Lecture 1

Peroxisome: homeostasis and human biogenesis disorders

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A. Distinct roles of acyl/alkyl dihydroxyacetonephosphate reductase in peroxisomes and ER.

Plasmalogen biosynthesis is initiated in peroxisomes. At the third synthesis step, acyl/alkyl dihydroxyacetonephosphate reductase (ADHAPR) reduces DHAP to 1-alkyl-*sn*-glycero-3-phospate, where the enzyme activity was found in both peroxisomes and microsomes. We herein address functional roles of ADHAPR in peroxisomes and ER. ADHAPR targets to peroxisomes via a Pex19p-dependent pathway, while ADHAPR is also inserted into the ER by SRP. The N-terminal domain-deleted ADHAPR mutant preferentially targets to the ER, restoring the ethanolamine plasmalogen (PlsEtn) synthesis in ADHAPR-deficient cells. In contrast, the expression of full-length ADHAPR in the mutant cells elevates the synthesis of phosphatidylethanolamine, but not PlsEtn. Collectively, the third step of plasmalogen synthesis is most likely mediated by ER-localized ADHAPR (1).

B. Phosphorylation of Pex14 induced by H_2O_2 suppresses peroxisomal import of catalase to counteract oxidative stress.

Most of peroxisomal matrix proteins including a hydrogen peroxide (H2O2)-decomposing enzyme, catalase, are imported in a peroxisome-targeting signal type-1 (PTS1)-dependent manner. Here, we address that Pex14, a central component of the protein import machinery, is phosphorylated in response to oxidative stresses such as H2O2 in mammalian cells. The H2O2-induced phosphorylation at Ser232 of Pex14 spatiotemporally regulates peroxisomal import of catalase, functioning in counteracting reaction against oxidative stress by the increase of cytosolic catalase (2).

C. Mammalian homologue NME3 of DYNAMO1 regulates peroxisome division.

Peroxisomes proliferate by sequential processes comprising elongation, constriction, and scission of peroxisomal membrane. The constriction step is mediated by a GTPase, dynamin-like protein 1 (DLP1). Mechanism of fuelling GTP to DLP1 remains unknown in mammals. We here identified that nucleoside diphosphate kinase 3 (NME3), a mammalian homologue of DYNAMO1 found in a red alga, *Cyanidioschyzon merolae* (3), localizes to peroxisomes. Elongated peroxisomes are observed in cells by *NME3* suppression and fibroblasts from an NME3-deficient patient. In the wild-type cells expressing catalytically-inactive NME3, peroxisomes are elongated. Taken together, NME3 plays an important role in peroxisome division in a manner dependent on its NDP kinase activity (4).

References:

1. Honsho, M. et al. *Front. Cell Dev. Biol.* 8: e855 (2020).
2. Okumoto, K. et al. *eLife* 9: e55896 (2020).
3. Imoto, Y. et al. *Nat. Commun.* 9: e4634 (2018).
4. Honsho, M. et al. *Int. J. Mol. Sci.* 21: e8040 (2020).

Lecture 2

Peroxisome: an essential organelle for neural development and memory function

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The peroxisome is a subcellular organelle essential for various metabolic pathways, such as biosynthesis of plasmalogens, β-oxidation of very-long-chain fatty acids, and degradation of hydrogen peroxide. The patients with peroxisome biogenesis disorders (PBDs) manifest severe neurodegenerative symptoms in the central nervous system (CNS), including disturbance of cortical laminar structure and abnormal cerebellar development. However, the pathogenic mechanisms underlying PBDs remain largely unknown.

To investigate how peroxisome deficiency causes malformation of CNS in the patients with PBDs, we recently established a PBD model mouse defective in Pex14, a key peroxin in the peroxisomal matrix import machinery, termed *Pex14*$^{\Delta C/\Delta C}$ mouse. Malformation of cerebellum is observed in the *Pex14*$^{\Delta C/\Delta C}$ mouse, especially the defect of Purkinje cell arborization is evident. Brain-derived neurotrophic factor (BDNF) is elevated in the cerebellum of this mutant mouse together with the upregulation of TrkB-T1, a dominant-negative isoform of the BDNF receptor. Downstream of BDNF-TrkB signaling pathway is also attenuated. Therefore, peroxisome deficiency induces the dysregulation of the BDNF-TrkB pathway, giving rise to the malformation of the cerebellum in PBDs.

