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Special Issue: Singularity Biology and Beyond

Commentary and Perspective (Invited)

Research on the molecular mechanism of singularity phenomenon in neurological disorders

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In Alzheimer's disease (AD), toxic oligomers of tau proteins originate from a small number of cells deep inside the brain and propagate to other regions of the brain, ultimately causing cell death in up to 50% of neurons in the cerebral cortex. Based on the pathological observations of over 2000 brains [1,2], it has been hypothesized that the phosphorylated tau, the primary cause of tauopathy, initially appears in the locus coeruleus (LC) in the brainstem and entorhinal cortex (EC), spreading from there to the hippocampus and cerebral cortex. Integrating results from these human pathological anatomy and animal experiments [3] suggests that tau aggregation begins in a small number of neurons in LC and/or EC, and rapidly expands to affect the entire brain at some point. This phenomenon is considered a "singularity phenomenon", and neurons in LC and EC that acquired an ability to propagate toxic tau are referred to as "singularity cells". Interestingly, the formation of protein aggregates and their propagation from one brain region to another are common pathogenesis mechanisms shared by various neurodegenerative diseases. Amyloid β (Aβ), α-synuclein, and TAR DNA-binding protein 43 (TDP-43), which are found as aggregates in the patients of AD, Parkinson's disease as well as dementia with Lewy bodies, and amyotrophic lateral sclerosis (ALS), respectively, are also characterized by the expansion of large-scale neurodegeneration starting from a few cells [4]. These neurodegenerative diseases can also be considered "singularity phenomena". However, traditional research has not viewed neurodegenerative diseases as having a "singularity", or the cells forming toxic protein aggregates and gaining the ability to propagate aggregates have not been recognized as "singularity cells" in the onset of neurodegenerative diseases. To understand the mechanisms underlying these neuronal diseases and to advance prevention and treatment, it is essential to seamlessly analyze the process of accumulation of aggregated proteins, which gives rise to singularity cells and causes damage to neurons and the entire brain, across molecular, cellular, and tissue levels.

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In the Singularity Biology A03-1 group (Table 1), we focused on tau proteins, as the aggregation of tau protein is involved in multiple neurodegenerative diseases such as AD, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), chronic traumatic encephalopathy (CTE) that are generally referred as "Tauopathy". Our objective is to answer biological questions that when and where "singularity cells" acquire the ability to propagate and accumulate toxic tau oligomers. This involves understanding the biological challenges of when and how tau oligomers released from singularity cells rapidly propagate to the cerebral cortex. Here, we report the research activities by the Singularity Biology A03-1 group to understand the "singularity" of neurodegenerative diseases, revealing the singularity phenomena at the cellular and neuronal circuit levels by developing novel technologies to discover" singularities".

Table 1 A03-1 group composition and collaborators in the Singularity Biology

A03-01 group	
Principal Investigator	Hiroko Bannai (Waseda University)
Co-Investigator (CI)	Akihiko Takashima (Gakushuin University) Michio Hiroshima (RIKEN, Osaka University) Gen Matsumoto (Nagasaki University) Keiko Matsuda (Keio University)
Collaborating Researcher (CR)	Yoshiyuki Soeda (Gakushuin University)
Collaborators in the Singularity Biology	
A01-2 PI	Takeharu Nagai (Osaka University)
A01-2 CI	Hideaki Yoshimura (University of Tokyo)
A01-2 CR	Mitsuru Hattori (Osaka University)
A03 PI	Naruhiko Sahara (National Institutes for Quantum Science and Technology

