Keynote Lecture

Lessons from Yeast -Autophagy as a Cellular Recycling System-

Yoshinori Ohsumi
Tokyo Institute of Technology, Japan
Honorary Professor

Every cellular event is achieved through a balance between synthesis and degradation. The cellular degradation process is highly regulated and plays critical roles in cell physiology. There exist two major pathways of intracellular degradation, the lysosome/vacuole- and ubiquitin/proteasome- systems. The former is mediated mainly via autophagy and facilitates bulk and non-selective degradation. Almost 30 years ago I first observed under a light microscope that the yeast S. cerevisiae induces massive protein degradation within the vacuole under nutrient starvation. Electron microscopy revealed that membrane dynamics during this process are similar to known macroautophagy in mammals. Using the yeast system, many autophagy-defective mutants were successfully obtained. Now we know that 18 ATG genes are essential for starvation-induced autophagy. These Atg proteins concertedly function in the sequestration of cytoplasmic constituents into a specialized membrane structure, the autophagosome. The Atg proteins consist of six functional units, including an Atg1 kinase complex, the PI3 kinase complex and two unique ubiquitin-like conjugation systems. Soon we found that most ATG genes are well conserved from yeast to mammals. The identification of ATG genes completely changed the landscape of autophagy research. Genetic manipulation of the ATG genes unveiled a truly broad range of physiological functions of autophagy. Autophagy plays critical roles not only in nutrient recycling, but also intracellular clearance through the elimination of harmful proteins and damaged organelles. It is becoming clear that autophagy is relevant to many diseases and has become one of the most popular field in cell biology.

Our recent works on the mechanisms of the unique membrane dynamics during autophagy and physiological roles of autophagy in yeast will be discussed. Even in yeast there are many fundamental questions that remain to be answered.

Further comprehensive and biochemical analyses are required from various points of view.

Since discovery of lysosome and coining autophagy as self-eating process by C. de Duve, for long time not much progress had been made about its molecular mechanism.

Spatial Transcriptomics in Neurodegenerative Disease

Joakim Lundeberg

SciLifeLab/KTH - Royal Institute of Technology

Group Leader/Professor

In standard bulk RNA sequencing whole tissue biopsies are homogenized and average representations of expression profiles within the entire sample are obtained. Consequently, information on spatial patterns of gene expression is lost and signals from small regions with deviant profiles are obscured. To overcome these deficiencies, a protocol employing Spatial Transcriptomics (ST) technology has been developed, which enables spatial analysis of specific gene expression patterns within tissue sections. In order to establish conditions for retrieval and attachment of mRNA in situ with positional information, a spatially barcoded microarray is used to facilitate identification of specific parts of the investigated tissue. Each feature on the array contains more than 200 million probes, all sharing a unique DNA sequence (barcode) specific to that feature. The barcode is used in the downstream analysis to link each feature's position within the tissue to the mRNA captured at that position. Finally, following reverse transcription and tissue removal, barcoded cDNA is enzymatically released from the array and used to generate sequencing libraries. We have also developed an open, available software (www.spatialtranscriptomicsresearch.org) which combines images of tissue sections with information from the sequencing, that is, which genes are expressed and at what level. In this presentation some recent spatial transcriptomics work on Alzheimer mouse models (in collaboration with Dr Bohr, NIH, USA) and ALS mouse models (in collaboration with Dr Phatani and Dr Maniatis, NYGC, USA) will be demonstrated.

Transcriptional Control of Human Embryo Genome Activation Yields Clues for Reprogramming

Juha Kere

Karolinska Institutet/King's College London/RIKEN Center for Life Science Technologies (CLST)

Professor/Senior Fellow of JSPS

After fertilization of the egg cell, the embryonal development starts with its individual genome activation (Embryo Genome Activation, EGA). EGA is especially amenable to transcriptomic analysis. To understand human EGA, we performed single-cell transcriptome sequencing of over 340 cells, including oocytes, zyogtes and single blastomeres from 4-cell and 8-cell embryos, obtained by informed consent as donations after in vitro fertilization treatments[1]. The total content of mRNA molecules remained essentially unchanged between oocytes and zygotes, but revealed an increase of *DUX4* repeat-sequence transcripts[2]. Comparison of the transcriptomes of oocytes and 4-cell stage blastomeres identified the first 32 embryonally transcribed genes and a further 129 genes upregulated at 8-cell stage[1]. Our transcription start site targeted data allowed also the identification of critical regulators of EGA as 36 bp and 35 bp conserved promoter elements at the two stages of EGA, respectively. These data constitute a resource for understanding the earliest steps of human embryonal development and provide new genes of interest for study of pluripotency and stem cell technologies. To that end, we have recently used guide RNA molecules (gRNAs) directed to the EGA-associated regulatory sequences along with *OCT4, SOX2, KLF4, MYC* and *LIN28A* to achieve fully CRISPRa based reprogramming with high efficiency[3].

