific inhibition of complex I$^{117,142}$. The bioenergetics studies conducted in mouse primary cortical neurons using a Seahorse Extracellular Flux Analyzer (Agilent Technologies, Inc.) demonstrated that CP2 improved cellular bioenergetics by augmenting spare respiratory capacity, an indicator of mitochondrial ability to produce energy under conditions of increased workload or stress, which is essential for long-term survival and function$^{35}$. Similarly, CP2 increased mitochondrial respiratory control ratio and reduced proton leak, suggesting better coupling efficiency of the neuronal ETC, greater bioenergetic reserve, and enhanced ability to withstand stress. In vivo efficacy of chronic CP2 administration was examined in independent cohorts of male and female mice that express mutant human amyloid precursor protein (APP), human presenilin 1 protein (PS1), mutant APP and PS1 (APP/PS1) or mutant APP, PS1 and human Tau protein (3xTgAD) starting in utero for 14 months, at pre- or symptomatic stages of the disease$^{141-143}$. In all studies, chronic CP2 treatment did not induce toxicity or affect development. In all treatment paradigms, animals were allowed to have CP2 in drinking water ad lib. Remarkably, in all treatment groups, CP2 improved energy homestasis in the brain and periphery (glucose uptake and utilization, glucose tolerance, and insulin resistance), synaptic activity, long-term potentiation, dendritic spine maturation, cognitive function and proteostasis (reduced Aβ and pTau levels), and reduced oxidative stress and inflammation in the brain and periphery, ultimately blocking the ongoing neurodegeneration (Fig. 4)$^{32,143}$. We observed increased levels of ATP consistent with improvement of brain energy homestasis and reduced levels of ceramides, indicative of the release of the ER stress prominent in patients with AD$^{32}$. Therapeutic efficacy was monitored using translational in vivo biomarker fluorodeoxyglucose-positron emission tomography (FDG-PET), phosphorous-31 magnetic resonance imaging (31P MRI), and blood-based metabolomics. Interestingly, this treatment augmented mitochondrial dynamics and function, including restoration of axonal trafficking in neurons from CP2-treated PS1 and APP/PS1 mice$^{117}$. While CP2 was demonstrated to be selective and specific complex I inhibitor that lacks the off-target activities$^{32,142}$, it was shown to interfere with
the formation of Aβ aggregates, which could also contribute to its beneficial properties.

Further translational support for this therapeutic strategy was provided by the cross-validation of transcriptomic data generated in CP2-treated AD mice with the human brain transcriptome data available through the co-expression meta-analysis in the Accelerating Medicines Partnership Program for Alzheimer’s disease database (ampadportal.org). Beneficial changes in gene expression associated with CP2 treatment in APP/PS1 mice overlap with signatures established in patients with AD, female patients in particular, supporting high translational potential of this approach. Major translational targets included the immune system response and multiple pathways involved in synaptic function and neurotransmission, which underlie early pathology in patients with AD. Since CP2 improved axonogenesis and dendritic spine morphology and function, it is feasible that this treatment could also induce neuronal regeneration.

Molecular mechanisms of neuroprotection converged on the AMPK activation and the downstream signaling that resulted in increased resistance to oxidative stress, augmented mitochondrial bioenergetics, improved glucose uptake and utilization, increased production of sirtuins 1 and 3, reduction of glycogen synthase kinase 3 beta (GSK3β) activity, significant reduction in levels of pTau and Aβ, and increased autophagy and levels of BDNF and synaptic proteins in vivo. With CP2-inhibited complex I activity, the overall energy levels in the brain measured using 31P MRI were not decreased, which could be attributed to enhanced mitochondrial biogenesis and bioenergetics and improved brain energy homeostasis. The translational relevance of this approach is emphasized by the fact that the intervention was started after the onset of Aβ neuropathology, cognitive symptoms, bioenergetic dysfunction, and progressive neurodegeneration. These data provide further support for brain energy rescue as a novel concept for treatment of neurodegenerative diseases of aging. Furthermore, similar to metformin and resveratrol, CP2 also enhanced health and life span in chronologically aged wild-type mice and mice fed with a high-fat diet (our unpublished observations), implying that the activation of mitochondria-induced ISR using complex I as a small molecule druggable target could delay the onset or block the progression of age-related neurodegenerative diseases.