Takashima team in A03-1 confirmed in SH-SY5Y cells that hyper-excitation enhanced the translation of tau protein depending on the strength of stimulation, without altering tau mRNA levels [5]. This result suggests the possibility of local translation induction in dendrites leading to an increase in tau concentration, and possibly the increase in tau oligomers. Additionally, Soeda and Bannai team of A03-1 and Yoshimura of the A01-2 group established a technique to control the state and localization of tau within cells using optogenetics. We developed the optogenetic tool "OptoTau", consisting of a P301L mutant human tau fused with the light-sensitive protein CRY2olig, which formed homo-oligomers upon blue light stimulation [6]. Twenty-four hours of blue-light stimulation increased the phosphorylation of OptoTau and resulted in tau translocation into the aggresomes [7]. In brains of AD and other tauopathies, N-terminally deficient tau has been identified [8]. When cells overexpressed OptoTau-ΔN, a Cry2Olig fused to one of these N-terminally deficient tau fragments, they were found to form liquid-liquid phase-separated droplets of tau seeds upon stimulation with blue light [7]. These results suggest that tau liquid droplet formation and N-terminal truncation are necessary for the formation of neurofibrillary tangles (NFT) in neurodegenerative diseases, representing a cellular-level singularity known as tau seed formation.

Groups led by Sahara from the publicity offered research group A03 and Matsumoto from the A03-1 group revealed the molecular mechanism of selective autophagy, which continuously degrades tau oligomers [9]. They proposed that the imbalance between tau generation and degradation could become a cellular-level singularity.

From the research activities by the A03-1 group, several novel imaging technologies contributing to detecting the singularity in neurological diseases have been developed. Before this study was initiated, Hiroshima in the A03-1 group had developed a fully automated in-cell single-molecule imaging system (AiSIS) using computer operations, robotics, and artificial intelligence (AI) [10]. Using AiSIS, Hiroshima analyzed the dynamics of plasma membrane molecules in cells expressing OptoTau with blue-light illumination and the dynamics of tau molecules at the single-molecule resolution, aiming to reveal the impact of various tau supramolecular complexes on neural activity and survival.

Another ongoing challenge is the development of new probes to detect the moment of tau oligomer formation in the brain. In collaboration with the A01-2 group's Hattori and Nagai, the A03-1 team led by Bannai found the prototype of luminescent probes to detect tau seeds within OptoTau knock-in cells. In the future, we would like to improve this probe and develop a probe that can detect tau oligomers that appear in earlier stages in the pathogenesis of tauopathy. This will allow us to ask when and where "singularity cells", which acquire the ability to propagate and accumulate toxic tau oligomers, arise within an individual animal.

Finally, we introduce research aimed at detecting the singularity phenomenon that causes tauopathy and other neurodegenerative diseases within neural circuits or animals and humans. The EC in the brain serves as a hub connecting several regions of the cerebral cortex and hippocampus, playing a crucial role in spatial recognition and navigation. Cognitive impairments observed in AD and tauopathies are considered to be associated with the accumulation of NFT, preceding the onset of impairments [11]. A03-1 Takashima team developed a 3D virtual reality (3DVR) task reflecting the activity of grid cells responsible for spatial recognition in the EC [12]. Conducting this 3DVR task with 177 dementia-

free volunteers aged 20 to 89 revealed an age-related increase in error rates in navigation tasks involving the EC. Given the correlation between NFT accumulation in the EC and age [13], errors in this 3DVR task may reflect the accumulation of NFT in the EC. Since NFT accumulation in the EC precedes awareness of cognitive impairment [11], this 3DVR task could be a method for early identification of persons who may develop AD.

In the research where A03-1 Matsumoto joined, an innovative technology was developed to amplify and detect α-synuclein aggregate cores in the blood of Parkinson's patients and those with Lewy body dementia using super-resolution microscopy [14]. This method holds the potential for application in detecting Tau seeds present in the cerebrospinal fluid of patients.

In summary, the A03-1 group in Singularity Biology has considered tauopathy, a neurodegenerative disease, as a "singularity phenomenon" and has developed methods to discover and manipulate "singularity cells". In the future, we would like to further develop the results of this research to understand the cellular level singularity of tauopathy, which will lead to the elucidation of the pathogenesis mechanism and the solution of diagnostic methods.