We also examined the role of peroxisomes in astrocytic functions by using primary co-culture system. From a peroxisome-deficient astrocytic cell line, secretion of BDNF is elevated, resulting in the promotion of axonal collaterals of co-cultured primary hippocampus neurons. We show that the cytosolic reductive condition caused by a mislocalized catalase, but not the defects of peroxisomal β-oxidation and plasmalogen biosynthesis, is responsible for the upregulation of BDNF.

Moreover, we investigated the peroxisomal functions in adulthood brain using tamoxifen-inducible conditional *Pex2*-knockout mouse. Peroxisome deficiency in the conditional *Pex2*-knockout adult mouse notably causes memory disturbance and upregulations of BDNF and TrkB-T1 in hippocampus. Therefore, peroxisome deficiency results in the dysfunction of hippocampal circuit through the attenuation of BDNF signaling.

Taken together, peroxisome deficiency impairs BDNF-TrkB signaling, giving rise to abnormal CNS development and neurological deficits.

Lecture 3

Free radical scavenging ability of plasmalogen by ESR and neuroprotective effects of scallop-derived plasmalogen in an ischemic stroke mouse model

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Plasmalogen (Plas) has both anti-oxidative stress and anti-inflammation activities, but its exact free radical scavenging ability has not been reported in *in-vitro* studies. On the other hand, its efficacy has not been investigated in ischemic stroke models where oxidative stress accelerates the pathophysiological progression. Therefore, in the present study, we assessed the scavenging effects of scallop (Hotate) and ascidian (Hoya) – derived Plas on methyl radical ($\cdot$CH3), hydroxyl radical ($\cdot$OH), and superoxide anion (O^{2-}) using electron spin resonance (ESR) in *in-vitro* study and the neuroprotective effects of Plas in ischemic stroke using a transient middle cerebral occlusion (tMCAO) mouse model. Our results showed that Hotate and Hoya-derived Plas suppressed 3 free radicals in relative to the control group with a small dose-dependent manner in H_2O_2/NaOH/DMSO chemical reaction system and protected experimental mice from ischemia/reperfusion injury via the suppression of oxidative stress, which provides a potential of Plas in preventing and treating oxidative stress-associated neurological diseases.

Lecture 4

Stress and inflammation reduced plasmalogen in brain microglial cells

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Neuroinflammation characterized by activation of glial cells is common in neurodegenerative diseases. Although the reduction of ether-type glycerophospholipids, plasmalogens (Pls), in the brain is found in Alzheimer's disease (AD) patients, the mechanism of the reduction and the effect on neuroinflammation remained unknown. We found for the first time that inflammatory stimuli reduced Pls in murine glial cells via NF-κB activation, which then downregulated a Pls-synthesizing enzyme, glycerone phosphate O-acyltransferase (Gnpat) through the increased c-Myc recruitment onto the Gnpat promoter. We also found that systemic injection of lipopolysaccharide, aging, and chronic restraint stress reduced brain Pls in mice, which were associated with glial NF-κB activation, an increase in c-Myc expression, and downregulation of Gnpat. Interestingly, the reduction of Pls contents in the murine cortex itself could increase the activated phenotype of microglial cells and the expression of proinflammatory cytokines, suggesting further acceleration of neuroinflammation by reduction of brain Pls. A similar mechanism of Gnpat reduction was also found in human cell lines, triple-transgenic AD mouse brain, and postmortem human AD brain tissues. These findings suggest a novel mechanism of neuroinflammation that may explain the progression of AD and could help us to explore preventive and therapeutic strategies to treat neurodegenerative diseases.

Lecture 5

The effect of plasmalogen on TNF-α and ICAM-1 expression by periodontal bacteria-derived lipopolysaccharide

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Periodontal disease is characterized by the chronic inflammation of periodontal tissues, which is mainly caused by the infection of gram-negative bacteria. Severe periodontal disease demonstrates the extensive loss of tooth-supporting tissues and results in the tooth extraction. Periodontal disease is now the primary reason of tooth extraction in Japan. Moreover, it has been revealed that periodontal disease and various systemic diseases are related each other, including diabetes mellitus and cerebrovascular disease, indicating the importance of periodontal disease treatment in terms of systemic health. The aim of this study was to investigate the effect of plasmalogen on inflammation-related gene expression including tumor necrosis factor–α (TNF-α) and intercellular adhesion molecule-1 (ICAM-1), which was elicited by periodontal bacteria lipopolysaccharide (LPS) *in vitro*.