5. Conclusions

We summarized here evidence for a novel therapeutic approach to exploit the incredible ability of mitochondria to engage multifaceted neuroprotective stress response triggered by partial complex I inhibition. This approach promises relief for multiple human conditions, including, but not limited to mitochondrial diseases, HD, PD, and amyotrophic lateral sclerosis, and to promote healthy aging to delay the onset of neurodegenerative diseases, AD in particular, where age is the greatest risk factor. There is a mounting body of evidence generated in model organisms and humans in support of the safety of chronic application of complex I inhibitors. However, a better understanding of the molecular mechanisms is required to establish safety in translation to humans, including the development of biomarkers that inform on mitochondrial function and the capacity to induce the beneficial stress response. Further therapeutic developments should produce selective and specific complex I inhibitors capable of penetrating the BBB with excellent safety profile.

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Author contributions

Eugenia Trushina conceptualized the manuscript, Eugenia Trushina, Sergey Trushin, and Md Fayad Hasan wrote the manuscript, Md Fayad Hasan developed figures, all authors approved the manuscript.

Conflicts of interest

Eugenia Trushina is an inventor on the patent US20180044295A1 (“Compounds for modulating mitochondrial function”). The authors declare no conflict of interest.
References

1. Alzheimer’s Association. 2017 Alzheimer’s disease facts and figures. Alzheimer’s Dement 2017;13:325–73.

2. Mayeux R, Stern Y. Epidemiology of Alzheimer disease. Cold Spring Harb Perspect Med 2012;2:a006239.

3. Talwar P, Sinha J, Grover S, Rawat C, Kushwaha S, Agarwal R, et al. Dissecting complex and multifactorial nature of Alzheimer’s disease pathogenesis: a clinical, genomic, and systems biology perspective. Mol Neurobiol 2016;53:4833–64.

4. Deming Y, Dumitrescu L, Barnes LL, Thambisetty M, Kunkle B, Gifford KA, et al. Sex-specific genetic predictors of Alzheimer disease biomarkers. Acta Neuropathol 2018;136:857–72.

5. Toledo JB, Arnold M, Kastenmüller G, Chang R, Baillie RA, Han X, et al. Metabolic network failures in Alzheimer’s disease: a biochemical road map. Alzheimers Dement 2017;13:965–84.

6. Swerdlow RH. Mitochondria and mitochondrial cascades in Alzheimer’s disease. J Alzheimers Dis 2018;62:1403–16.

7. Tonnies E, Trushina E. Oxidative stress, synaptic dysfunction, and Alzheimer’s disease. J Alzheimers Dis 2017;57:1105–21.

8. Mosconi L, Papi A, De Leon MJ. Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer’s disease. Ann N Y Acad Sci 2008;1147:180–95.

9. Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Toledo JB, Arnold M, Kastenmüller G, Chang R, Baillie RA, Han X, et al. Metabolic network failures in Alzheimer’s disease: a biochemical road map. Alzheimers Dement 2017;13:965–84.

10. Steinhubl SR. Why have antioxidants failed in clinical trials?. Am J Cardiol 2008;101:S140–9D.

11. Wang W, Karamanlidis G, Tian R. Novel targets for mitochondrial medicine. Sci Transl Med 2016;8:326rv3.

12. Andersen SL. Centenarians as models of resistance and resilience to Alzheimer’s disease and related dementias. Adv Geriatr Med Res 2020;2:e200018.

13. Seto M, Weiner RL, Dumitrescu L, Hohman TJ. Protective genes and metabolic pathways in Alzheimer’s disease: moving towards precision interventions. Mol Neurodegener 2021;16:29.

14. Dyall SD, Brown MT, Johnson PJ. Ancient invasions: from endosymbionts to organelles. Science 2004;304:253–7.

15. Bélanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab 2011;14:724–38.

16. Vacanti NM, Divakaruni AS, Green CR, Parker SJ, Henry RR, Charaldi TP, et al. Regulation of substrate utilization by the mitochondrial pyruvate carrier. Mol Cell 2014;56:425–35.

17. Mookerjee SA, Gromeser AA, Nicholls DG. Brand MD. Quantifying intracellular rates of glycolytic and oxidative ATP production and consumption using extracellular flux measurements. J Biol Chem 2017;292:7189–207.

18. Zhang L, Trushina E. Chapter 5. In: Strack S, Usachev Y, editors. Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov 2020;19:609–33.