References

1) Töhönen V, Katayama S & al. Novel PRD-like homeodomain transcription factors and retrotransposon elements in early human development. Nature Commun 6:8207 (2015)

2) Töhönen V, Katayama S & al. Transcription activation of early human development suggests DUX4 as an embryonic regulator. Biorxiv.org. doi: https://doi.org/10.1101/123208

3) Weltner J & al. Human pluripotent reprogramming with CRISPR activators. Biorxiv.org. doi: https://doi.org/10.1101/206144

RIKEN Ageing Resource Project: Of Mice and Super-centenarian Men

Aki Minoda

RIKEN Center for Life Science Technologies (CLST)

Unit Leader

We are preparing to generate resource datasets to study ageing. Bulk analyses of high-throughput genomic technologies have produced invaluable publicly available data resource on ageing. However, these data are limited in terms of accurately defining cellular and disease states. In order to understand how the process of ageing affects tissues at the cellular state level, we are generating single cell transcriptome datasets of selected tissues from different ages of mice and blood from super-centenarians. One unique aspect of our analysis will be a comparison between SPF (“standard”) and germ-free mice, which may reveal key regulatory pathways that are activated by the microbiome that may affect the process of ageing. For a selected cell types, we will carry out bulk multi-omics, such as DNA methylation, chromatin openness (ATAC-seq), translatome (ribosome profiling), proteomics and metabolomics to increase our understanding of the ageing process at many levels of cellular processes. We present here our initial single cell transcriptomic results produced from a couple of mouse tissues.

Losing Chromosome Y in Leukocytes Matters!

Jan Dumanski
Uppsala University
Professor and Group leader

We have recently reported a new method to predict risk of cancer and Alzheimer's disease based on analyses of mosaic **L**oss **O**f chromosome **Y** (LOY) in leukocytes of aging males. LOY in leukocytes is associated with shorter survival and risk for cancer in many organs. Males with LOY-affected leukocytes had 5.5 years shorter median survival time and LOY can be induced by smoking.

Our leading hypothesis explaining phenotypic effects of LOY is that LOY negatively affects specific types of leukocytes, disturbing the functions of the immune system that are normally eliminating abnormal cells (such as cancer cells and cells forming amyloid plaques in the brain) throughout the body. This disease preventive process is called immune-surveillance.

Our research is designed to advance the above discoveries, focusing on development LOY as a clinically useful early marker of cancer and Alzheimer's disease risk. We further develop new methods increasing the predictive power of LOY as a biomarker. We also work on the functional consequences of LOY on cellular and systemic level with focus on the transcriptome and proteome.

Current methods treatment of cancer and Alzheimer's disease are focused on management of advanced disease. To shift medicine of these disorders towards a more preventive paradigm, we need new robust tools to find individuals with increased risks of life threatening forms of sporadic cancers and Alzheimer's disease, years before the earliest symptoms, and this is the long-term goal of this project.

Molecular and Neural Circuit Mechanisms Underlying Stress and Resilience

Tomoyuki Furuyashiki
Kobe University
Professor

Stress is caused by adverse social environments and lifestyles, and affects our mental and physical functions in multiple ways. In general, brief or moderate stress promotes adaptation and resilience to stress, whereas prolonged or excessive stress induces emotional and cognitive dysfunctions and precipitates mental and physical illnesses. Using social defeat stress in mice, we identified novel molecular and neural-circuit mechanisms underlying stress and resilience. Single social defeat stress activates the dopaminergic pathway projecting to the medial prefrontal cortex (mPFC). This dopaminergic response activates dopamine D1 receptor subtype and induces the growth of apical dendrites in superficial layer pyramidal neurons in mPFC, leading to resilience to social defeat stress. By contrast, repeated social defeat stress attenuates this dopaminergic response through prostaglandin E_2 (PGE_2), an inflammation-related bioactive lipid, and its receptor called EP1. COX1, a PG synthase enriched in microglia, is critical for repeated social defeat stress-induced social avoidance as well as brain PGE_2 synthesis, suggesting the involvement of PGE_2 derived from microglia in this behavioral change. Recently it has been postulated that innate immune receptors such as Toll-like receptors (TLRs) sense endogenous damage-associated molecules to induce sterile inflammation. We found that TLRs are critical for social avoidance and elevated anxiety induced by repeated social defeat stress as well as concomitant dendritic atrophy and reduced responsiveness of pyramidal neurons and microglial activation in mPFC. Notably, knockdown of TLRs selectively in mPFC microglia abolishes social avoidance induced by repeated social defeat stress, indicating that TLR-mediated activation of mPFC microglia mediates this behavioral change. Collectively our findings suggest that brief stress promotes stress resilience through dopamine D1 receptor signaling associated with dendritic growth in mPFC pyramidal neurons, whereas prolonged stress activates microglia, which in turn reduce this stress resilience and induces dendritic atrophy of mPFC neurons through inflammation-related molecules, leading to emotional dysfunctions. Therefore, we pave the way for identifying molecular targets to specifically regulate distinct stress-activated pathways towards therapeutic development for stress-related disorders.

