EURON-Japan double degree program

International Workshop

New Developments applied to Neuroscience

January 25-26, 2017

Place: Kyoto Prefectural University of Medicine
Kitayama campus (Inamori Bldg) 211 room

Key note speakers:
Hiroshi Kiyama (Nagoya University)
Ryosuke Takahashi (Kyoto University)

President: Toshiki Mizuno (Kyoto Pref. Univ. Med)
Director of the EURON-Japan DDP program: Harry M.W. Steinbusch (Maastricht University)

Secretary general: Katsuhiko Ono (Kyoto Pref. Univ. Med)
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PROGRAM

WEDNESDAY, January 25

Opening remark

Toshikazu Yoshikawa (president of KPUM)

9:10~9:40 **Masaki Sone** (Faculty of Science, Toho University)

Effect of specific inhibition of synaptic delivery of APP by loss-of-function of *yata* in the *Drosophila* Alzheimer's disease model

9:40~10:10 **Christophe Lefebvre** (Lille University)

The medicinal leech as a model of dialog between resident microglia and neurons in a context of neuroprotection

10:10~1040 **Takashi Kasai** (Department of Neurology, Research Institute for Geriatrics, Kyoto Prefectural University of Medicine)

Dementia in Down syndrome: Down syndrome in Dementia.

11:00~11:30 **Bart PF Rutten** (Neuroscientist and Psychiatrist; Head of the Division of Neuroscience, School for Mental Health and Neuroscience, Maastricht University Medical Centre)

Neuroepigenetics in mental health and illness: from discovery using longitudinal studies to experimental neuroscience

11:30~12:00 **Paole Mellini** (Graduate School of Medical Science, Kyoto Prefectural University of Medicine)

From NCO-141 to PMA02-073, molecular design behind the discovery of potent SIRT2 selective inhibitors with antidepressant-like and neurite outgrowth activities

Keynote speaker

13:15~14:25 **Hiroshi Kiyama** (Department of Functional Anatomy & Neuroscience, Nagoya University, Graduate School of Medicine)

A new horizon in nerve regeneration research -Consequences of interactions between neuron and non-neuronal cells in nerve regeneration -

14:40~15:10 **Hitoshi Gotoh** (Department of Biology, Kyoto Prefectural University of Medicine)

Glycogen metabolism in neural stem cell regulation

15:10~15:40 **Fumika Nanto** (Department of Orgain Anatomy, Tohoku University Gradulate School of Medicine.)

Evaluating therapeutic effects of Mitochonic acid 5 on mitochondrial disease in a mouse model of Leigh syndrome

15:40~16:10 **JSH Vles** (Department of Neurology, Maastricht University Medical Centre, Maastricht, the Netherlands)

DUCHENNE MUSCULAR DYSTROPHY, COGNITIVE DEFICITS & EPILEPSY: EVIDENCE FOR A TRIANGULAR ENCEPHALOPATHY

THURSDAY, January 26

9:00~9:30 **Yoshiki Tsuchiya** (Department of Physiology and Systems Bioscience, Kyoto Prefectural University of Medicine)

Disruption of MeCP2 attenuates circadian rhythm in CRISPR/Cas9-based Rett syndrome model mouse

9:30~10:00 **Boris W. Kramer** (Maastricht University Medical Center)

CELL-BASED THERAPY FOR HYPOXIC-ISCHEMIC INJURY IN THE PRETERM BRAIN

10:20~10:50 **Katsutoshi Taguchi** (Department of Anatomy and Neurobiology, Graduate School of Medical Science)

Differential expression profiles of α-synuclein, a key molecule in Parkinson's disease and dementia with Lewy bodies

10:50~11:20 **Takahiro Fujimoto** (Department of Pathology and Applied Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine)

Degradation mechanism and molecular function of brain-type dystrophin, Dp71.

11:20~11:50 **Harry WM Steinbusch** (Dept. Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University Medical Centre)

Epigenetic changes and Brainstem Dysfunction in Neuropsychiatric Disorders – AD/PD/Anx

Keynote speaker

13:00~14:10 **Ryosuke Takahashi (** Department of Neurology, Kyoto University Graduate School of Medicine**)**

Animal models of Parkinson's Disease

14:30~15:00 **Noriko Osumi** (Department of Developmental Neuroscience, Tohoku University School of Medicine)

Crosstalk between a genetic risk and paternal aging in the mouse model for neurodevelopmental diseases

15:00~15:30 **Kazuhiko Yanai** (Department of Pharmacology, Tohoku University School of Medicine)

Advances in the Development of Tau PET Radiotracers and Their Clinical Applications

16:00 Ali Jahanshah

(The title will be available at the registration desk)

Keynote Speakers

Keynote speaker

A new horizon in nerve regeneration research -Consequences of interactions between neuron and non-neuronal cells in nerve regeneration -

Hiroshi Kiyama
Department of Functional Anatomy & Neuroscience, Nagoya University, Graduate School of Medicine

Proper nerve regeneration is allowed under spatiotemporally restricted environment. Amongst nervous systems peripheral nervous system (PNS) could provide injured axons with a suitable environment for regeneration. Exploring the mechanisms underlying this higher potential seen in PNS would be useful for therapeutic strategies of injured CNS repair. During last two decades we have identified several molecules, which are involved in nerve regeneration, and revealed biological and pathological consequences of those molecules. Along with recent advance of molecular and genetic methods evidence highlights that positive interactions between injured neurons and glial cells would be critical for damaged-neurons to survive and regenerate, whereas the failure of the communication or a devastating signal released from surrounding non-neuronal cells causes exacerbation of neuron damages. Among non-neuronal cells, microglia in brain and Schwann cells and macrophage in periphery play critical roles in survival and regeneration of injured neurons. In this talk I would like to introduce involvement of some molecules, which are recently identified as critical molecules for the neuron-glia interactions. I also address recent methodological advances in nerve regeneration studies: a manipulation of microglial morphology and function, and a transgenic strategy in which a specific gene expression is regulated specifically in injured neurons. By exploring new molecular mechanisms underlying nerve regeneration using the latest technologies, a field so-called the nerve regeneration biology, which is still in the process of being formed, would be established shortly.

Keynote speaker

Animal models of Parkinson's Disease

Ryosuke Takahashi
Department of Neurology, Kyoto University Graduate School of Medicine

Parkinson's disease (PD) is a second common neurodegenerative disorders among elderly people. PD is a progressive movement disorder characterized by tremor, rigidity and bradykinesia. The pathological hallmark of PD is selective degeneration of dopaminergic neurons in the substantia nigra accompanied by formation of cytoplasmic aggregates termed Lewy bodies. Lewy bodies are composed of α-synuclein, a brain-specific 140 amino-acid protein. Missense or multiplication mutations in the *α-synuclein* gene leads to familial form of PD, suggesting that α-synuclein plays a key role in the pathogenesis of PD. The most robust risk gene for idiopathic PD is the *GBA* gene encoding glucocerebrosidase, which is responsible for Gaucher disease. To elucidate the relationship between *GBA* and PD, we have created *GBA* deficient medaka fish. *GBA* deficient medaka fish displayed abnormal movement accompanied by massive neuroinflammation, non-selective neuronal death and α-synuclein aggregate formation, suggesting that loss of GBA function contributes to Lewy body pathology..