19. Steinhubl SR. Why have antioxidants failed in clinical trials?. Am J Cardiol 2008;101:S140–9D.

20. Wang W, Karamanlidis G, Tian R. Novel targets for mitochondrial medicine. Sci Transl Med 2016;8:326rv3.

21. Andersen SL. Centenarians as models of resistance and resilience to Alzheimer’s disease and related dementias. Adv Geriatr Med Res 2020;2:e200018.

22. Seto M, Weiner RL, Dumitrescu L, Hohman TJ. Protective genes and metabolic pathways in Alzheimer’s disease: moving towards precision interventions. Mol Neurodegener 2021;16:29.

23. Brand MD, Chien LF, Ainscow EK, Rolfe DF, Porter RK. The causes and functions of mitochondrial proton leak. Biochim Biophys Acta 1994;1187:132–9.

24. Yellen G. Fueling thought: management of glycosylation and oxidative phosphorylation in neuronal metabolism. J Cell Biol 2018;217:2235–46.

25. Chandel NS. Evolution of mitochondria as signaling organelles. Cell Metab 2015;22:204–6.

26. Mottis A, Herzig S, Auwerx J. Mitochondria to bedside. EMBO J 2017;36:2670–83.

27. Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Toledo JB, Arnold M, Kastenmüller G, Chang R, Baillie RA, Han X, et al. Metabolic network failures in Alzheimer’s disease: a biochemical road map. Alzheimers Dement 2017;13:965–84.
Partial inhibition of mitochondrial complex I induces neuroprotective stress response

47. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* 2007;8:74–85.

48. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol* 2012;13:251–62.

49. Herzog S, Shaw RJ. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol* 2018;19:121–35.

50. Peixoto CA, Oliveira WH, Araújo SMDR, Nunes AKS. AMPK activation: role in the signaling pathways of neuroinflammation and neurodegeneration. *Exp Neurol* 2017;298:31–41.

51. Ruderman NB, Xu XJ, Nelson L, Cacicedo JM, Saha AK, Lan F, et al. AMPK and SIRT1: a long-standing partnership?. *Am J Physiol Endocrinol Metab* 2010;298:E751–60.

52. van de Ven RAH, Santos D, Haigis MC. Mitochondrial sirtuins and molecular mechanisms of aging. *Trends Mol Med* 2017;23:320–31.

53. Flannery PJ, Trushina E. Mitochondrial dysfunction in Alzheimer’s disease and progress in mitochondria-targeted therapies. *Curr Behav Neurosci Rep* 2019;6:88–102.

54. Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* 2012;337:587–90.

55. Merkwirth C, Jovaisaite V, Durieux J, Matilainen O, Jordan SD,큐르스 MJ, et al. Distinct mitochondrial defects trigger the integrated stress response depending on the metabolic state of the cell. *Elife* 2020;9:e49178.

56. Lee SS, Lee BY, Fraser AG, et al. Rates of behavior and aging specified by mitochondrial molecular mechanisms of aging. *Exp Gerontol* 2019;115:23–34.

57. Mick E, Titov DV, Skinner OS, Sharma R, Jourdain AA, Mootha VK. Targeting mammalian sirtuin 1-integrin pathway. *Sci Rep* 2018;8:13190.

58. Lee SS, Lee RY, Fraser AG, et al. Two conserved histone demethylases regulate mitochondrial ATP synthase by IF1. *Cell Metab* 2019;30:35–49.

59. Hamilton B, Dong Y, Shindo M, Liu W, Odell I, Ruvkun G, et al. Extension of Drosophila lifespan assessed by urinary F2-isoprostanes: the CALERIE 2 study. *Aging Cell* 2015;14:230–41.

60. Ma L, Wang R, Dong W, Zhao Z. Caloric restriction can improve oxidative status assessed by urinary F2-isoprostanes: the CALERIE 2 study. *Aging Cell* 2015;14:230–41.

61. Heinz S, Freyberger A, Lawrenz B, Schladt L, Schmuck G, Ellinger-Ziegelbauer H. Mechanistic investigations of the mitochondrial complex I inhibitor rotenone in the context of pharmacological and safety evaluation. *Sci Rep* 2017;7:45465.

62. Raule N, Sevini F, Li S, Barbieri A, Tallaro F, Lomartire L, et al. The occurrence of mtDNA mutations on different oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human longevity and is population specific. *Aging Cell* 2014;13:401–7.

63. Rea SL, Ventura N, Johnson TE. Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in Caenorhabditis elegans. *PLoS Biol* 2007;5:e259.