Brain Degeneration Caused by Abnormal Calcium Signaling

Katsuhiko Mikoshiba
RIKEN Brain Science Institute (BSI)
Senior Team Leader

G protein-coupled receptor (GPCR) signal is linked to the production of IP_3 which leads to Ca^{2+} release from ER to exert various physiological function. IP_3 receptor (IP_3R) works as a signal converter to convert IP_3 signal to Ca^{2+} signal. We reported dysfunction of IP_3R causes cerebellar ataxia (***Nature*** 1996), spinocerebellar ataxia (***J.Neurol.*** 2017), ER stress induced brain damage (Chaperone GRP78 (***Neuron*** 2010), ERp44 (***Cell*** 2005)), ER stress inducible enzyme Transglutaminase 2 blocking-IP_3R1 in Huntinton disease (***PNAS*** 2014). Disrupted-in-schizophrenia 1 (*DISC1*), a susceptibility gene for schizophrenia binds UTR of mRNA of IP_3R1 (***Nature Neuroscience*** 2015). Genetic ablation of IP_3R2 decreases survival of $SOD1^{G93A}$ (ALS mice) (***Human Molecular Genetics*** 2016). Newly identified pseudo-ligand of IP_3R, IRBIT (***Mol. Cell*** 2006) is involved in apoptosis regulation (***eLife*** 2016). Bcl2 family is involved in apoptosis (***Cell Death and Diseases*** 2013)(***Cell Calcium*** 2017) and is closely associated with IP_3R. Transcranial direct current stimulation-induced plasticity (***Nature Communications*** 2016) and mechanical allodynia (***J. Clinical Invest.*** 2016) are found to be regulated by the IP_3R in astrocytes. In addition, there are many reports to show IP_3R is involved in cellular senescence, apoptosis and anti-tumor suppression. Dysregulation of IP_3R caused by the deranged proteins association results in abnormal Ca^{2+} signaling. By X-ray crystallography of the large cytosolic domain with 2217 amino acid residues long in the presence and absence of IP_3, we identified an IP_3-induced long-range allosteric structural change to the channel through the “leaflet” structure which is the essential relay region to open the channel (***PNAS*** 2017). Apoptosis related Cytochrome C, BCL-XL binding site and phosphorylation site by Akt/PKB locate near the “leaflet” region suggesting that these molecules should regulate channel gating. The 3D structures of IP_3R provides a new structural basis for gating transmission through the “leaflet” to understand the core mechanism of IP_3-gated Ca^{2+} release, which will surely provide us new drug targets for IP_3R-relating diseases. The mapping of regulatory sites for associating proteins and posttranslational modifications provide us great information of the leaflet-mediated gating transmission that should be regulated by associated molecules and by posttranslational modifications.

Nanomedicine for Infection and Neuroscience

Agneta Richter-Dahlfors

Karolinska Institutet

Professor, Director

Modern advances in biomedical research call for dynamically controllable systems. The unique attributes of organic bioelectronics make this class of materials particularly interesting. Owing to the structural and functional similarities of conducting polymers and biological systems, a novel class of biologically compatible devices is being created that enable exceptional control over cells and tissues. This presentation will describe our work on the development of conducting polymer devices that are able to sense, modify, and interact with the microenvironment in order to study how minute environmental changes influences the outcome of bacterial infections. Our sensors and devices are either applied to study the infected host tissue, or to study the specialized bacterial community termed biofilm. The latter is specifically important for the ageing population, since bacterial biofilms are major causes of catheter- and device-associated infections. In neuroscience, accumulation of protein aggregates is associated with many neurodegenerative diseases. This presentation will report on the development of novel optoelectronic nanoprobes, which are applied as molecular fluorescent ligands able to detect protein aggregates, the pathological hallmarks of Alzheimer's disease pathology.

iPS-based Regenerative Medicine-Retinal Diseases

Masayo Takahashi
RIKEN Center for Developmental Biology (CDB)
Project Leader

The first in man application of iPS-derived cells started in September 2014, targeted age-related macular degeneration (AMD). AMD is caused by the senescence of retinal pigment epithelium (RPE), so that we aimed to replace damaged RPE with normal, young RPE made from iPS cells. We judged the outcome 1 year after the surgery. Primary endpoint was the safety, mainly the tumor formation and immune rejection. The grafted RPE cell sheet was not rejected nor made tumor after two years. The patient's visual acuity stabilized after the surgery whereas it deteriorated before surgery in spite of 13 times injection of anti-VEGF in the eye.