Clinicopathological studies indicate that Lewy body pathology in PD brain is initiated several years before the onset of motor symptoms and correlates with non-motor signs and symptoms in the prodromal stage. We have created a prodromal PD model based on a) injection of α-synuclein preformed fibrils (PFF) into mouse brain and b) α-synuclein bacterial articficial chromosome (BAC) transgenic mouse crossbred with *GBA* mutant mouse. When α-synuclein PFF is injected into the olfactory bulb, they form aggregates spreading through neural connections between olfactory structures. In the double mutant mice, we observed a similar α-synuclein aggregates distribution pattern. These prodromal PD mouse models may represent useful tools to develop a disease modifying therapy for PD.

Faculty session

Effect of specific inhibition of synaptic delivery of APP by loss-of-function of *yata* in the *Drosophila* Alzheimer's disease model.

Masaki Sone

Faculty of Science, Toho University

APP (Amyloid Precursor Protein) is a causative molecule of Alzheimer's disease. APP is synthesized in the cell bodies of neurons and is transported towards synaptic terminals. We have identified the *Drosophila yata* gene as a molecule that is required for the intracellular trafficking of APPL that is the *Drosophila* homologue of APP. In the null mutants of *yata*, APPL is accumulated aberrantly in cell bodies. *yata* mutants also show phenotypes such as shortened lifespan, progressive brain volume reduction, and developmental abnormalities. Loss of human and murine orthologues of *yata* causes neurodegeneration, indicating that *yata* is an evolutionally conserved molecule that is required to prevent neurodegeneration. Furthermore, it has been shown that there is a genetic interaction between *yata* and *Appl*. In this study, we examined whether *yata* mutation affects pathology of the *Drosophila* model of Alzheimer's disease. We induced expression of the human APP in the third instar larval motor neurons that is a model of glutamatergic neurons. Immunostaining with anti-APP antibody revealed the localization of APP in the neuromuscular synaptic boutons, whereas introduction of *yata* mutation resulted in the decreased synaptic delivery of APP. On the other hand, synaptic expression of other synaptic proteins such as Synaptotagmin and Cysteine string protein was not altered. Previous studies have shown that overexpression of human or *Drosophila* APP causes excessive formation of the small-sized synaptic boutons called “satellite boutons”. In our study, expression of APP caused increase of satellite boutons, which was suppressed by the *yata* mutation. Thus our data identified *yata* as a molecule that can modify synaptic pathology caused by APP.

The medicinal leech as a model of dialog between resident microglia and neurons in a context of neuroprotection

Raffo Antonella, Arab Tanina, Lemaire Quentin, Van Camp Christelle, Sautière Pierre-Eric, Le Marrec-Croq Françoise, Vizioli Jacopo, Wisztorski Maxence, Gimeno Jean-Pascal, Salzet Michel and Lefebvre Christophe.

The leech *Hirudo medicinalis* is an interesting model in neurobiology because it undergoes synapse regeneration as a natural and functional process. Within 24 hours following a lesion in the CNS, resident microglial cells migrate at the injury site. Microglia are essential for the natural repair of injured axons in leech since we know that the inhibition of their accumulation induces a significant reduction in axonal sprouting. This model allows the study of resident microglia exclusively in order to elucidate the crosstalk between activated microglia and damaged neurons. Our results showed that the microglial mobility involves specific factors including leech homologs for C1q, EMAPII and/or Interleukin-16. In addition the early phase of leech microglial recruitment to the lesion would be driven by neuroprotective cytokine TGF-□ leading to the neuronal survival. A massive production of extracellular vesicles including exosomes and ectosomes has been observed from recruited microglia allowing a crosstalk with injured axons. These microglial vesicles are collected, purified and analyzed to identify their RNA and protein contents. These characterizations are crucial since we know that microglial vesicles alone can mediate a part of neurite outgrowth instead of entire microglial cells. Thus some strategies are developed in the leech CNS to focus on neuroprotective microglia subpopulations and their vesicular products allowing the initiation of a nerve repair.

Keywords: extracellular vesicles, microglia, neuroinflammation, nerve repair.

Dementia in Down syndrome: Down syndrome in Dementia.

Takashi Kasai[1*], Takahiko Tokuda[12], Takuma Ohmichi[1], Ryotaro Ishii[1], Harutsugu Tatebe[3], Toshiki Mizuno[1]

1 Department of Neurology, Research Institute for Geriatrics, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan
2 Department of Molecular Pathobiology of Brain Diseases, Research Institute for Geriatrics, Kyoto Prefectural University of Medicine
3 Department of Zaitaku (Homecare) Medicine, Kyoto Prefectural University of Medicine

Down syndrome is famous as the most common cause of intellectual disabilities in pediatric field, while problems in adult Down syndrome have been little recognized by general physicians for decades. However, we can no longer ignore adult Down syndrome because their average life span has been dramatically improved owing to progress of perioperative managements in pediatric surgery. For neurologists, early dementia in Down syndrome would be the most important and interesting.

It has been reported that the disease is a great risk of dementia over 40 years old. Its mechanism is believed to be due to overexpression of amyloid precursor protein (*APP*) gene that is coded on chromosome 21. Actually, aged Down's brains have abundant Alzheimer's pathological changes that are senile plaques and neurofibrillary tangles. In this context, the adults with Down syndrome can be said as a group strongly vulnerable for Alzheimer's disease.

Actual situation of adult Down syndrome has been little known in Japan, thus we have been investigating them for several years. Our current purpose is to establish a dementia scale suitable for them. But, the final goal is set to develop objective fluid biomarkers reflecting Alzheimer's pathology in this high-risk group. We expect that such biomarkers would one day lead to effective interventions for people not only with Down syndrome but with preclinical Alzheimer disease.

In the workshop, we will show our recent data.

Neuroepigenetics in mental health and illness: from discovery using longitudinal studies to experimental neuroscience

Bart PF Rutten, MD PhD

Neuroscientist and Psychiatrist; Head of the Division of Neuroscience, School for Mental Health and Neuroscience, Maastricht University Medical Centre, Maastricht, the Netherlands

Identifying the mechanisms underlying differential sensitivity to environmental exposures in relation to the onset and course of mental disorders is a key goal for current interdisciplinary neuroscience. Epigenetic mechanisms have been proposed to be centrally involved in mediating the impact of environmental exposures on brain functioning and mental health. The scientific frontiers in neuroepigenetic research are currently challenged by difficulties in capturing robust and relevant environmental exposures in a longitudinal manner, which would allow for investigations of changes over time. Neuroepigenetic research is furthermore challenged by the very limited accessibility of the primary tissue type of interest, i.e. the brain, as well as by the complexity of the numerous and dynamic cell populations that are present in a given brain region. The field is therefore in search for innovative ways that enable scientists to tap into the epigenetic mechanisms underlying brain (dys) functioning in the aftermath of environmental exposure. Our recent research has discovered that distinct blood-based epigenetic profiles can be linked to differential sensitivity to environmental exposures in relation to longitudinal exposure-related alterations in mental health. This was done by performing human studies on longitudinal changes in epigenetic profiles as measured in blood samples over the period of exposure to robust environmental factors, i.e. 1) combat trauma exposure during military deployment, 2) the administration of (therapeutic) seizures to the brain (ECT) for patients with treatment-resistant depression. The studies consisted of a multistep validation and replication cycle consisting of technical validation, replication in independent cohorts, methylation-mRNA analyses, bioinformatics, human blood-brain correlations and cross-species validation. The results of these translational studies enhance our understanding on how epigenetic mechanisms may mediate sensitivity to environmental factors in mental health.