64. Copeland JM, Cho J, Lo Jr T, Hur JH, Bahadorani S, Arabyan T, et al. Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. *Curr Biol* 2009;19:1591–9.

65. Miwa S, Jow H, Barty K, Johnson A, Czapiewski R, Saretzki G, et al. Low abundance of the matrix arm of complex I in mitochondria predicts longevity in mice. *Nat Commun* 2014;5:3837.

66. Lapointe J, Heikimi S. Early mitochondrial dysfunction in long-lived *M. b.1e*−/− mice. *J Biol Chem* 2008;283:26217–27.

67. Dell’agnello C, Leo S, Agostino A, Szabadkai G, Tiveron C, Zulian A, et al. Increased longevity and refractoriness to Ca2+−−dependent neurodegeneration in *Sur1* knockout mice. *Hum Mol Genet* 2007;16:431–44.

68. Fukui H, Díaz F, Garcia S, Moraes CT. Cytochrome c oxidase deficiency in neurons decreases both oxidative stress and amyloid formation in a mouse model of Alzheimer’s disease. *Proc Natl Acad Sci U S A* 2007;104:14163–8.

69. Pinto M, Pickrell AM, Fukui H, Moraes CT. Mitochondrial DNA damage in a mouse model of Alzheimer’s disease decreases amyloid beta plaque formation. *Neurobiol Aging* 2013;34:2399–407.

70. Garcia-Aguilar A, Cuevasa JM. A review of the inhibition of the mitochondrial ATP synthase by IF1 in vivo: reprogramming energy metabolism and inducing mitohormesis. *Front Physiol* 2018;9:1322.

71. Ross E, Ata R, Thavarajah T, Medvedev S, Bowden P, Marshall JG, et al. AMP-activated protein kinase regulates the cell surface proteome and integrin membrane traffic. *PLoS One* 2015;10:e0128013.

72. Rossmeisl M, Barbatelli G, Flachs P, Brauner P, Zingaretti MC, Marelli M, et al. Expression of the uncoupling protein 1 from the ap2 gene promoter stimulates mitochondrial biogenesis in unilocular adipocytes in *vivo*. *Eur J Biochem* 2002;269:19–28.

73. Birch AV, Chao WM, Bracken C, Warren JD, Szeto HH. Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. *Br J Pharmacol* 2014;171:2017–28.

74. Desquaire V, Loiseau D, Jacques C, Douay O, Malthiéry Y, Ritz P, et al. Dinitrophenol-induced mitochondrial uncoupling in vivo triggers respiratory adaptation in HepG2 cells. *Biochim Biophys Acta* 2006;1757:21–30.

75. Urra FA, Muñoz F, Córdova-Delgado M, Ramírez MP, Peña-Ahumada B, Rios M, et al. FR5812a: a new uncoupler of OXPHOS that inhibits migration in triple-negative breast cancer cells via SirT1/AMPKβ1-integrin pathway. *Sci Rep* 2018;8:13190.

76. Vaughan RA, Garcia-Smith R, Bisoffi M, Trujillo KA, Conn CA. Effects of caffeine on metabolism and mitochondria biogenesis in rhabdomyosarcoma cells compared with 2,4-dinitrophenol. *Nutr Metab Insights* 2012;5:59–70.

77. Baumgart M, Pribe S, Groth M, Hartmann N, Menzel U, Pandolfi L, et al. Longitudinal RNA-Seq analysis of vertebrate aging identifies mitochondrial complex I as a small-molecule-sensitive modifier of lifespan. *Cell Syst* 2016;2:122–32.

78. Heinz S, Freyberger A, Lawrenz B, Schladt L, Schmuck G, Ellinger-Ziegelbauer H. Mechanistic investigations of the mitochondrial complex I inhibitor rotenone in the context of pharmacological and safety evaluation. *Sci Rep* 2017;7:45465.

79. Raule N, Sevini F, Li S, Barbieri A, Tallaro F, Lomartire L, et al. The occurrence of mtDNA mutations on different oxidative phosphorylation subunits, not detected by haplogroup analysis, affects human longevity and is population specific. *Aging Cell* 2014;13:401–7.

80. Salminen A, Kaarniranta K. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Aging Cell* 2012;11:e259–70.

81. Chen A, Raule N, Chomyn A, Attardi G. Decreased reactive oxygen species production in cells with mitochondrial haplogroups associated with longevity. *PLoS One* 2012;7:e46473.

