2018

HIROSAKI UNIVERSITY RESEARCH HIGHLIGHTS

Establishing a Global Identity

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HIROSAKI UNIVERSITY

Autophagy in neurodegenerative disorders

Purpose and Background of the Research

Synucleinopathy comprises a group of neurodegenerative disorders that share abnormal alpha-synuclein in selected vulnerable neurons and glial cells. Abnormal alpha-synuclein is accumulated and fibrillated in the neuronal cytoplasm and processes as Lewy bodies (LBs) and Lewy neurites, respectively, in the brains of patients with Parkinson's disease (PD) and dementia with LBs (DLB), as well as in glial cytoplasmic inclusions (GCIs) in multiple system atrophy (MSA).

Macroautophagy (herein referred to as autophagy) is a highly conserved degradation pathway whereby not only cytosolic components but also aberrant proteins are sequestered within double-membraned vesicles, known as autophagosomes. We have previously shown that downstream autophagosomal proteins are incorporated into LBs and GCIs. Therefore, we elucidated the role of upstream proteins of autophagy in the pathogenesis of Lewy body disease (PD and DLB) and MSA.

Research Results

In human specimens, LBs were positive for upstream autophagosomal proteins (ULK1, ULK2, Beclin1, VPS34 and AMBRA1). Western blotting of fractionated brain lysates showed that upstream autophagosomal proteins were detected in the soluble and insoluble fraction in DLB, corresponding to the bands of phosphorylated alpha-synuclein. However, Western blot analysis of total brain lysates in PD and DLB showed that the increase of upstream autophagosomal proteins was only partial. Furthermore, we demonstrated that AMBRA1 is a novel hub binding protein of alpha-synuclein and plays a central role in the pathogenesis of MSA through the degradative dynamics of alpha-synuclein. These results raise the possibility that molecular modulation targeting AMBRA1 can be a promising candidate for the treatment of synucleinopathy. We further performed a whole-transcriptome assay by microarray, quantitative RT-PCR and Western blot analysis using peripheral blood mononuclear cells of patients with PD and age-matched controls. We provided evidence that autophagy in peripheral blood mononuclear cells could detect a feature confirmed by the neuropathology of PD and this alteration of autophagy is a fundamental aspect of PD.

Future Prospects

We believe that the activation of autophagy may have a place in the therapy of synucleinopathy. Recently, we have generated a mouse model of MSA, using the Cre-loxP system, to express inducible alpha-synuclein. Beginning in adulthood, these MSA model mice exhibit clinical and pathological features of MSA. These findings suggest that this new mouse model of MSA represents a useful tool for analyzing the pathophysiological alterations that underlie the progression of this disease.

Funding (Direct Cost)

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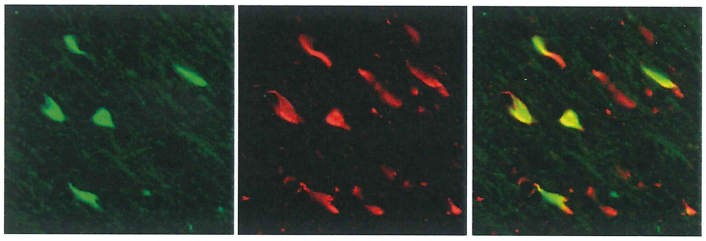
13,200,000 Yen



Double immunofluorescence labeling showing immunoreactivity of AMBRA1 (green) and alpha-synuclein (red) in GCIs of patients with MSA. Yellow indicates the colocalization of AMBRA1 and alpha-synuclein.

PROFILE

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Analysis of three-dimensional morphogenesis in multicellular organisms with application of local gene manipulation

Purpose and Background of the Research

Each multicellular organism has a unique shape. "Morphology" is the field of biology that focuses on the shape and structure of organisms. The formation of shape (morphogenesis) in plants and animals involves sophisticated mechanisms but its molecular mechanisms remain largely unclear. The most critical problem for morphological study in modern biology is that researchers are so far unable to induce an expression of an interested gene in a desired region of an organism except for a few limited model organisms. To solve this problem, we applied a heat shock-inducible system to induce gene expression at the target region of the organisms (Figure). By adding a local heat shock, we aim to induce gene expression and to elucidate the molecular mechanisms of three-dimensional morphogenesis in both animals and plants.