From NCO-141 to PMA02-073, molecular design behind the discovery of potent SIRT2 selective inhibitors with antidepressant-like and neurite outgrowth activities

Paolo Mellini,[a] Yukihiro Itoh,[a] Ying Li,[a] Natsuko Tokuda,[b] Rosa M. Tordera,[c] and Takayoshi Suzuki[a, d]

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The NAD^+ dependent deacetylase SIRT2 a member of sirtuin family (SIRT1-7) has gained growing attention for its involvement in various cellular processes, such as chromatin condensation, DNA repair, cell cycle, apoptosis, metabolism and aging. Activities strongly linked with the ability of SIRT2 to deacetylate histone and non-histone proteins. Growing body information identified SIRT2 as potential drug target of neurodegenerative disease and depressive disorders. SIRT2 inhibition was found effective in reducing α-synuclein toxicity, brain cholesterol levels, increasing brain derived neurotrophic factor (BDNF) and neurite outgrowth. Nowadays potent and isotype selective SIRT2 inhibitors are urgently needed. [1, 2, 3]

In 2012 we performed an extensive structure activity relationship investigation on our 2-anilinobenzamide prototype that led the identification of NCO-141 a drug-like selective SIRT2 inhibitor (IC_{50} = 0.57μM) [4]. Recent outputs, thanks to joint collaboration with Dr. Tordera evidenced that subchronic treatment of C57BI6 mice with NCO-141 increased the levels of both GluN2A and GluN2B NMDA receptors subunits as well serotonin levels in PFC. In addition, chronic treatment reverted anhedonia and social avoidance induced by chronic mild stress. Thus, highlighting the therapeutic potential of SIRT2 as new target for the treatment of major depression.

In order to improve the inhibitor potency, we solved X-ray crystal structure of SIRT2 in complex with 33a (a derivative of NCO-141). Then, following a fragment based approach to target SIRT2 substrate binding site we identified PMA02-073 a submicromolar (IC_{50} = 0.056μM) mechanism based inhibitor with a remarkable activity in inducing neurite outgrowth in N2a cells.

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Glycogen metabolism in neural stem cell regulation

Hitoshi Gotoh

Department of Biology, Kyoto Prefectural University of Medicine

The energy metabolism regulates various aspects of development such as cell proliferation, differentiation, or survival. The regulatory mechanism of neural development by energy metabolism remains largely unknown. Glycogen is an energy source that is formed by glucose and is reported to regulate the proliferation of cancer cell line as an essential energy source (Favaro et al., Cell Metab 2012). Glycogen is present in radial glial cells, neural stem cell (NSC) in the central nervous system. However, its significance has not been elucidated.

I found that glycogen was present in early embryonic cerebral cortex (E12.5) and the amount of glycogen increases at later embryonic stages (E18.5). Glycogen was mainly present in Pax6+ neural stem cells but not in Tuj1+ neurons or Tbr2+ intermediate neuronal progenitors. Then, I focused on glycogenin (Gyg) protein, an enzyme essential for glycogen synthesis, to analyze the role of glycogen in neural stem cell proliferation and differentiation. I introduced shRNA against Gyg by in utero electroporation and found that the neural stem cell prematurely exited the cell cycle and differentiated into neuron.

These results suggest that the glycogen energy metabolism pathway is important for the maintenance of neural stem cells, thus regulating their development at perinatal stage.

Evaluating therapeutic effects of Mitochonic acid 5 on mitochondrial disease in a mouse model of Leigh syndrome

Fumika Nanto[1, 2], Angelina Misiou[1], Takaaki Abe[2], Yuji Owada[1]

1 Department of Orgain Anatomy, Tohoku University Gradulate School of Medicine.

2 Department of Medical science, Tohoku University Gradulate School of Biomedical Engineering

Mitochondrial diseases are largely untreatable because of the lack of effective drugs. We found that mitochonic acid 5 (MA-5: 4-(2,4-difluorophenyl)-2-(1H-indol-3-yl)-4-oxobutanoic acid) targets the mitochondrial protein mitofilin at the crista junction of inner membrane and changes the conformation of the mitochondrial inner membrane organizing system, increasing ATP and cell survival. MA-5 could provide a completely new direction for searching drugs for mitochondrial diseases.

We previously reported that MA-5 improved mitochondrial COX activity and respiration in damaged heart and kidney in the mitochondrial DNA defect disease model “Mitomice” and also prolonged the life span. However, compared to disease model mice generated by manipulation of the nuclear genome, Mitomice are not always suitable for large-scale studies. Recently, a constitutive knockout for the complex I subunit component NADH dehydrogenase Fe-S protein 4 (Ndufs4) was reported. Ndufs4 knockout mice manifested encephalomyopathy including a retarded growth rate, lethargy, loss of motor skill, blindness, and elevated serum lactate, causing a human Leigh-like phenotype.

In this study, to confirm the effectiveness of MA-5, we will use the Ndufs4 knockout mouse model to examine the life span, mitochondrial respiration in heart, skeletal muscle and neural function. Furthermore, we will also measure the serum level of growth differential factor 15 (GDF-15) in the Ndufs4 knockout mouse with or without MA-5 to evaluate the feasibility of GDF-15 as a useful biomarker to predict the effectiveness of MA-5 treatment. Our results will be helpful to establish the non-clinical proof of concept of MA-5 for the upcoming clinical trial review and facilitate the process.

DUCHENNE MUSCULAR DYSTROPHY, COGNITIVE DEFICITS & EPILEPSY: EVIDENCE FOR A TRIANGULAR ENCEPHALOPATHY

Ruben G.F. Hendriksen, Jos G.M. Hendriksen, Marlien W. Aalbers, Sandra Schipper, Govert Hoogland & Johan S.H. Vles

Department of Neurology, Maastricht University Medical Centre, Maastricht, the Netherlands

Duchenne muscular dystrophy (DMD) is a severe X-chromosomal linked genetic disorder that is primarily characterised by progressive muscular degeneration (1, 2). The DMD gene is vast; it compromises 0.1% of the human genome, and contains the code for the dystrophin protein. This partly contributes to the fact that DMD is the most common form of muscular dystrophy as it affects approximately 1 in 3,500-5,000 live male births, hence making it the second most common single gene disorder in Western countries (2).

In the eighties of the previous century extremely low levels of the newly discovered full-length isoform (i.e. Dp427) were discovered in the brain (3). In the discussion the authors describe a potential role of dystrophin in mental retardation, as this was seen in approximately 30% of patients with DMD. Now, anno 2017, it is known that males with DMD may not only have intellectual disabilities but also learning disorders, particularly those involving language and reading (4), and neurobehavioural comorbidites (autism spectrum disorder, attention deficit hyperactivity disorder and obsessive compulsive disorder) (5, 6), which is supposed to be related to the absence of dystrophin in specific brain regions.