Although autologous RPE sheet transplantation is scientifically best approach, it is time consuming and expensive and it is necessary to prepare allogeneic transplantation to establish a standard treatment. RPE cells are suitable for allogeneic transplantation because they suppress the activation of the T-cell. From in vitro and in vivo study, it is possible that the rejection is considerably suppressed by using the iPS cell with matched HLA. Our new protocol has accepted by ministry in Feb 2017. We are planning transplantation using allogeneic iPS-RPE cell suspension & sheet, and also autologous iPS-RPE. For the cell suspension transplantation we will not combine CNV removal and apply to milder cases than sheet transplantation.

In Japan, pharmaceutical law has been changed and a new chapter for regenerative medicine was created for clinical trial. Also the separate law for safety of regenerative medicine for clinical research (study) was enforced in 2015. These laws made the suitable condition for the brand new field of regenerative medicine. We are making regenerative medicine in co-operation with ministry & academia.

Stem Cell-based Therapy for Parkinson's Disease

Jun Takahashi

Center for iPS Cell Research and Application, Kyoto University

Professor

Human induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease (PD). Towards clinical application of iPSCs, we have developed a method for 1) scalable DA neuron induction on human laminin fragment and 2) sorting DA progenitor cells using a floor plate marker, CORIN. The grafted CORIN^{+} cells survived well and functioned as midbrain DA neurons in the 6-OHDA-lesioned rats, and showed minimal risk of tumor formation. In addition, we performed a preclinical study using primate PD models. Regarding efficacy, human iPSC-derived DA progenitor cells survived and functioned as midbrain DA neurons in MPTP-treated monkeys. Regarding safety, cells sorted by CORIN did not form any tumors in the brains for at least two years. Finally, MRI and PET imaging was useful to monitor the survival, expansion and function of the grafted cells as well as immune response by the host brain. These results suggest that human iPSC-derived DA progenitors generated by our protocol are clinically applicable to treat PD patients.

Ageing-related Alteration by the Changing of Murine Intestinal Environment

Naoko Satoh-Takayama
RIKEN Center for Integrative Medical Science (IMS)
Researcher

“Ageing” means the limit of cell-renewal, which cannot be avoidable for anyone. It will be necessary for us to understand how ageing is regulated and can be modified for our future to spend a high quality of life. Recent reports have indicated that gut commensal microbiota play some important roles as an immune-regulator for keeping homeostasis of the gut ecosystem directly or indirectly. Epithelium is important as first mucosal barrier, which directly interacts with microbiota. However, questions remain unanswered whether epigenetic changes of intestinal epithelium can be caused by microbiota composition as well as ageing. We are therefore focusing on the gene expression associated with epigenetic changes in the intestinal epithelium along ageing in mice with or without commensal microbiota.

In order to pick up the gene(s) affected by ageing, intestinal epithelium from the small and large intestine of specific pathogen-free (SPF) and germ-free (GF) mice at 3 weeks (wean-young), 18 weeks (middle age) and 2 years (aged) were analyzed. Concomitantly, epithelial organoids (ex vivo primary culture of epithelium) of these mice were established to see whether epigenetic changes are preserved or not. Further, metabolome analysis of intestinal contents and feces were also performed. We were then able to determine the gene expression profile of the collected samples with RNAseq.

Structures and Functions of Misfolded Alzheimer's Amyloid-beta: Solid-state NMR Studies

Yoshitaka Ishii

Tokyo Institute of Technology/RIKEN Center for Life Science Technologies (CLST)
Professor

This work involves two separate topics on structural biology of amyloid-β and other proteins using solid-state NMR (SSNMR). First, we discuss structural studies of misfolded 42-residue Alzheimer's amyloid β (Aβ). Misfolded fibrillar aggregates of Aβ are a primary component of senile plaque, a hallmark of a brain affected by Alzheimer's disease (AD). Increasing evidence suggests that formation and propagation of misfolded aggregates of 42-residue Aβ42, rather than the more abundant 40-residue Aβ40, provokes the Alzheimer's cascade. Our group recently presented the first detailed atomic model of Aβ42 amyloid fibril based on SSNMR data.[1] The result revealed a unique structure that was not previously identified for Aβ40 fibril. Based on the results, we discuss how amyloid fibril structures affect "prion-like" propagation across different Aβ isoforms. We also present our ongoing efforts to analyze a structural conversion in misfolding of Aβ42 from oligomeric intermediates to fibrils. [2,3]