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References

[1] Braak, H., Thal, D. R., Ghebremedhin, E., Del Tredici, K. Stages of the pathologic process in Alzheimer disease: Age categories from 1 to 100 years. J. Neuropathol. Exp. Neurol. 70, 960–969 (2011). https://doi.org/10.1097/NEN.0b013e318232a379

[2] Elobeid, A., Soininen, H., Alafuzoff, I. Hyperphosphorylated tau in young and middle-aged subjects. Acta Neuropathol. 123, 97–104 (2012). https://doi.org/10.1007/s00401-011-0906-z

[3] Holmes, B. B., Furman, J. L., Mahan, T. E., Yamasaki, T. R., Mirbaha, H., Eades, W. C., et al. Proteopathic tau seeding predicts tauopathy in vivo. Proc. Natl. Acad. Sci. U.S.A. 111, E4376–E4385 (2014). https://doi.org/10.1073/pnas.1411649111

[4] Jucker, M., Walker, L. C. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature 501, 45–51 (2013). https://doi.org/10.1038/nature12481

[5] Kobayashi, S., Tanaka, T., Soeda, Y., Takashima, A. Enhanced tau protein translation by hyper-excitation. Front. Aging Neurosci. 11, 322 (2019). https://doi.org/10.3389/fnagi.2019.00322

[6] Taslimi, A., Vrana, J. D., Chen, D., Borinskaya, S., Mayer, B. J., Kennedy, M. J., et al. An optimized optogenetic clustering tool for probing protein interaction and function. Nat. Commun. 5, 4925 (2014). https://doi.org/10.1038/ncomms5925

[7] Soeda, Y., Yoshimura, H., Bannai, H., Koike, R., Takashima, A. Intracellular tau fragment droplets serve as seeds for tau fibrils. bioRxiv (2023). https://doi.org/10.1101/2023.09.10.557018

[8] Taniguchi-Watanabe, S., Arai, T., Kametani, F., Nonaka, T., Masuda-Suzukake, M., Tarutani, A., et al. Biochemical classification of tauopathies by immunoblot, protein sequence and mass spectrometric analyses of sarkosyl-insoluble and trypsin-resistant tau. Acta Neuropathol. 131, 267–280 (2016). https://doi.org/10.1007/s00401-015-1503-3

[9] Ono, M., Komatsu, M., Ji, B., Takado, Y., Shimojo, M., Minamihisamatsu, T., et al. Central role for p62/SQSTM1 in the elimination of toxic tau species in a mouse model of tauopathy. Aging Cell 21, e13615 (2022). https://doi.org/10.1111/acel.13615

[10] Hiroshima, M., Yasui, M., Ueda, M. Large-scale single-molecule imaging aided by artificial intelligence. Microscopy (Oxf) 69, 69–78 (2020). https://doi.org/10.1093/jmicro/dfz116

[11] Takashima, A., Koike, R., Soeda, Y. Can the entorhinal cortex help distinguish healthy aging brains from pathological aging brains? Aging Brain 2, 100026 (2022). https://doi.org/10.1016/j.nbas.2021.100026

[12] Koike, R., Soeda, Y., Kasai, A., Fujioka, Y., Ishigaki, S., Yamanaka, A., et al. Path integration deficits with phosphorylated tau accumulation in the entorhinal cortex in mice and humans. Brain Commun. **6**, fcad359 (2024). https://doi.org/10.1093/braincomms/fcad359

[13] Llamas-Rodríguez, J., Oltmer, J., Greve, D. N., Williams, E., Slepneva, N., Wang, R., et al. Entorhinal subfield vulnerability to neurofibrillary tangles in aging and the preclinical stage of Alzheimer's disease. J. Alzheimers Dis. 1379–1399 (2022). https://doi.org/10.3233/JAD-215567

[14] Okuzumi, A., Hatano, T., Matsumoto, G., Nojiri, S., Ueno, S. I., Imamichi-Tatano, Y., et al. Propagative alpha-synuclein seeds as serum biomarkers for synucleinopathies. Nat. Med. 29, 1448–1455 (2023). https://doi.org/10.1038/s41591-023-02358-9