In the decades to follow it turned out that dystrophin seemed primarily located in three structures within the human brain: hippocampus, prefrontal cortex and cerebellum. Likewise, three dystrophin protein (Dp) isoforms have been identified in the brain: i) Dp427, localized post-synaptically; ii) Dp140, a minor component found in brain extracts, which seems amongst others linked to microvascular glia cells; and iii) Dp71, the most abundant expressed isoform, that is found in both neurons and glia cells. The functions of these proteins vary; that is, Dp427 clusters gamma-aminobutyric acid type A ($GABA_A$) receptors at the post-synaptic membrane. The function of Dp140 is, however, unknown. Its expression seems limited to the stages of foetal development where it appears to be developmentally regulated. Later in life, after this initial high expression phase in prenatal stages, it is found at very low levels at the same locations as Dp71, i.e. vascular endothelium and astroglial endfeet processes. Aside from the clustering of water and potassium channels in the astrocyte membrane, the expression of Dp71 at such perivascular endfeet suggests amongst others a role in blood brain barrier functioning (7)

Intriguingly, the brain pathology in DMD appears not to be limited to the abovementioned spectrum of cognitive- and behavioural deficits, thereby further complicating the supposed role of dystrophin in the brain. That is, clinical, theoretical and molecular evidence is indicating a possible relationship between a lack of dystrophin and hyperexcitation, a process characterized by tremendous excitatory brain signalling by means of the spread of large neuronal currents. This can subsequently result in seizures.

The evidence for a potential relationship between epilepsy and DMD has only recently been slightly, yet slowly emerging. In 1997 the first clinical epidemiological study on the increased prevalence of epilepsy in a muscular dystrophy population appeared, and was followed by a small study in 2004 and, finally, by a rather elaborative one in 2013. The prevalence of

epilepsy ranged from 3,1% to 12,3% (8-10). The subsequent average epilepsy prevalence based on these three studies is 5.3% (N = 477). In contrast, in the normal population this is approximately 0.5-1%. In addition, our research group recently also studied the prevalence of epilepsy in an international population of 228 boys and men with DMD and found a prevalence of 7.9% (13), thereby confirming the increased prevalence. It is furthermore particularly intriguing to realise that both DMD, as summarized above, and epilepsy, are associated with cognitive and behavioural deficits. Similarly, we found a statistically increased prevalence of ADHD, OCD and anxiety disorders in DMD males with epilepsy compared to those without epilepsy. This may reflect an underlying (triangular) relationship. The subsequent question that emerges provides the rationale for the title of this chapter: might there be an underlying mechanism or could we, alternatively, perhaps speak of a new triad within (paediatric) neurology?

A possible underlying mechanism for the increased prevalence of epilepsy (and cognitive/behavioural deficits) in DMD has been extensively studied and described by our research group. In summary, if Dp71 is absent, potassium and water channels function less well, which impairs, via different mechanisms, the extracellular potassium buffering capacity. Increased potassium concentrations may lead to hyperexcitation because of the influence on action potential propagation. Next, because Dp71 also appears to have a function in the blood-brain barrier, its absence may result in leaky blood vessels, which can expose the brain to serum components, hence giving rise to seizures. Dp427, on the other hand, clusters $GABA_A$ receptors post-synaptically. Aberrant or defective clustering of the most important inhibitory neurotransmitter receptor system results in less inhibitory (counterbalance) effects and hence potentially hyperexcitable brain networks (7). In fact, it has been known for quite some time that such defective GABA-ergic mechanisms could give rise to epilepsy. Similarly, it has been postulated more than once that this could also constitute the cause of the neuropsychological problems often observed in DMD.

Finally, in order to (indirectly) test the abovementioned hypothesis we studied dystrophin distribution and expression in rat- and human brain tissue by means of immunohistochemistry and Western blot analysis respectively. In rat hippocampus and cerebellum there were no differences between epileptic and control animals. However, in human hippocampus, Dp427 levels were about 60% higher in temporal lobe epilepsy patients compared to post-mortem controls. This upregulation may indicate a compensatory mechanism in the chronic epileptic human brain to occur over time, as more Dp427 implies more GABA-input and hence a possible restoration of the seizure threshold. Since DMD patients are not able to make use of such potential mechanisms their seizure threshold may be lower, thus possibly clarifying the increased prevalence of epilepsy (11).

However, as shown here, not much research on this specific and rather new association has been performed. Consequently, in daily, clinical practice, awareness seems to be lacking. This could be attributed to a lack of insight in the fact that DMD could additionally be considered a brain disorder, even though the scientific interest for this specific matter has substantially increased during the past decade(s). Additionally it should be noted that a diagnosis of epilepsy might be challenged by the wide spectrum of possible associated features seen in DMD (e.g. ADD), also referred to as diagnostic overshadowing (12). Because of the huge impact of epileptic seizures on the quality of life, even more in males with a severe and progressive disorder such as DMD, more research is required. Not least as this may generally also give insight in the pathogenesis of epilepsy, cognition or into the possible existence of a common neuropaediatric triad as suggested here.

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Disruption of MeCP2 attenuates circadian rhythm in CRISPR/Cas9-based Rett syndrome model mouse

Yoshiki Tsuchiya[1], Yoichi Minami[1], Yasuhiro Umemura[1], Hitomi Watanabe[2], Daisuke Ono[3], Wataru Nakamura[4], Tomoyuki Takahashi[5], Sato Honma[3], Gen Kondoh[2], Toyojiro Matsuishi[5], Kazuhiro Yagita[1]

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Methyl-CpG-binding protein 2 (*Mecp2*) is an X-linked gene encoding a methylated DNA binding nuclear protein which regulates transcriptional activity. The mutation of *MECP2* in humans is associated with Rett syndrome (RTT), a neurodevelopmental disorder. RTT patients frequently exhibit abnormal sleep patterns and sleep-associated problems, in addition to autistic symptoms, raising the possibility of circadian clock dysfunction in RTT.

In this study, we investigated circadian clock function in *Mecp2*-deficient mice. We successfully generated both male and female *Mecp2*-deficient mice on the wild-type C57BL/6 background and $PER2^{Luciferase}$ ($PER2^{Luc}$) knock-in background by utilizing the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. Generated *Mecp2*-deficient mice recapitulated reduced activity in mouse models of RTT, and their activity rhythms were diminished in constant dark conditions. Furthermore, real-time bioluminescence imaging revealed that the amplitude of $PER2^{Luc}$ driven circadian oscillation was significantly attenuated in *Mecp2*-deficient SCN neurons.

Together, these results demonstrate that *Mecp2* deficiency abrogates the circadian pacemaking ability of the SCN, which may be a therapeutic target to treat the sleep problems of RTT patients.

CELL-BASED THERAPY FOR HYPOXIC-ISCHEMIC INJURY IN THE PRETERM BRAIN

Boris W. Kramer

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Background and aims

Preterm infants are at risk for hypoxic-ischemic encephalopathy. Unfortunately, no therapy exists to treat the brain injury in this patient population. The aims of this translational study were to assess the neuroprotective effect of exogenous administration of mesenchymal stem cells (MSC) and extracellular vesicles from MSCs, the mobilization of endogenous hematopoietic stem cells in the preterm brain after global hypoxia-ischemia.