Secondly, we briefly discuss recent development in biomolecular SSNMR in a high magnetic field (^{1}H frequency: 750-900 MHz). Major challenges in biomolecular SSNMR are limited sensitivity and resolution. Our data on protein microcrystal GB1 and amyloid-β (Aβ) fibril show that traditionally time-consuming 3-4D biomolecular SSNMR is feasible for signal assignments and structural elucidation of sub-mg of proteins with this approach using ultra-fast magic-angle spinning (MAS). [4,5]

References

[1] Xiao, Y., Ma, B., McElheny, D., Parathasarathy, S., Hoshi, M., Nussinov, R. & Ishii, Y. *Nat. Struct. Mol. Biol.* **22**, 499-505 (2015).
[2] Noguchi, A., Matsumura, S., Dezawa, M., Tada, M., Yanazawa, M., Ito, A., Akioka, M., Kikuchi, S. et al. *J. Biol. Chem.* **284**, 32895-32905 (2009)
[3] Parthasarathy, S., Inoue, M., Xiao, Y., Matsumura, Y., Nabeshima, Y., Hoshi, M. & Ishii, Y. *J. Am. Chem. Soc.* **137**, 6480–6483 (2015)
[4] Wickramasinghe, N.P., Parthasarathy, S., Jones, C.R., Bhardwaj, C., Long, F., Kotecha, M., Mehboob, S., Fung, L.W.M., Past, J., Samoson, A. & Ishii, Y. *Nat. Methods* **6**, 215-218 (2009).
[5] Parathasarathy, S., Nishiyama, Y. & Ishii, Y. *Acc. Chem. Res.* **46**, 2127-2135 (2013).

Precision Systems Cancer Medicine

Olli Kallioniemi

Science for Life Laboratory/Karolinska Institutet
Director/Professor

Making cancer care more effective, safe and individually optimized is a central aim for cancer researchers and oncologists worldwide. A common strategy to achieve this is based on sequencing tumor genomes with the aim to identify oncogenic driver mutations whose effects could be blocked by specific drugs with a predicted therapeutic gain. Our precision medicine strategy is based on the integration of genomic, transcriptomic and proteomic profiling data as well as insights from direct high-throughput testing of *ex vivo* efficacies of a panel of cancer drugs on patient-derived cancer cells. This approach started in acute myeloid leukemias and other hematological malignances (*Pemovska et al., 2013; 2015*), and is now being expanded to to solid tumors. This approach can help to reposition existing cancer drugs to new indications, prioritize emerging drugs for clinical testing in molecularly defined subgroups of patients, identify biomarkers and mechanisms of action of drugs as well as help to design tailored drugs and drug combinations for precision patient treatment in the clinic.

Advanced Optical Noninvasive Sensing Technology for Health Science

Satoshi Wada
RIKEN Center for Advanced Photonics (RAP)
Group Director

With Japan's super-aging society, the research and development of new healthcare and diagnosis technologies, contributing to improved healthy life expectancy, are desired. Among them, the research and development of noninvasive diagnosis technology such as human-breath analysis is receiving much attention.

Human breath contains several hundred gaseous substances such as inorganic gases and volatile organic compounds (VOCs). Gaseous substances associated with diseases are included in human breath, meaning that breath diagnosis can be realized by the quantitative analysis of substances as biomarkers. It is also possible to apply to the new technology to healthcare, leading to preventive medicine and reduced medical costs.

We are promoting research on finding the biomarkers of diseases and monitoring health on the basis of breath component analysis, targeting the elderly. The number of elderly, people providing breath is planed to be 1000 people per year. We have already collected breath samples from over 100 elderly people and analyzed gaseous substances contained in the breath using gas chromatography–mass spectrometry (GCMS). Various gaseous substances including ethanol and acetone were included in the breath, and it was found that the substances also differed among elderly people (Fig. 1). We are searching for the biomarkers of presymptomatic diseases peculiar to elderly people based on correlation analysis between the breath components and the health of elderly people. In the future, we aim to develop an optical noninvasive breath analysis system using our mid-infrared laser spectroscopy technology. The system will provide on-site and rapid analysis of relevant biomarkers. In this presentation, we discuss the present state of breath component analysis and future plans for optical sensing research.