Research Results

First, we examined the time course of gene induction after whole-body heat shock in an amphibian (Xenopus laevis) by recording the fluorescence of the GFP (green fluorescent protein) in a time-lapse manner [for reference, please watch the video at http://cshprotocols.cshlp.org/content/2018/12/pdb.prot101014/suppl/DC1]. Then, we succeeded at gene induction at the single-cell-level in X. laevis by laser irradiation. We also induced a specific gene expression in a wider area by the application of a heated metal probe and succeeded in altering the morphogenesis in developing and regenerating limbs in X. laevis. We expanded local gene induction by infrared laser irradiation to a tunicate (Ciona intestinalis) and a plant (Arabidopsis thaliana) and succeeded in local gene induction.

Future Prospects

So far, we have succeeded in both single-cell-level gene induction and wider gene induction by heated metal probe. While single-cell-level gene induction is useful for cell lineage tracing, local but wider gene induction is often needed for the study of organ morphogenesis. The application of a heated metal probe is useful for wider gene induction, but this method requires a high level of manual skills. To improve the reproducibility of local gene induction, we need to improve the laser-irradiation method of gene induction. While the gene induction range of laser irradiation is narrow at present, gene induction by laser is reproducible. In theory, we should be able to bring together the individual narrow gene inductions by laser into a wider gene induction in a precise and reproducible manner. This is our most important task for the near future and we are now making efforts to achieve this task.

Funding (Direct Cost)

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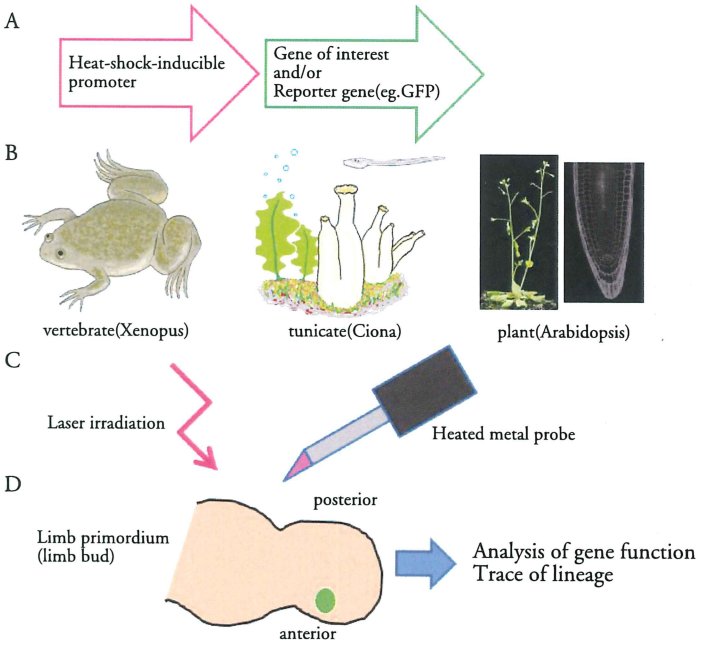
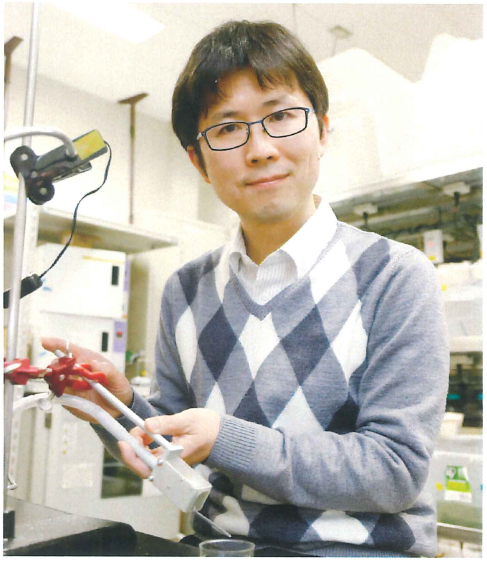


Local gene induction in multicellular organisms by heat shock. (A) A gene of interest is placed under the control of a heat-shock promoter. (B) A heat shock-inducible system is applied to various multicellular organisms — Xenopus, Ciona and Arabidopsis (courtesy of Dr. Michiko Sasabe) — . (C) Two methods for local heat shock. (D) An example of local gene induction. The expression of GFP is induced on the anterior side of a limb primordium in a Xenopus tadpole.