Methods

Instrumented preterm sheep were subjected to global hypoxia-ischemia by 25 minutes of umbilical cord occlusion at a gestational age of 104 (term is 150) days. During a 7 day reperfusion period all vital parameters, including (amplitude-integrated) electroencephalogram, were recorded. At the end of the experiment, the preterm brain was assessed using histology and magnetic resonance imaging (diffusion tensor imaging (DTI)). In three separate experimental set-ups, exogenous MSCs, extracellular vesicles from MSCs or granulocyte-colony stimulating factor (G-CSF) were administered intravenously with the appropriate control groups.

Results

Administration of exogenous MSCs and extracellular vesicles from MSCs reduced cerebral inflammation and white matter injury. MSCs and extracellular vesicles from MSCs induced T-cell tolerance, which was paralleled with diminished mobilization and invasion of these cells in the preterm brain. In addition, MSCs and extracellular vesicles from MSCs established functional improvement, as shown by decreased number of seizures after global hypoxia-ischemia.

Similarly, mobilization of endogenous stem cells using systemic granulocyte-colony stimulating factor (G-CSF) reduced cerebral inflammation and white matter injury. However, G-CSF did not

reduce the number of seizures after global hypoxia-ischemia.

Conclusion

We have shown for the first time in a translational animal model that cell-based therapy (MSCs) and cell-derived therapy (extracellular vesicles from MSCs) is effective in protecting the preterm brain against the cerebral and peripheral inflammatory responses which are involved in the etiology of white matter injury in the preterm brain after global hypoxia-ischemia. Our studies form the basis for future clinical trials studying feasibility of cell-based therapy in preterm infants with hypoxic-ischemic encephalopathy.

Biography

Boris W. Kramer has completed his MD at the University of Tübingen/ Germany, and his P.h.D at the University of Maastricht. Dr. Kramer performed postdoctoral studies at the Cincinnati Children's Hospital/Ohio/USA. He is a neonatologist, professor and the director of pediatric research at Maastricht University Medical Center. He has published more than 235 papers in reputed journals and serving as an editorial board member of international journals.

Differential expression profiles of α-synuclein, a key molecule in Parkinson's disease and dementia with Lewy bodies

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α-Synuclein is a major constituent of Lewy bodies (LB) and Lewy neurites (LN), which are pathological hallmarks of synucleinopathies including Parkinson's disease (PD) and dementia with Lewy bodies. Several missense mutations, as well as duplicate and triplicated regions in α-synuclein gene are responsible for familial PD. Accumulated evidence suggests that abundant intracellular expression of α-synuclein is one of the risk factors for pathological protein-aggregation such as LB and LN. On the other hand, α-synuclein is normally expressed in presynapses and is involved in synaptic function although physiological function of α-synuclein remains to be fully elucidated. Recently, we reported brain region-dependent differential expression of α-synuclein because we think it is important to determine the precise expression profiles of α-synuclein to understand the mechanisms of formation of the pathological protein-aggregation (Taguchi K et al, J Comp Neurol, 2016). This report demonstrated that some neurons in early PD-affected human brain regions express high levels of perikaryal α-synuclein in the mouse brain. Synaptic expression of α-synuclein is mostly accompanied by the expression of vesicular glutamate transporter-1, an excitatory presynaptic marker protein. In contrast, expression of α-synuclein in GABAergic inhibitory synapses is different among brain regions. These results suggest that expression of α-synuclein is regulated in cell-type dependent manner. In this talk, I will present our latest findings including the neuronal differentiation-related roles of α-synuclein in the olfactory bulb and discuss its novel physiological function.

Degradation mechanism and molecular function of brain-type dystrophin, Dp71.

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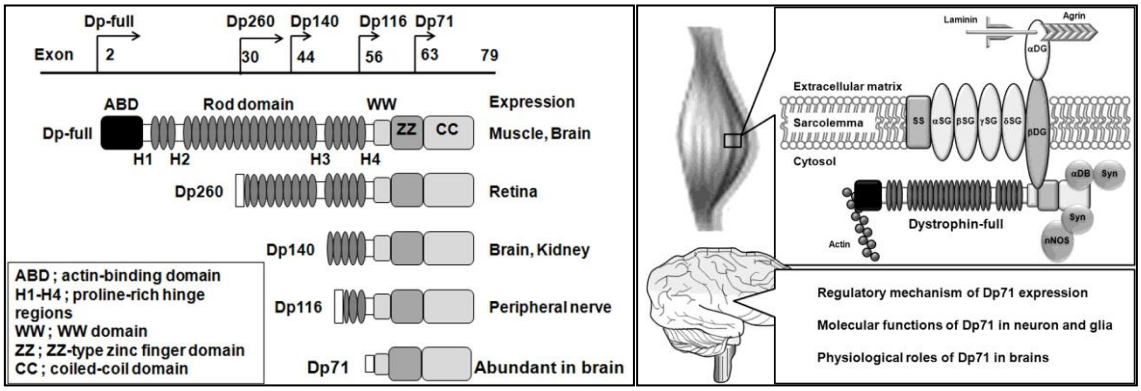
Duchenne muscular dystrophy (DMD) is an X-linked hereditary disease characterized by progressive muscular degeneration. About one third of all DMD patients manifest variable degrees of cognitive impairment. *DMD* gene produces multiple dystrophin (Dp) isoforms due to the presence of several promoters and alternative splicing. Because of complexities in the *DMD* gene expression and in the brain structure and functions, the regulatory mechanisms underlying the cognitive impairment in DMD patients remain to be elucidated. Since Dp71 isoform is most abundant among Dp products in the brain, precise understanding of the regulatory mechanism of its expression and function will unveil the molecular bases of the cognitive impairment in DMD patients.

We herein report a novel mechanism in which Dp71 protein degradation is modulated by post-translational modification. In addition, we provide an insight into a molecular function of Dp71from the perspective of the protein-protein interaction.



Epigenetic changes and Brainstem Dysfunction in Neuropsychiatric Disorders – AD/PD/Anx

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Despite the fundamental role of the brainstem in regulating vital functional abilities such as arousal, breathing, autonomic nervous system activity as well as regulating all higher cerebral functions via neurotransmitter projections systems originating in the brainstem, the role of the brainstem has received relatively little attention in most neuropsychiatric disorders. Besides the dorsal and median raphe nuclei complex comprising mainly serotonin-producing neurons, the brainstem also contains noradrenalin, dopamine and histamine-producing nuclei, i.e. resp. the locus coeruleus, the substantia nigra and the mamillary bodies. The brainstem is furthermore the relay station of afferent and efferent projections between the autonomic nervous system in the peripheral body and higher cerebral brain regions. The current presentation aims to review the neuroanatomy of the brainstem as well as the current status on findings, derived from a wide range of studies using molecular, cellular and imaging technologies, of brainstem involvement in neurodevelopmental (i.e. autism, schizophrenia) and neurodegenerative disorders (Alzheimer's and Parkinson's disease).