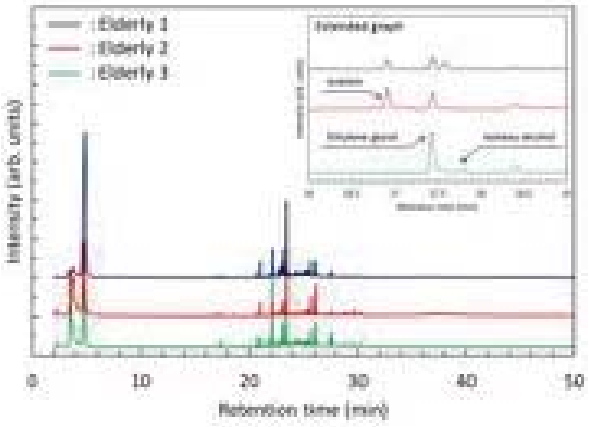
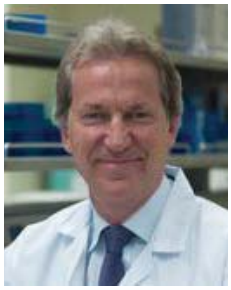


Fig. 1 Examples of gas chromatographs of elderly people's breath.



Translational PET Neuromaging and Radioligand Development

Christer Halldin

Karolinska Institutet

Professor

PET provides a new way to image the function of a target and by elevating the mass, to pharmacologically modify the function of the target. The main applications of radioligands in brain research concern human neuropsychopharmacology and the discovery and development of novel drugs to be used in the therapy of psychiatric and neurological disorders. A basic problem in PET brain receptor studies is the lack of useful radioligands with ideal binding characteristics. During the past decade more than hundred neurotransmitters have been identified in the human brain. Most of the currently used drugs for the treatment of psychiatric and neurological disorders interact with central neurotransmission. Several receptor subtypes, transmitter carriers, and enzymes have proven to be useful targets for drug treatment. Molecular biological techniques have now revealed the existence of hundreds of novel targets for which little or no prior pharmacological or functional data existed. Due to the lack of data on the functional significance of these sites, pharmacologists are now challenged to find the physiological roles of these receptors and identify selective agents and possible therapeutic indications. During the past decade various ^{11}C- and ^{18}F-labeled PET radioligands have been developed for labeling some of the major central neuroreceptor systems. There is still a need to develop pure selective PET radioligands for all the targets of the human brain. This presentation will review recent examples in translational PET neuroimaging and radioligand development. A basic problem in the discovery and development of novel drugs to be used in for example the therapy of neurological and psychiatric disorders is the absence of relevant *in vitro* or *in vivo* animal models that can yield results to be extrapolated to man. Drug research now benefits from the fast development of functional imaging techniques such as PET. Drug industry is heavily involved in PET for drug development in collaboration with academia.

Neuroscience of Primate Brain Evolution

Atsushi Iriki
RIKEN Brain Science Institute (BSI)
Team Leader

Human evolution has involved a continuous process of learning new kinds of cognitive capacity, including those relating to manufacture and use of tools and to the establishment of language. The dramatic expansion of the brain that accompanied additions of new functional areas would have supported such continuous evolution. Extended brain functions would have driven rapid and drastic changes in the human ecological niche, which in turn demanded further brain resources to adapt to it. In this way, human primate ancestors have constructed a novel niche in each of the ecological, cognitive and neural domain, whose interactions accelerated their individual evolution through a process of the “Triadic Niche Construction”. Human higher cognitive activity can therefore be viewed holistically as one component of the earth’s ecosystem. The primate brain’s functional characteristics of learning capabilities seem to play a key role in this triadic interaction.

Species’ behavioral repertoire has evolved so as to match the environmental demands through adaptation of bodily morphologies, and their functions controlled by the brain. Tool use behavior represents an ideal model to explore such interaction between phylogenetic and environmental effects through acquisition of novel behavioral repertoire. Various types of tool use are observed in various species not necessarily related to the phylogenetic similarity. Thus, a sort of convergent evolution, perhaps guided by some unique environmental factors, matters for tool-use induction. On the other hand, not all the primate species can use tools – while humans, chimpanzees, capuchin monkeys, and cynomolgus monkey use tools, others such as Japanese macaques rarely and common marmosets do not use tools, but they were able to acquire tool use behavior to retrieve the food items, through short- (macaque) or long-term (marmoset) trainings.

Animals acquire new behavior or technology in reaction to fluctuating environments. Acquisition of new behavior requires formation and activation of new brain networks. Several recent studies report laboratory-raised, nonhuman primates exposed to tool use can exhibit intelligent behaviors, such as gesture imitation and reference vocal control, that are never seen in their wild counterparts. Tool-use training appears to forge a novel cortico–cortical connection that underlies this boost in capacity, which normally exists only as latent potential in lower primates. Although tool-use training is patently non-naturalistic, its marked effects on brain organization and behavior could shed light on the evolution of higher intelligence in humans.