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High Throughput Screening of Alzheimer's Disease-preventive Materials Using *Drosophila*.

Purpose and Background of the Research

Alzheimer's disease (AD) is one of the major forms of dementia. Because there is no effective therapy for AD after onset at present, the prevention or delaying of the progression of AD is main target of study. If some foods have AD-preventive functions, intake of such foods is one of the most reasonable approaches against AD. For these reasons, it is important to screen foods or food ingredients for AD prevention. However, when the conventional mouse model is applied for this purpose, it is difficult to perform a high throughput assay because it costs time and money. On the other hand, *Drosophila* has a relatively short lifespan (approx. 2 months); it is ideal for studying the prevention of aging-related diseases, including dementia. Therefore, we chose *Drosophila* as a model organism for the screening of AD-preventive materials.

Research Results

Tauopathy is one of the key features of AD. We focused on the accumulation of phosphorylated tau protein in the neurons of the human tau-expressing mutant *Drosophila* and tauopathy-induced movement disorder. The former was assessed by Western blotting and latter was assessed by climbing assay (Mohideen *et al.*, Sci. Rep. (2015) 5:10821) with slight modifications. To develop these assay systems, we generated neuron-specific human tau protein-expressing *Drosophila* using GAL4-UAS system (Mohideen *et al.*, Sci. Rep. (2015) 5:10821) and optimized screening conditions. More than 40 foods or food ingredients were screened. One of screened foods was subjected to the next experiment using SAMP8 mice, which are commonly used in aging studies.

Future Prospects

To investigate the mechanism of action of the candidate AD-preventive foods, DNA microarray analysis of the foods ingested *Drosophila* will be performed. The *Drosophila* screening system will be applied not only in aging studies but also in other research about food function in order to reduce mouse experiments.

Funding (Direct Cost)

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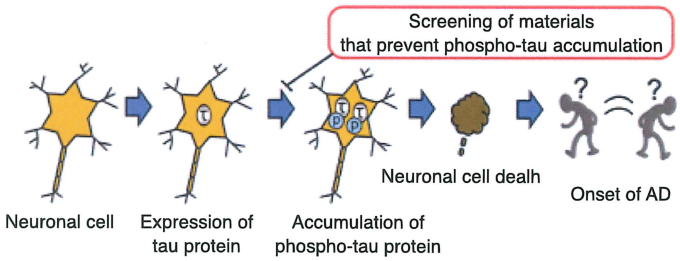


Figure 1. Outline of this study.

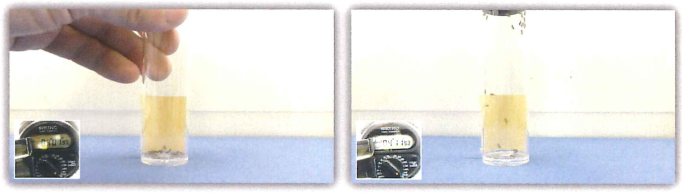
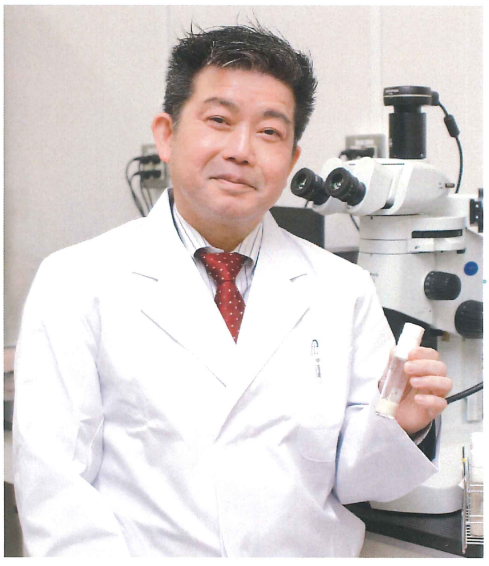


Figure 2. Climbing assay.
Flies were placed in an empty plastic vial. The vial was then gently tapped to knock all of the flies to the bottom. The number of flies who climbed above the line (30 mm above the bottom of the vial) was counted after 10 seconds.



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