様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 １８ 日現在

機関番号：３８００５
研究種目：基盤研究(C)（一般）
研究期間：2021～2023
課題番号：２１Ｋ０６４００

研究課題名（和文）ATP-dependent liquid phase separation during aging and neurodegeneration

研究課題名（英文）ATP-dependent liquid phase separation during aging and neurodegeneration

研究代表者
Ｇｕｉｌｌａｕｄ　Ｌａｕｒｅｎｔ（Guillaud, Laurent）

沖縄科学技術大学院大学・分子神経科学ユニット・グループリーダー

研究者番号：９０５９６２２２

交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：私の研究プロジェクトは、加齢および神経変性疾患における細胞粘弾性および病的なタンパク質凝集の調節における ATP 向水性活性の重要な役割を実証しました。私の結果は、加齢および神経変性に関連するミトコンドリア活性および ATP 合成の低下が、タンパク質凝集の大幅な増加および軸索粘弾性の喪失につながり、NADH 経路を介した ATP 合成の促進によって回復できることを示しました。全体として、生涯を通じて高レベルの ATP を維持することで、ニューロンにおけるタンパク質の病的な凝集を軽減または緩和し、加齢関連タンパク質症の進行または発症を予防または遅らせることができることを示しています

研究成果の学術的意義や社会的意義
My research achievements revealed the novel and critical hydrotropic function of ATP in the regulation of neuronal protein aggregation during aging and neurodegeneration. These results open the door to the development of new anti-aging and neuroprotective interventions

研究成果の概要（英文）：My research project demonstrated the critical role of ATP hydrotropic activity in the regulation of cellular viscoelasticity and pathological protein aggregation during aging and in neurodegenerative diseases. My results indicated that the reduction in mitochondrial activity and ATP synthesis associated with aging and neurodegeneration lead to significant increase in protein aggregation and loss of axoplasmic viscoelasticity and can be rescued by boosting ATP synthesis via the NADH pathway. Altogether I show that preserving hight levels ATP throughout lifespan can reduce or alleviate the pathological aggregation of proteins in neurons and potentially protect against or slow down the progression or development of aged-related proteinopathies

研究分野：Neuroscience

キーワード：Neurodegeneration　Aging　ATP synthesis　Protein aggregation　Liquid phase separation

１．研究開始当初の背景

Recently protein liquid phase separation (LPS) at the cellular level has been shown to play import roles in various biological functions and implicated in neurodegenerative diseases often associated with aging. Protein aggregation and mitochondrial disfunctions have been shown to be biological hallmarks of Parkinson (PD), Alzheimer (AD) and Amyloid Lateral Sclerosis (ALS) diseases. Deciphering the mechanisms underlying soluble/insoluble phase transition in physiological and pathological conditions will thus improve our ability to understand and potentially develop treatment for these diseases often associated with aging. In addition, it is widely reported that mitochondrial activity, and thus ATP production, are significantly altered during aging and mitochondrial disfunctions often associated with neurodegenerations. Very importantly, ATP has been shown to be one of the most potent hydrotropic factors, playing a critical role in the regulation of protein aggregation in vitro. Thus, it is critical to characterize how aging affects LPS in neurons through the gradual impairment in mitochondria activity and the decrease ATP synthesis, as well as to demonstrate that these age-related perturbations in ATP-dependent LPS might also be major factors promoting the pathological aggregation of proteins during neurodegeneration.

２．研究の目的

In our modern society, extension of individual life span is also associated with an increasing occurrence of neurodegenerative disorders. Understanding the cellular mechanisms underlying or promoting the early onset of PD, AD or ALS became one of the most challenging research topics in the field of life sciences. Thus, I propose to analyze how mitochondrial activity and ATP concentration can modify both intracellular and extracellular biocondensate LPS during aging and decipher how perturbations in LPS of several key proteins play critical roles in neuronal homeostasis and in the physiopathology of neurodegenerative diseases. The outcome of my research will demonstrate that, in addition to its energetic property, ATP mainly regulates LPS in vivo. I hypothesize that the variation or perturbation in ATP synthesis, associated with mitochondrial disfunction during aging, and the concomitant disturbance in LPS might represent an early marker or aggravating factor in the onset of ALS and PD. These results might lead to the development of new approaches for the detection and/or treatment of neurodegenerative diseases, in which protein aggregations could be prevented or reverted by restoring or enhancing mitochondrial activity or increasing ATP concentration genetically or pharmacologically.
Thus, my research objective is to elucidate the cellular mechanisms underlying protein LPS in neurons during aging and neurodegeneration. I aim to provide physiological and biological evidence showing that mitochondrial activity and the hydrotropic property of ATP are playing central roles in the regulation of protein solubility during aging, and that LPS are critical for the maintenance of cellular functions in healthy young neurons and are impaired in elder diseased neurons. The main objectives of this proposal are: 1) Demonstrate and establish the correlation between mitochondrial activity, ATP concentration and protein LPS in cultured neurons from different ages. 2) Analyze whether protein LPS, mitochondrial activity and ATP concentration are also altered in hiPSC-derived neurons from ALS and PD patients. 3) Rescue and restore normal LPS in neuronal culture models by increasing intracellular ATP concentration. 4) Analyze ATP-dependent LPS of pure proteins in vitro.