Primate Brain Connectomics Using Cutting-edge MRI and PET Technologies

Takuya Hayashi

RIKEN Center for Life Science Technologies (CLST)
Team Leader

The cerebral cortex plays inevitable and diverse roles in motor control, cognition, perception, and emotion. The cerebral cortical area of human is 10-fold greater than the intensively studied macaque and 100-fold greater than the marmoset which is recently focused in Japan. Resolving the pattern of evolutionary change should provide invaluable insights into what makes us uniquely human. Many neuroscientists have argued that human evolutional expansion was pronounced in prefrontal cortex, but little is known about how it is associated with functional specialization, sophistication, and emergence. We are attempting to address these issues by using multi-modal, macroscale neuroimaging technologies such as MRI and PET. Our strategy involves acquiring high-quality in-vivo data in primates, and bringing data into common-spatial, surface-based template, as adopted in the Human Connectome Project (HCP). We are recently finding cortical profiles of myelin, resting state networks, neurite and connectivity of macaque and marmoset. A sophisticated algorithm in HCP for multi-modal surface-based registration could be effective in aligning cross-species differences in cortical convolutions and functional variabilities, needed to map structural-functional profiles on the common cortical model. Our strategy may enable to homologize cortical parcellations and connectomics across species, and could address the issue of human evolutional expansion. It also provides benefits for understanding lifespan brain changes and disease progression by finding translatable biomarkers that capture common pathophysiology between human and primate model.

Genetic, Environmental and Age Effects on Human Brain Neurotransmission

Lars Farde
Karolinska Institutet
Professor

The initial discovery of neuroreceptors was a result of experimental pharmacological studies using inbred animal strains, where variability in receptor density is not a major concern. When translating this field of experimental research to humans, a different picture emerged. For instance, in a study of more than 200 human brains post mortem, a nearly four-fold range was reported for the striatal D2-dopamine receptor (D2R) density. This finding of a large interindividual variability was later replicated in vivo using PET . Similar ranges of variability have been reported also for other neuroreceptors. In addition, an age effect on receptor density has been reported for several G-protein coupled receptors. For instance, about 8% of the D2R are lost per decade, an effect which has been linked to the age-related decline of episodic memory.

Despite the high interest for the serotonin and dopamine neurotransmission systems in psychiatry research, little is known about the regulation of receptor and transporter density levels. Considering the high heritability of major psychiatric disorders, it is of fundamental interest to understand if the density in adult life is genetically determined or influenced by the environment. In a recent attempt to elucidate this issue, we used PET in a twin design to estimate the relative contribution of genetic and environmental factors, respectively, on dopaminergic and serotonergic markers in the living human brain. Heritability, shared environmental effects and individual specific non-shared effects were estimated for 5-HT_{1A} receptor availability in serotonergic projection areas and for D2R in striatum. We found a major contribution of genetic factors (0.67) on individual variability in striatal D2R binding and a major contribution on environmental factors (pair-wise shared and unique individual; 0.70-0.75) on neocortical 5-HT_{1A} receptor binding. Interestingly, the heritability for D2-R was in the similar range as has previously been reported for the presynaptic marker [^{18}F]DOPA. These results confirm that both genetic and environmental factors should be taken into account in disease models of psychiatric or cognitive disorders that are based on aberrations in the brain neurotransmission systems.

Precision Health/Medicine with Integrated Multi-modal Imaging Technologies

Yasuyoshi Watanabe

RIKEN Center for Life Science Technologies (CLST), and
The "Compass to Healthy Life" Research Complex Program
Center Director/Program Director

Integrated multi-modal imaging technologies with Omics analyses and precise biomarker analyses give us the chance to create Precision Medicine and Precision Health. Especially, Positron Emission Tomography (PET) technologies open up a new era of 4-dimentional analyses of molecular events in human body. In combination with functional and anatomical imaging with MRI (Magnetic Resonance Imaging) and MEG (Magnetoencephalogram), total understanding of human dysfunction such as chronic fatigue could be obtained.

Here, we will present recent achievements of Division of Bio-function Dynamics Imaging, CLST, RIKEN, then the contribution of multi-modal imaging to Precision Medicine and Health. To extend this, we are now promoting the Research Complex program under Japanese Government.

The "Compass to Healthy Life" Research Complex aims to serve as a "compass" to help people lead healthier and more fulfilling lives by developing a "virtual-self" tool that will offer accurate guidance for better maintenance and promotion of health. To this end, we are bringing together leading researchers from RIKEN and other research institutes and universities both in Japan and overseas at the Kobe Biomedical Innovation Cluster (an advanced medical technology R&D hub) to combine life sciences, nanotechnology, measurement science, and device and computer sciences in a way that advances our understanding of the human body and enables the building of a computer-based virtual-self tool that people can use to predict their future health status.

The new knowledge and data obtained through this initiative will serve as a substantial foundation for health-related industries and lead to the development of new services and products in various industries. In addition to joint research and development, every effort will be made to build an international hub for health sciences-related business by establishing mechanisms for generating and supporting the rapid implementation of new business ideas and nurturing entrepreneurs.