Human monocytes (THP-1) and mouse endothelial cells (ms-1) were used in this study. Cells were stimulated with LPS from periodontal bacteria, *Porphyromonas Gingivalis (P.g.).* Gene expression of TNF-α and ICAM-1 was determined by real-time PCR. Protein level of ICAM-1 was examined using flow cytometry analysis.

LPS from *P.g.* (*P.g.*-LPS) induced gene expression of TNF-α in THP-1 and ICAM-1 in ms-1. When the cells were pre-treated with various doses of plasmalogen before *P.g.*-LPS stimulation, TNF-α expression in THP-1 and ICAM-1 expression in ms-1 were reduced in accordance with plansmalogen concentration. Flow cytometry analysis demonstrated that the protein level of ICAM-1 in ms-1 was also inhibited by exogenous addition of plasmalogen.

These data suggested the potential of plasmalogen to inhibit the chronic inflammation of periodontal disease through the regulation of TNF-α and ICAM-1 expression. Findings of this study may provide useful information for new therapeutic intervention of periodontal disease using plasmalogen.

Lecture 6

Change in phospholipid composition of erythrocyte membrane in chronic inflammatory disease: a decrease of ethanolamine plasmalogen and no change of phosphatidyl ethanolamine

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Alzheimer’s disease, Parkinson’s disease and coronary arteriosclerosis are all considered as chronic inflammatory diseases although they are different diseases. In these diseases, plasmalogen levels are decreased in the peripheral blood, and their change patterns in phospholipid composition of the erythrocyte membrane are similar to one another. To be specific, ethanolamine plasmalogen (PlsPE) and phosphatidylcholine (PC) are decreased whereas phosphatidyl ethanolamine (PE) remains unchanged. Therefore, it is possible that PlsPE and PE are present at different locations in the erythrocyte membrane. The cell membrane contains lipid rafts, which are clusters of lipids and are rich in plasmalogens. Some studies reported that lipid rafts are involved in both intracellular and extracellular singling. It is also reported that G-protein coupled receptors (GPCR) reside in lipid rafts and that plasmalogens act on cells through GPCR. Considering these reports, decreased PlsPE and unchanged PE may suggest that plasmalogens residing in lipid rafts are reduced in chronic inflammatory diseases and that major changes are made in lipid rafts. Such changes in the erythrocyte membrane might also reflect changes in the cell membranes of other tissues including neural cells or coronary artery endothelial cells.

Lecture 7

Effects of plasmalogen on psycho-behavioral conditions of college athletes

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Background: Plasmalogen supplementation has been shown to improve cognitive function and neuro-degenerative pathology. Plasmalogens may also be potentially beneficial with respect to psycho-behavioral aspects. We explored the effects of scallop-derived purified plasmalogen on psycho-behavioral conditions in a randomized placebo-controlled trial of college athletes in Japan.

Methods: Eligible subjects were male unmarried students aged 18–22 years who belonged to university athletic clubs. They were randomly allocated to either plasmalogen (2 mg per day) or placebo treatment of 4 weeks. The primary outcome was the Total Mood Disturbance (TMD) T-score of the Profile of Mood States (POMS) 2–Adult Short. The secondary outcomes included the 7 individual scales of POMS 2 and other psycho-behavioral measures. The trial was registered at the Japan Registry of Clinical Trials (CRB7180004).

Results: A total of 42 subjects participated in the trial and completed the 4-week treatment. The TMD T-score showed no measurable difference in the change from baseline between the two groups. The anger-hostility and fatigue-inertia scores decreased substantially in plasmalogen treatment, but not in placebo treatment. The decreases were statistically significantly greater in plasmalogen than in placebo ($P = 0.04$ for anger-hostility and $P = 0.01$ for fatigue-inertia). While the vigor-activity score also showed a statistically significant difference in the 4-week change between the two groups ($P = 0.02$), the score rather decreased in plasmalogen treatment. Performance in the Uchida-Kraepelin test increased in the two groups. An increased performance in the plasmalogen group was more notable in the middle and toward the end of the 10-minute task.

Conclusion: Plasmalogen exerted favorable effects on specific components of mood status, but not on the overall mood status. Plasmalogen may mitigate anger-hostility and fatigue-inertia mood states and also may be beneficial at least partially in work performance.