３．研究の方法

My research strategy and methods relied on fluorescence live cell imaging to assess cytosolic viscoelasticity and mitochondrial activity, in vitro measurement of ATP and NADH by luciferase assays, and quantification of protein aggregation (TDP-43 and G3BP1)

by immunofluorescence microscopy in cultured human iPSCs, hiPSC-derived neurons and mouse sensory neurons. Protein aggregation was also quantified by in vitro protein LPS assays.Cell viability and proliferation, as well as DNA methylation level were also quantified in cells from different ages. Various pharmacological treatments were used to boost ATP synthesis in cultured cells and check their effect on cell viability and proliferation, viscoadaption and protein aggregation, and intracellular levels of ATP and NADH.

4．研究成果

(1) Impairment in ATP synthesis and perturbation of cellular viscoadaptation in mouse sensory neurons during aging

Using mouse sensory neurons from 8-10 weeks and 50-60 weeks old animals, I found significant reduction in the number of active mitochondria in axons from older mice compared to younger mice. This decrease in active mitochondria was also associated with a reduction in ATP synthesis. The loss of intracellular ATP reduced cytosolic viscoelasticity by promoting the aggregation of G3BP1 stress granules in older neurons compared to younger ones.
Conversely, boosting ATP synthesis by chronic treatment with NMN, a precursor of the NAD+/NADH pathway, significantly reduced protein aggregation and increase cytosolic fluidity in older neurons comparable to the levels observed in younger neurons.

(2) Mitochondrial and ATP synthesis impairment affect cell viability and proliferation of human iPSCs from individuals of different age

Using human iPSCs from 20 and 80 y/o individuals, I showed that iPSCs from older individual retained aged-specific phenotypes such as mitochondrial dysfunction (lower ATP synthesis) and higher DNA methylation, indicating higher levels of methylation and lower intracellular levels of ATP in aged iPSCs compared to younger iPSCs.In addition, iPSCs from 80 y/o individual showed significantly lower cell viability and reduced cell proliferation than iPSCs from 20 y/o individual. Preliminary observation also indicated a reduction in cytosolic viscoelasticity in older iPSCs compared to younger ones.
Treatment with NMN or creatine which boosted ATP synthesis also improved cell viability and proliferation from old iPSCs comparable to the levels observed in younger iPSCs.

(3) ATP-dependent perturbation of axoplasmic viscoelasticity and axonal protein aggregation in hiPSC-derived neurons from ALS and PD patients

Using human iPSC-derived neurons from healthy and ALS or PD patients, I first demonstrated that neurons from ALS patients showed a decrease in the number of active mitochondria along their axons compared to neurons from healthy individuals, a reduction in ATP synthesis and an increase in the number and size of pathological TDP-43 protein aggregates. This increase in pathological protein aggregation was also correlated with a significant perturbation of axoplasmic viscoelasticity.
Chronic treatment of iPSC-derived neurons from ALS patients with NMN also rescued both intracellular levels of ATP and axoplasmic viscoelasticity to the levels observed in neurons from healthy individuals, as well as reduced the number of pathological TDP-43 aggregates.
Secondly, I also confirmed similar observations in human iPSC-derived neurons from PD patients indicating that the ATP-dependent hydrotropic regulation of protein aggregation is a common and share mechanism among various degenerative diseases.

(4) ATP-dependent decondensation of protein aggregates in vitro

Using purified proteins, I showed that the condensation and decondensation of protein aggregates in vitro is highly dependent on the concentration of ATP, confirming the observations collected in mouse and human neurons. Both TDP-43 (ALS), a-synuclein (PD) and Tau (AD) can undergo ATP-dependent LPS and increasing concentration of ATP can prevent the formation of aggregates as well as solubilize pre-formed aggregates.

Taken together, my current results clearly established a correlation between mitochondrial activity, ATP synthesis and protein aggregation during aging and in neurodegenerative diseases such as ALS and PD. This work uncovered and confirmed the essential role of ATP as a cellular hydrotrope in mouse and human neurons, and its critical implication in the regulation of neuronal homeostasis throughout our lifespan, and in the protection against the pathological aggregation of proteins observed in neurodegenerative diseases often associated with aging.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　3件）

〔学会発表〕　計3件（うち招待講演　2件／うち国際学会　0件）

１．発表者名 Laurent Guillaud
２．発表標題 Nicotinamide mononucleotide rescues ALS pathological phenotypes in TDP- 43N390D human iPSC-derived motor neurons
３．学会等名 Axonal Degeneration Regeneration International Workshop（招待講演）
４．発表年 2022年

１．発表者名 Laurent Guillaud
２．発表標題 Nicotinamide mononucleotide rescues ALS pathological phenotypes in TDP-43 N390D human iPSC-derived motor neurons
３．学会等名 Gordon Research Conference
４．発表年 2023年

１．発表者名 Laurent Guillaud
２．発表標題 ATP hydrotropic regulation of neuronal proteins aggregation in synapse organization and neurodegeneration
３．学会等名 Axonal Degeneration Regeneration International Workshop（招待講演）
４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関