Over the past decades, the incidence of age-related, neurological and psychiatric disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), but also depression has considerably increased. Mood disorders are strongly related to the exposure to stress. The hippocampus and other forebrain structures are the apex of the stress hormone control mechanism and damage to them may be one way in which stress hormone secretion escapes from inhibitory control in depression. In turn, stress, probably through toxic effects of glucocorticoids, decreases neurogenesis and cell survival while antidepressants enhance these processes in experimental animals. Therefore, since treatment strategies are not yet available, primary prevention in these age-related and stress related neurological disorders is of importance. As mentioned before most of the focus on neurobiological questions on above mentioned disease are related to forebrain structures since they are often associated with cognitive dysfunction. The brainstem is a highly

neglected brain area in neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's (PD) disease and frontotemporal lobar degeneration. Likewise, despite a long-standing recognition of brainstem involvement, relatively few studies have addressed the exact mechanisms that underlie brainstem autonomic dysfunction. Improved insight in the cellular and molecular characteristics of brainstem function is pivotal to study the developmental origins. As brainstem dysfunction also poses health issues in several other, neurodegenerative, disorders (like AD and PD), progress in these neurological fields will benefit from scientific advancement in the current proposal as well. In the area of depression, several observations have been made in relation to changes in one particular brain structure: the Dorsal Raphe Nucleus (DRN). In addition dysfunction of the cerebellum is also observed in AD and associated with pulmonary deregulation. The DRN is also related in the circuit of stress regulated processes and cognitive events.

In order to gain more information about the underlying mechanisms that may govern the neurodegeneration, e.g. amyloid plaques, neurofibrillary tangles, and impaired synaptic transmission in AD, a rat dissociation culture model was established that allows mimicking certain aspects of our autopsy findings. We observed a similar phenomenon in brains from patients suffering from neurodegenerative disease since this also related to changes in BDNF levels. The ascending projections and multitransmitter nature of the DRN in particular and the brainstem in general stress its role as a key target for AD/PD research and autonomic dysfunction. It also points towards the increased importance and focus of the brainstem as key area in various neurodevelopmental and age-related diseases.

Crosstalk between a genetic risk and paternal aging in the mouse model for neurodevelopmental diseases

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In rodent, advanced paternal age induces some abnormal behaviors, and how paternal aging affect phenotypes of their offspring is an intriguing issue. We previously demonstrated that the offspring derived from old sires showed significant decrease in average number of ultrasonic vocalization (USV) that is known as communicative call of mice. Histological analysis suggested that this trans-generational effect of advanced paternal age had its molecular basis on epigenetic changes during spermatogenesis. Here, we report that paternal *Pax6* mutation accelerates the decrease in USV calls caused by advanced paternal age. To examine the influence of paternal *Pax6* mutation and paternal aging to their offspring, spontaneous *Pax6* mutant (*Sey*) sire mice at each stage of young (3-month-old), middle-aged (6-8-month-old), and old (>12-month-old) were mated with young (3-month-old) wiled-type (WT) females. Their offspring were separated from the dams at postnatal-day 6 and the number of USV calls was measured during 5 minutes separation. Although the number of USV calls was comparable between *Sey* offspring and WT litter when their *Sey* sire was young, Sey offspring derived from middle-age *Sey* sire showed significant decrease in the number of USV calls compared to WT litter. The number of USV calls was decreased in both genotypes of offspring when their *Sey* sire was old. We also found the expression of Pax6 in spermatocyte, suggesting that *Pax6* haploinsufficiency accelerates epigenetic changes in spermatocyte caused by advanced paternal age. We are currently working hard on underlying epigenetics in this model.

Advances in the Development of Tau PET Radiotracers and Their Clinical Applications

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Objective: Recent progress in the development of tau-selective PET tracers enabled non-invasive visualization of neurofibrillary pathology in the human brain. The amount and spatial distribution of tau tracer binding in the brain is closely associated with neurodegeneration and cognitive symptom of dementia. Therefore, tau PET imaging is expected to be useful for tracking disease progression, assessing disease severity, and accurately predicting dementia prognosis. The purpose of this study was to assess the clinical usefulness of THK tau PET tracers.

Methods: Subjects with Alzheimer's disease, mild cognitive impairment and healthy controls (Number of each group is more than 10) underwent [^{18}F]THK-5351 and [^{11}C]PiB PET scans. Standard uptake value ratios between 50-60 minutes post injection for THK-5351 was calculated using the cerebellar cortex as a reference region.

Results: Subjects with mild cognitive impairment showed higher THK retention in the fusiform gyrus, inferior temporal and parietal cortices than healthy control subjects. Patients with Alzheimer's disease showed higher and more extensive neocortical THK retention than subjects with mild cognitive impairment. In some cognitively normal individuals, THK retention was mildly elevated in the inferior temporal area. THK retention in the parahippocampal and fusiform gyrus, inferior temporal and parietal cortices was correlated with clinical severity of dementia.

Conclusion: THK-5351 enables sensitive and selective detection of neurofibrillar pathology in Alzheimer's disease. Tau PET imaging with this tracer could be employed to study longitudinal tau deposition in normal aging and pathological process of Alzheimer's disease.

Student session

Project title: Mechanistic Study of Neurite Outgrowth Induction by Epigenetic Inhibitors

Alexander van der Wiel [1], Ying Li [1], Yukihiro Itoh [1], Toshifumi Tojo [1], Takayoshi Suzuki [1,2]

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Neurodegenerative diseases, such as Alzheimer's disease (AD), form a major health problem and prevalence is expected to rise dramatically in the next years. Effective treatment is still not available and current focus is on the suppression of symptoms rather than treating the underlying mechanisms of disease.

In these neurodegenerative diseases, a characteristic change reported is a decrease in neurite formation, a process which is critically involved in the formation of synapses and hence the normal functioning of the brain. Studies have shown that epigenetic mechanisms, especially histone acetylation status, are crucial in regulating this neurite outgrowth. The balance between histone acetylation and deacetylation determines the expression of several genes involved in neurite formation. In neurodegenerative diseases, this balance is disturbed, favouring a state of deacetylation and hence suppression of gene expression. I therefore hypothesize that administration of pharmacological agents inhibiting histone deacetylation restores the decrease in neurite outgrowth observed in neurodegenerative diseases.

The aim of this research is to identify compounds that have positive effects on neurite outgrowth, and to characterize their properties. In this study, neurons will be treated with histone deacetylase 2 (HDAC2)-selective inhibitors, and the neurite outgrowth activity of the inhibitors will be evaluated. To examine the neurite outgrowth mechanisms of the inhibitors, gene expression analysis and ChIP analysis will be performed.

Knowledge obtained from this project will lead to the identification of novel compounds that can be used as therapeutic agents for neurodegenerative diseases, as the need for effective treatment is higher than ever.

Project title: RNF213 susceptibility for intracranial artery stenosis/ occlusion (ICASO) in CADASIL

W.T.E. Yeung

MA Biomedical Science

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is considered as one of the most common hereditary small vessel diseases caused by mutations in the *NOTCH3* gene. CADASIL patients suffer from migraines at a young age before developing other symptoms, most commonly recurrent ischaemic events, cognitive impairment and dementia. To date, no therapy is available to prevent or even delay the symptoms of CADASIL.

The onset ages and severity of the clinical features vary substantially among patients, but it can be characterised by 5 main symptoms: migraine with aura, subcortical ischaemic events, mood disturbances, apathy and cognitive impairment. Since clinical presentation vary significantly among patients, it is thought that not only the gene mutation, but also other modifying factors affect the disease.