82. D’Aquila P, Bellizzi D, Passarino G. Mitochondria in health, aging and diseases: the epigenetic perspective. *Biogerontology* 2015;16:569–85.
89. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017;60:1577–85.

90. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 1998;352:854–65. Erratum in: Lancet 1998;352:1558.

91. Cameron AR, Logie L, Patel K, Erhardt S, Bacon S, Middleton P, et al. Metformin selectively targets redox control of complex I energy transduction. Redox Biol 2018;14:187–97.

92. Zhang CS, Li M, Ma T, Zong Y, Cui J, Feng JW, et al. Metformin activates AMPK through the lysosomal pathway. Cell Metab 2016;24:521–2.

93. Wang Y, An H, Liu T, Qin C, Seshak H, Guo S, et al. Metformin improves mitochondrial respiratory activity through activation of AMPK. Cell Rep 2019;29:1511–1523.e5.

94. Cao B, Rosenblat JD, Brielzke E, Park C, Lee Y, Musial N, et al. Comparative efficacy and acceptability of antidiabetic agents for Alzheimer’s disease and mild cognitive impairment: a systematic review and network meta-analysis. Diabetes Obes Metab 2018;20:2467–71.

95. Koenig AM, Mechanic-Hamilton D, Xie SX, Combs MF, Cappola AR, Us theirs AM, et al. Effects of the insulin sensitizer metformin in Alzheimer disease: pilot data from a randomized placebo-controlled crossover study. Alzheimer Dis Assoc Disord 2017;31:107–13.

96. Luchsinger JA, Perez T, Chang H, Mehta P, Steffener J, Pradabhan G, et al. Anti-inflammatory effects of metformin irrespective of diabetes status. J Alzheimers Dis 2016;51:501–14.

97. Herath PM, Cherbuin N, Eramudugolla R, Anstey KJ. The effect of diabetes medication on cognitive function: evidence from the PATH through life study. BioMed Res Int 2016;2016:7208429.

98. Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. Ageing Res Rev 2017;40:31–44.

99. Cameron AR, Morrison VL, Levin D, Mohan M, Forteath C, Beall C, et al. Anti-inflammatory effects of metformin irrespective of diabetes status. Circ Res 2016;119:652–65.

100. Handschin C. Caloric restriction and exercise “mimetics”: ready for prime time?. Pharmacol Res 2016;103:158–66.

101. Anisimov VN, Berstein LM, Egoruin PA, Piskunova TS, Popovich IG, Zabezhinski MA, et al. Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice. Exp Gerontol 2005;40:685–93.

102. Anderson RM, Shannuganayagam D, Weindruch R. Caloric restriction and aging: studies in mice and monkeys. Toxicol Pathol 2009;37:47–51.

103. Witte AV, Fokker M, Gellner R, Knecht S, Floel A. Caloric restriction improves memory in elderly humans. Proc Natl Acad Sci U S A 2009;106:1255–60.

104. Campbell JM, Stephenson MD, de Courten B, Chapman I, Bellman SM, Aronotarios E. Metformin use associated with reduced risk of dementia in patients with diabetes: a systematic review and meta-analysis. J Alzheimers Dis 2018;65:1225–36.

105. Chen Y, Zhou K, Wang R, Liu Y, Kwak YD, Ma T, et al. Antidiabetic drug metformin (GlucophageR) increases biogenesis of Alzheimer’s amyloid peptides via up-regulating BACE1 transcription. Proc Natl Acad Sci U S A 2009;106:3907–12.

106. Infeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer’s disease: a population-based case-control study. J Am Geriatr Soc 2012;60:916–21.

107. Moore EM, Mander AG, Ames D, Kotowicz MA, Carne RP, Brodaty H, et al. Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care 2013;36:2981–7.

108. Maitre-Coello G, Courchet J, Pieraut S, Courchet V, Maximo A, Polleux F. The CAMKK2-AMPK kinase pathway mediates the synaptotoxic effects of Aβ oligomers through Tau phosphorylation. Neuron 2013;78:94–108.

109. Cai Z, Yan LJ, Li K, Quazi SH, Zhao B. Roles of AMP-activated protein kinase protein in Alzheimer’s disease. NeuroMolecular Med 2012;14:1–14.

110. Chapman LE, Darling AL, Brown JE. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes: a systematic review and meta-analysis. Diabetes Metab 2016;42:316–27.

