



2023 IMPACT CIRCLE

Project Title: **Brain on a dish: Uncovering the role of senescent cells to the decline of neuronal proteostasis during aging.**

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Unmet Need/Primary Question: A loss in the buffering capacity of the proteostasis network is a central pillar of aging, contributing to the decay in the function of the grain. There is a lack in our understanding of the molecular mechanisms explain why brain proteostasis fails during aging, preventing us from developing new and effective therapeutic interventions to reverse or ameliorate aged-associated diseases. We plan to tackle the following fundamental questions: What drives the breakdown of brain proteostasis during aging? What is the contribution of senescent cells in this process? Which brain cell types are more affected by the presence of senescent cells and their associated secretory phenotype?

Novel Hypothesis: Senescent cells accelerate the decline in brain proteostasis during aging through the senescence-associated secretory phenotype (SASP).

Project Proposal: Decades of research have defined the hallmarks of aging and underscored the biological processes that determine when and how organisms age [1, 2]. Brain aging is the main risk factor to develop neurodegenerative diseases such as Alzheimer's and Parkinson's, which are associated with an abnormal deposition of protein aggregates. Consistent with this idea, the signaling pathways that maintain protein homeostasis or proteostasis are significantly altered in aged organisms, a phenomenon believed to be a primary pillar of brain aging [3, 4]. Maintaining proteostasis requires the dynamic coordination of efficient folding of newly synthesized proteins, and quality control and protein degradation mechanisms to reduce the burden of misfolded/unfolded proteins and prevent abnormal protein aggregation [4].

Senescent cells, which are in a state of essentially irreversible cell cycle arrest but remain viable, can accumulate with aging in multiple organs and at pathogenic sites of multiple disorders and diseases and limit longevity and healthy life span (healthspan) across multiple species. Senescent cells are characterized by a distinct proinflammatory terminal state caused by the secretion of multiple soluble factors, including cytokines, chemokines, proteases, and growth factors, and collectively known as the *senescence-associated secretory phenotype* (SASP) [5]. The decline in proteostasis has been demonstrated in nematode and human senescent cells [6]. However, the paracrine effect of the SASP itself in regulating proteostasis in different brain cells and regions during aging has not been studied.

The brain is the most sophisticated and complex organ that nature has evolved, in which several different specialized cell types including many types of neurons, astrocytes, and microglia interact in temporally and spatially precise ways to coordinate its many vital functions. The influence of the complex three-dimensional structure of the brain and its role in disease development during aging cannot be fully assessed using traditional two-dimensional cell culture techniques. The development of cell culture techniques to grow whole brains in a dish (so-called “mini-brains” or brain organoids), using human pluripotent stem cells (hPSC) has revolutionized the field of neuroscience [7, 8]. These brain organoids show a highly advanced cellular composition, maturation, architecture and interconnectivity than two-dimensional cell cultures, serving as an interface between *in vitro* and *in vivo* models. Our labs are experts in brain organoids, aging and proteostasis and aim to join forces to synergize and develop a unique project. In this proposal, we plan to investigate the paracrine effects of the SASP in the regulation of proteostasis and protein aggregation in the whole brain using mini brains as an experimental model. Brain organoids will allow us to simultaneously assess the effects of SASP in specific brain cell types (neurons, glia, etc.) and brain regions (cortex, hippocampus). Moreover, these cell subpopulations can be further isolated and cultured for additional *in vitro* mechanistic experiments (see Figure 1). To this end, we will generate mini-brains using human induced pluripotent stem cells (iPSC) and co-culture them with senescent cells (Figure 1, experimental set-up). After brain organoids have been exposed to the SASP, we will determine proteostasis competence in target cells by analyzing different quality control mechanisms involved in proteostasis maintenance, such as the unfolded protein response (UPR) and autophagy, two quality control mechanisms that are inhibited during brain aging. Unbiased studies using single cell sequencing and quantitative proteomics will be performed to assess central nodes of the proteostasis network globally, the communication between cell types and its relation to senescence. Additionally, to assess the relevance of SASP to age-related neurological disease, we will take advantage of a recently generated mini brain derived from patients with Alzheimer’s disease (AD). These patients have a mutation in the APOE4 gene, which is the strongest genetic risk factor associated with late-onset AD [9]. These brain organoids exhibit increased accumulation of amyloid β plaques and phosphorylated Tau protein aggregates, two hallmarks of Alzheimer’s disease that reflect a loss of proteostasis. Together, these experiments will allow us to study the impact of senescent cells to proteostasis and neurodegeneration in a more physiological model that considers the complexity of brain composition and 3D architecture.

Description of Potential Impact: During brain aging, the collapse of proteostasis is at the heart of many age-related pathologies and associated conditions. The molecular mechanisms involved in the decline of proteostasis during aging are not well understood. Here, we propose to a connection between two fundamental pillar of aging: proteostasis and senescence. We envision that removing or modulating of senescent cells in the brain will restore proteostasis and reduce the accumulation of protein aggregates associated with some neurological disorders. We also plan to investigate the putative differential effects on different brains cell types and regions induced by a sustained SASP. This project will open the door to the development of novel strategies to sustain brain health during aging.

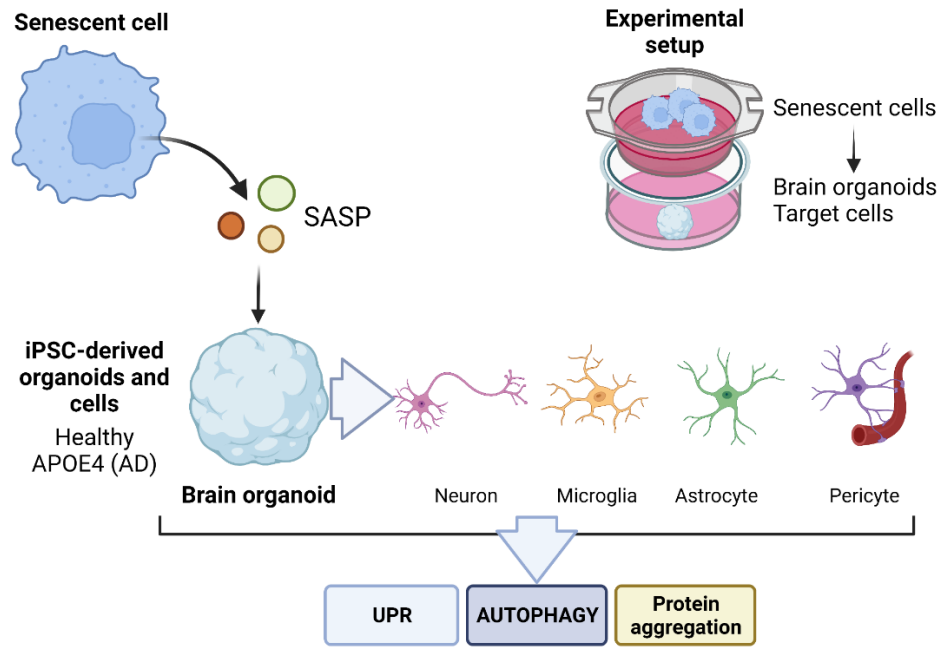


Figure. Idea and workflow: Senescent cells will be co-cultured with iPSC-derived brain organoids from healthy and APOE4-mutant patients (the most common genetic mutation in late-onset Alzheimer’s disease [AD]). Proteostasis competence in response to different stress conditions and protein aggregation burden will be determined from whole brain organoids or cell-specific cultures established from these organoids. UPR: unfolded protein response; iPSC: induced pluripotent stem cell.