CADASIL causes stenosis by primarily affecting the small cerebral arteries. However, involvement of middle to large cerebral arteries has been reported in some CADASIL patients. Recently, an association has been found between a variant of RING finger protein 213 (*RNF213*) and moyamoya disease (MMD) and intracranial artery stenosis/ occlusion (ICASO). In some CADASIL cases ICASO in the large cerebral arteries has been reported. Taken together, we hypothesise that *the RNF213 gene affects middle to large artery lesions in CADASIL patients.*

By using genotyping we will investigate the percentage of *RNF213* gene variant in CADASIL patients. Afterwards, genotype data will be compared to MRA data to determine the relationship of *RNF213* gene variant and stenosis/occlusion in CADASIL patients.

Project title: Expression of c-Fos in Sagittalis Nucleus of the Hypothalamus after Ovarian Steroid Hormone Manipulation in Female Rat

Ivaylo E. Balabanov, Ken Ichi Matsuda, Shunji Yamada, Masaki Tanaka

Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine

During extended observation of estrogen receptor (ER) α-immunoreactive neurons in the hypothalamus, we previously identified a novel nucleus, Sagittalis Nucleus of Hypothalamus (SGN), in the interstitial area between the Arcuate nucleus and Ventromedial nucleus. SGN exhibits sexual dimorphisms in its volume and cell numbers, and estrous cycle related variations in ERα-immunoreactivity. Treatment of neonatal females with testosterone eliminated this sexual dimorphism. These characteristics of SGN indicate implication in sexually determined brain functions and behaviors. In this study, we examined involvement of SGN in sexual arousal. Double-immunohistochemistry of c-Fos, a marker of neuronal activity, and calbindin, a marker of SGN, was performed after administration of estrus-inducing dose of estradiol benzoate and progesterone in female rats. Microscopic images showed the number of c-Fos-expressing neurons in SGN was increased, following hormonal manipulation. These results suggest that SGN has an important role in the control of female sexual behavior.

Targeting REV-ERB in the circadian clock to combat metabolic syndrome: a novel therapeutic approach

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Obesity is the major cause for metabolic syndrome. As a result of today's obese epidemic, a worrisome increase of the metabolic syndrome prevalence is observed. The syndrome significantly increases the risk for not only diabetes and cardiovascular diseases but also cognitive impairment due to metabolic malfunction and chronic inflammation. In order to suppress these complications, medical treatment accompanied with lifestyle changes is crucial. Effective medical treatment options are very limited. As such, investigating new therapeutic approaches for metabolic syndrome is essential.

The circadian clock regulates behavioural and physiological processes in a 24h cycle. Recent findings demonstrate that the circadian clock regulates metabolic processes. Research has also shown that shift work is associated with an increased risk for obesity and cardiac diseases. These findings suggest that alteration of the circadian rhythm can influence metabolism.

Transcription factor REV-ERB forms an essential link between the circadian clock and metabolism. REV-ERB is an important component of the circadian clock and it regulates the metabolism in a circadian and tissue-dependent manner. Therefore, we hypothesize that drug targeting REV-ERB can combat the metabolic syndrome.

In this research, a REV-ERB-deficient mouse embryonic stem cell model will be established. We will investigate the effect of REV-ERB deficiency on the circadian clock and examine the effect of REV-ERB drug targeting on the circadian molecular clock, metabolism and inflammation by using a synthetic ligand.

Our *in vitro* research will contribute to further understanding of circadian regulation of metabolism and the discovery of new drugs for metabolic syndrome treatment.

Project title: Exploring the role of Fabp7 and essential polyunsaturated fatty acids on astrocyte proliferation as a contributor to schizophrenia.

INES ANDREIA GONCALVES MAGRO DOS REIS
Supervisors: Dr. Osumi Noriko and Dr. Inada Hitoshi
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Schizophrenia is a neuropsychiatric disorder affecting nearly 1% of the world population. It impairs cognitive and emotional brain function, heavily burdening the life of patients. The pathology of schizophrenia is highly complex and has been investigated for decades, mostly from the perspective of neuronal dysfunction hypotheses; however, underlying mechanisms are not completely understood.

Recent studies have linked astrocytes and dysfunction of fatty acid binding protein 7 (Fabp7) to schizophrenia. Analysis of schizophrenic patients' brains shows decreased density and impaired spatial arrangement of astrocytes, suggesting that these cells are involved in neuropathophysiology of schisophrenia. In mice, the lack of *Fabp7* impairs neuronal functions and hinders prepulse inhibition response, a schizophrenia endophenotype. Schizophrenic patients also show differential expression of Fabp7 and lower brain levels of polyunsaturated fatty acids (PUFAs), i.e., main ligands for Fabp7. Relevantly, dietary supplementation with PUFAs has been shown to partially rescue several aspects of brain function, including prepulse inhibition deficits in rodents.

In this project, we further explore the role of Fabp7 and PUFAs in astrocyte dysfunction. We hypothesize that the lack of Fabp7 disrupts astrocyte proliferation, which will be rescued by adequate PUFA supplementation. We will compare the *in vitro* proliferation and differentiation of astrocytes from wild type and *Fabp7* KO mice and analyze whether it is improved by the administration of different PUFAs.

Our results will contribute to understand whether Fabp7 dysfunction reduces astrocyte numbers in brain disorders. Finally, we will explore the potential of PUFAs as therapeutical agents.

Project title: A future of mini-brains: Structural and molecular pathology of human-derived neural stem cell-based organoids.

Cristina Nardone and Kyoko Itoh
Department of Pathology and Applied Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine.

Thanatophoric dysplasia (TD) is a lethal form of chondrodysplastic dwarfism. The disease, caused by a genetic mutation in the fibroblast growth factor receptor 3 (FGFR3) gene, is characterized by severe skeletal dysplasia and brain malformations. The precise mechanism linking the FGFR3 mutation to these malformations is unclear and needs to be elucidated.

Although transgenic animal models are available or can be produced, these models differ in brain morphology compared to humans. The absence of a reliable TD-model has prompted us to develop a novel *in vitro* disease-model. This model should be relatively simple, reproducible, but authentic to human brain morphology and/or pathology.

Recent stem cell technology provided outstanding outcomes in basic sciences and clinical trials with regards to disease models. These pluripotent stem cells can differentiate into a wide variety of cell types. In that context, we have attempted to employ neural stem cells (NSCs) which can differentiate into specific brain tissue.

Recently, normal- and TD-patient-derived NSCs were established by our collaborator. We hypothesize that human-derived NSCs can commit to neural cell lineages and produce cerebral organoids that resemble mini-brains. We will investigate both normal- and TD-derived organoids with molecular assays to gain insights into the underlying mechanisms *in vitro*.

Project title: Transcriptional regulation of glycogen related genes in the embryonic motor-neuron, focusing on Gys1 and PygB.

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Department of Biology, Kyoto Prefectural University of Medicine

Glycogen a polysaccharide made from glucose monomers is mainly used for energy storage. Its cerebral concentration is highest in well differentiated astrocytes, which supply glycogen derived lactate to neurons during periods of brain activation where glucose alone cannot meet the high energy needs (1, 2). Glycogen metabolism has an increased turnover during ischemia and hypoglycaemia resulting in rapid depletion of stores. As such glycogen is thought to function predominantly as an emergency energy source(3). It's also a major contributor to neuronal growth and critical functions such as learning and memory consolidation, providing the energy substrates necessary for synaptic activity(4).

Regulated by the enzymes Glycogen Synthase and Glycogen Phosphorylase, glycogen metabolism contributes biomolecules necessary for systemic cellular and developmental processes (5). Mice deficient in muscle-GYS1 isoform demonstrated perinatal lethality coupled with cardiac mal-development; further reinforcing glycogen as an important energy source for physiological development (6).