111. Moore E, Mander A, Ames D, Carne R, Sanders W, Datters D. Cognitive improvement and vitamin B12: a review. Int Psychogeriatr 2012;24:541–56.

112. Bristol-Myers Squibb Company. GLUCOPHAGE® (metformin hydrochloride extended-release tablets). 2020. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/020357s031, 0212020106b1.pdf.

113. Hu D, Xie F, Xiao Y, Lu C, Zhon J, Huang D, et al. Metformin: a potential candidate for targeting aging mechanisms. Aging Dis 2021;12:840–93.

114. Arnold SE, Arvanitakis Z, Macauley-Rambel SL, Koenig AM, Wang YH, Ahima RS, et al. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. Nat Rev Neurol 2018;14:168–81.

115. Rotermund C, Machetanz G, Fitzgerald JC. The therapeutic potential of metformin in neurodegenerative diseases. Front Endocrinol (Lausanne) 2018;9:400.

116. Vingtdeux V, Dreses-Werringloer U, Zhao H, Davies P, Marambaud P. Therapeutic potential of resveratrol in Alzheimer’s disease. BMC Neurosci 2008;9 Suppl 2:S6.

117. Zhang L, Zhang S, Maezawa I, Trushin S, Minhas P, Pinto M, et al. Modulation of mitochondrial complex I activity averts cognitive decline in multiple animal models of familial Alzheimer’s disease. EBioMedicine 2015;2:294–305.

118. Trushina E, Rana S, McCormay CT, Hua DH. Tricyclic pyrone compounds prevent aggregation and reverse cellular phenotypes caused by expression of mutant huntingtin protein in striatal neurons. BMC Neurosci 2009;10:73.

119. Leifert WR, Ayewaderna MY. Cardioprotective actions of grape polyphenols. Nutr Res 2008;28:729–37.

120. Salehi B, Mishra AP, Nigam M, Sener B, Kilic M, Sharifi-Rad M, et al. Resveratrol: a double-edged sword in health benefits. Bio- medicines 2018;6:61.

121. Hubbard BP, Sinclair DA. Small molecule SIRT1 activators for the treatment of aging and age-related diseases. Trends Pharmacol Sci 2014;35:146–54.

122. Guengan N, Desquiéret-Dumas V, Leman G, Chupon S, Baron N, Nivet-Antoine V, et al. Resveratrol directly binds to mitochondrial complex I and increases oxidative stress in brain mitochondria of aged mice. PLoS One 2015;10:e0144290.

123. Zini R, Morin C, Bertelli A, Bertelli AA, Tillement JP. Effects of resveratrol on the rat brain respiratory chain. Drugs Exp Clin Res 1999;25:87–97.

124. Zheng J, Ramirez VD. Inhibition of mitochondrial proton FOF1-ATPase/ATP synthase by polyphenolic phytochemicals. Br J Pharmaco 2000;130:1115–23.

125. Tellone E, Galtieri A, Russo A, Giardina B, Ficarra S. Resveratrol: a double-edged sword in health benefits. Nutr Res 2015;35:28.

126. Rege SD, Galtieri A, Russo A, Giardina B, Ficarra S. Resveratrol: a double-edged sword in health benefits. Nutr Res 2015;35:28.
Partial inhibition of mitochondrial complex I induces neuroprotective stress response

130. Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer’s disease. Mol Neurodegener 2020;15:40.

131. Wirth C, Brandt U, Hunte C, Zickermann V. Structure and function of mitochondrial complex I. Biochim Biophys Acta 2016;1857:902–14.

132. Fiedorczuk K, Letts JA, Degliesposti G, Kaszuba K, Skehel M, Choi WS, Kruse SE, Palmiter RD, Xia Z. Mitochondrial complex I.

133. Parey K, Haapanen O, Sharma V, Kofeler H, Zuullig T, Prinz S, et al. Partial inhibition of mitochondrial complex I induces neuroprotective stress response.

134. Fato R, Bergamini C, Bortolus M, Maniero AL, Leoni S, Ohnishi T, Yin Z, Burger N, Kula-Alwar D, Aksentijevich I, Martinez EA, Gomez-Pastor R. Mitochondrial dysfunction in Huntington’s disease; interplay between HSF1, p53 and PGC-1α transcription factors.

135. Intihar TA, Zhang Y, Zhou F, et al. Structural basis for a complex I mutation that blocks pathological ROS production.