Glycogenin (Gyg)-knockout mice demonstrated perinatal lethality characterised by abnormal neuromuscular junction formation revealing the significance of glycogen metabolism in vivo (unpublished data). Our group has demonstrated that Glycogen synthase 1 (Gys1) and Glycogen phosphorylase, brain isoform, (PygB) expression was exclusively localised in motor neurons. We therefore hypothesise that; glycogen metabolism is a major contributor to motor neuron development and function in physiological conditions. In order to reveal how and why motor neurones specifically acquire the glycogen metabolism pathway as compared among neuron subtype; we aim to determine the transcriptional regulators responsible for the expression of glycogen metabolising genes (Gys1/PygB) and their significance in neuronal development.

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Project title: Mechanisms of the On/Off regulation of the synaptic protein transport among different developmental stages

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Supervisor: Masaki Sone, Graduate School of Science, Toho University, Japan
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Many synaptic proteins are synthesized in the neuronal cell bodies and then transported to synaptic terminals by axonal transport. Synaptic transport of the *Drosophila* Hikaru genki (HIG) protein is regulated dynamically in an ON/OFF manner during synaptic development at the pupal stage. At mid-pupal stage, HIG is transported to synapses, whereas at late-pupal stage, it is retained in the neuronal cell bodies. HIG is a protein that is secreted to synaptic clefts of the cholinergic synapses. It has an RGD sequence, an immunoglobulin (Ig) domain and five complement binding (CB) domains.

Previous studies revealed that this protein is involved in the accumulation of acetylcholine receptor (AChR) subunits. *hig* mutant adult flies show remarkably reduced locomotor activity. Conditional rescue experiments using heat shock promoter showed that the behavioral phenotype of the adult *hig* mutants can be fully recovered by transient exogenous expression of *hig* at the mid-pupal stage but not at the late pupal stage.

In this study, a domain deletion analysis of HIG was performed to reveal underlying mechanisms of the transport of this protein. Expression of series of HIG deletion proteins was transiently induced in mid pupae using heat shock promoter and their subcellular localization was examined by using immunochemistry as well as the differential effect of its expression between males and females.

Thus, data suggest that the switching mechanism of synaptic transport of HIG is necessary for the proper control of synaptic assembly of AchR.

Project title: Alpha-synuclein in human erythrocytes

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Supervisor: Ayako OKADO-MATSUMOTO, Ph.D.
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Parkinson's Disease (PD) is the second most common neurodegenerative disease, affecting people worldwide and yet remains incurable. As many neurodegenerative diseases arise from protein misfolding/aggregation, particular enthusiasm is currently emerging around targeting alpha-synuclein (ASYN). ASYN is the main constituent of inclusion bodies "Lewy bodies" and "Lewy neurites", and is thought playing a role in neurodegeneration.

Research performed on the last decade provided pieces of evidence that neuronal cells of both PD and healthy patients, secrete ASYN into the surrounding brain regions in a way that seems physiological. It was hypothesized that PD might act as a prion-like disease where pathological forms of ASYN spread throughout the brain. One interesting possibility that emerges from these findings, is the potential study of ASYN and its derivatives in biological fluids. After secretion, ASYN can circulate to the cerebrospinal fluid and then to the blood. Thus, it is relevant to wonder whether extracellular ASYN forms and levels may witness the disease.

In blood, ASYN has been identified contained for 99% in erythrocytes, in which its physiological state is feeding an intense debate as it was demonstrated existing either as a monomer or a tetramer. Using chromatographic and electrophoresis techniques, we focused on elucidating ASYN's physiological forms in human erythrocytes. We tried to develop a suitable experimental model to investigate its detection, states, and interactions. These findings could contribute to the current research on targeting ASYN as a potential PD inducer, and could take part of the discovering of assays for the other synucleopathies.

Project title: Significance of FABP7 and Ndufs4 in the regulation of astrocytic mitochondrial dynamics and functions

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Mitochondria are the main intracellular organelles for energy production. Mitochondrial morphology is diverse and dynamic, varying between different cell types, and closely related to their functions. It has been recetly shown that mitochondrial dysfunction in brain cells is associated with various diseases such as neurodegenerative and psychiatric diseases. However, the significance of glial mitochondrial dynamics and functions in the pathology of such diseases is largely unknown.

Our previous functional analysis of fatty acid binding proteins (FABPs) demonstrated that FABP7, a cellular lipid chaperone expressed in astrocytes, is associated with human psychiatric diseases, such as schizophrenia, suggesting that lipid homeostasis in astrocytes, which is closely related to their mitochondrial dynamics, may underlie the pathology of these diseases.

Towards that, we will investigate the mitochondrial dynamics (fusion and fission) by live cell imaging, respiration activity by flux analyzer, and production of reactive oxygen and nitrogen species by fluorescence-based assay, in FABP7-KO astrocytes. Furthermore, the functional significance of mitochondrial respiratory chain will also be examined in astrocytes deficient for Ndufs4, aiming to find the link between cellular fatty acid homeostasis and mitochondrial function in astrocytes. Ndufs4 is a nuclear-encoded molecule, essential for the assembly and activity of Complex I (major complex of the mitochondrial respiratory chain) and involved in many human neural pathologies.

The results of this study will reveal the underlying mechanism by which FABP7 regulates mitochondria in astrocytes and may lead to the development of preventative drugs that target glial mitochondrial functions for neurodegenerative and psychiatric diseases.

Project title: SETTING UP A NOVEL PLATFORM FOR *IN VIVO* VALIDATION OF ANTI-PROPAGATION THERAPIES THAT TARGET α-SYNUCLEIN PATHOLOGY

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Lewy body dementias (LBDs), a group of diseases characterized by intracellular protein depositions, known as Lewy bodies, are currently the second most common type of neurodegenerative disease. Patient numbers keep rising and later disease stages are often debilitating, stressing the need for disease-modifying therapies and early diagnostic markers, which are currently unavailable.

An important player in LBDs is α-synuclein. α-synuclein fibrils form the main component of Lewy bodies. α-synuclein burden and deposition location correlate with disease severity and symptoms. Finally, α-synuclein deposits form early on and fuel disease propagation, making α-synuclein a therapeutic target. For validation of anti-propagation therapies, an animal model that accurately represents α-synuclein pathology is crucial. One such animal model exists, but its histopathology is not yet validated for imaging parameters during *in vivo* fibril propagation. Consequently, *in vivo* detection of α-synuclein pathology in animal models remains unavailable. This project aims to introduce a new platform for validation of anti-propagation therapies targeting α-synuclein, by charting *in vivo* imaging parameters in a mouse model of intracranial α-synuclein injection.

^{18}F-BF227, one of our previously developed PET tracers targeting amyloid-β plaques, showed unexpected affinity for α-synuclein in MSA patients and we confirmed high affinity for synthetic α-synuclein fibrils *in vitro*. Consequently, ^{18}F-BF227 can be used to chart imaging parameters for the mouse model using follow-up *in vivo* imaging. This approach will document *in vivo* consistency of disease model histopathology over time and provide a new validated platform to test anti-propagation therapies and diagnostic markers for LBDs using *in vivo* imaging.