136. Borsche M, Pereira SL, Klein C, Grünewald A. Mitochondria and Parkinson’s disease: clinical, molecular, and translational aspects. J Parkinsons Dis 2021;11:45–60.

137. Choi WS, Kruse SE, Palmter RD, Xia Z. Mitochondrial complex I inhibition is not required for dopaminergic neuron death induced by rotenone, MPP+, or paraquat. Proc Natl Acad Sci USA 2008;105:15136–41.

138. Flores IH, Fernandez-Vizarra E, Lykouri M, Braided B, Skehel GO, Miletic H, et al. Neuronal complex I deficiency occurs throughout the Parkinson’s disease brain, but is not associated with neurodegeneration or mitochondrial dysfunction. Acta Neuropathol 2018;135:409–25.

139. Intihar TA, Martinez EA, Gomez-Pastor R. Mitochondrial dysfunction in Huntington’s disease: interplay between HSF1, p53 and PGC-1α transcription factors. Front Cell Neurosci 2019;13:103.

140. Trushina E, Dyer RB, Badger 2nd JD, Ure D, Eide L, Tran DD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Mol Cell Biol 2004;24:8195–209.

141. Zhang L, Zhang S, Maezawa I, Trushin S, Minhas P, Pinto M, et al. Corrigendum to “Mutation of mitochondrial complex I activity averts cognitive decline in multiple animal models of familial Alzheimer’s disease” [EBioMedicine 2 (2015) 294-305]. EBioMedicine 2019;42:532. Erratum for: EBioMedicine 2015;2:294-305.

142. Stojakovic A, Trushin S, Sheu A, Khalili L, Chang SY, Li X, et al. Partial inhibition of mitochondrial complex I ameliorates Alzheimer’s disease pathology and cognition in APP/PS1 female mice.

143. Stojakovic A, Chang SY, Neshitt J, Pichurin NP, Ostroot MA, Aikawa T, et al. Partial inhibition of mitochondrial complex I reduces Tau pathology and improves energy homeostasis and synaptic function in 3xTg-AD mice. J Alzheimers Dis 2021;79:335–53.

144. Gao H, Tripathi U, Trushin S, Okremelidze L, Pichurin NP, Wei L, et al. A genome-wide association study in human lymphoblastoid cells supports safety of mitochondrial complex I inhibitor. Mitochondrion 2021;58:83–94.

145. Choi SW, Gerencser AA, Nicholls DG. Bioenergetic analysis of isolated cerebrocortical nerve terminals on a microgram scale: spare respiratory capacity and stochastic mitochondrial failure. J Neurochem 2009;109:1179–91.

146. Hong HS, Rana S, Barrigan L, Shi A, Zhang Y, Zhou F, et al. Inhibition of Alzheimer’s amyloid toxicity with a tricyclic pyrone molecule in vitro and in vivo. J Neurochem 2009;108:1097–108.

147. Jin LW, Hua DH, Shie FS, Maezawa I, Soper B, Martin GM. Novel tricyclic pyrone compounds prevent intracellular APP C99-induced cell death. J Mol Neurosci 2002;19:57–61.

148. Maezawa I, Hong HS, Wu HC, Battina SK, Rana S, Iwamoto T, et al. A novel tricyclic pyrone compound ameliorates cell death associated with intracellular amyloid-beta oligomeric complexes. J Neurochem 2006;98:57–67.

149. Johnson ECB, Dammer EB, Duong DM, Ping L, Zhou M, Yin L, et al. Large-scale proteomic analysis of Alzheimer’s disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation. Nat Med 2020;26:769–80.

150. Wengenack TM, Whelan S, Curran GL, Duff KE, Poduslo JF. Quantitative histological analysis of amyloid deposition in Alzheimer’s double transgenic mouse brain. Neuroscience 2000;101:939–44.

151. Holcomb LA, Gordon MN, Jantzen P, Hsiao K, Duff K, Morgan D. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: lack of association with amyloid deposits. Behav Genet 1999;29:177–85.

152. Trushina E, Nemutlu E, Zhang S, Christensen T, Camp J, Mesa J, et al. Defects in mitochondrial dynamics and metabolomic signatures of evolving energetic stress in mouse models of familial Alzheimer’s disease. PLoS One 2012;7:e32737.

153. Matchett BJ, Grinberg LT, Theofilas P, Murray ME. The mechanistic link between selective vulnerability of the locus coeruleus and neurodegeneration in Alzheimer’s disease. Acta Neuropathol 2021;141:631–50.