Ref. Fatigue Science for Human Health, edited by Watanabe, Y. et al., Springer, 2008.

Epigenetic Regulation of Acute Myeloid Leukemia

Andreas Lennartsson

Karolinska Institute
Senior Researcher

Acute myeloid leukemia (AML) has a poor prognosis in both adults and children, with a long-term survival of only 25% and 60% respectively. No major development has occurred of the treatment the last decades and the majority of treatments for AML consist of cytotoxic drugs with low specificity. AML is associated with perturbed epigenetic regulation, with early mutations in and chromosomal translocations of different epigenetic regulators. This indicates that epigenetic mechanisms may play an essential role in the development and AML and are potentially very potent drug targets. A network of epigenetic factors regulates DNA methylation, posttranslational histone modifications and chromatin structure, and relays information to the transcriptional program that dictates hematopoietic cell fate and differentiation. We have previous demonstrated the importance of epigenetic mechanisms in hematopoietic differentiation and AML development. Especially we have showed that epigenetic regulation of enhancer activity is crucial for normal myelopoiesis and AML (Rönnerblad et al. Blood 2014, Qu et al. Blood 2017). We have recently demonstrated that the generation of leukemic-specific gene expression involves an interplay of combinatorial epigenetic mechanisms at specific enhancer elements with their cognate promoters. Our results suggest that the normal epigenetic remodeling of enhancers, is perturbed during the evolution of leukemia and contribute to the leukemic phenotype (Qu et al. Blood 2017).

Transcriptional Response at Promoters and Enhancers After Drug Treatment

Erik Arner

RIKEN Center for Life Science Technologies (CLST)

Unit Leader

Drug response expression profiling has emerged as a powerful method for characterizing the cellular response to drug treatment at a molecular level. In this approach, cells are treated with various drugs and changes in expression compared to negative control are measured. Using this method, it is possible to gain insight into the mode of action (MOA) of drugs, distinguish direct from indirect targets, and also assess off target effects. It is also possible to use the data for drug repositioning, i.e. finding novel therapeutic targets for existing drugs. We here present ongoing research where we use CAGE, a sequencing-based unbiased approach for quantifying promoter and enhancer expression, to develop novel applications based on measuring the transcriptional response following drug treatment. In one project we develop a framework for systematic identification of on-/off-target pathways including adverse effects from drug treatment, by combining expression profiling after drug treatment with gene perturbation of the primary drug target. Using statins as a model system for the framework, expression profiles from statin-treated cells and HMG-CoA reductase knockdowns were analyzed, allowing for identification reported adverse effects but also novel candidates of off-target effects from statin treatment. In a second project we measure the transcriptional drug response at promoters and enhancers using C1 CAGE, a newly developed method for doing CAGE in single cells. By using C1 CAGE, we can address the shortcomings of currently used cell population and array based methods: achieving unbiased expression measurements with high genomic resolution, assess population response heterogeneity, and profile rare cell types. Using this technology we profile the response of HDAC inhibitors in cell lines as well as primary cells, and the response of BRD inhibitors in AML cells and leukemic stem cells. In a third project we show that the transcriptional response to combinatorial drug treatment at promoters is accurately described by a linear combination of the responses of the individual drugs at a genome wide scale, and exploit this to develop a method for identifying drug combinations that facilitate cell conversion.

Peptide Engineering for Therapeutic Applications

Shunsuke Tagami

RIKEN Center for Life Science Technologies (CLST)

Unit Leader

Peptides have been regarded as one of the most promising material for drug development these days because of their high and specific binding affinity to targets and low risks for side effects. In this talk, I will report our recent approach to engineer bacterial peptides with a special 3D structure for drug delivery and PET imaging application. I will also discuss our trial to develop new peptide drugs against bacterial and viral RNA polymerases, by mimicking mechanisms or structures of transcription regulatory factors from bacteria and bacteriophage.

Structure-functional Analysis of Novel Thiazole-oxazole Containing Antibiotic Peptides and Molecular Machines Involved in Their Synthesis

Konstantin Severinov

Skolkovo Institute of Science and Technology/Rutgers, the State University of New Jersey

Professor

Screening of the small-molecule metabolites produced by most cultivatable microorganisms often results in the rediscovery of known compounds. Alternative genome-mining strategies allow to harness much greater chemical diversity and could lead to discovery of new molecular scaffolds. By many criteria, ribosomally synthesized post-translationally modified peptide (RiPPs) are attractive lead compounds for design and development of new bioactive scaffolds. In the talk, results on genome-guided identification of a new RiPP antibiotic, klebsazolicin (KLB), from Klebsiella pneumoniae will be presented. Results of structural and functional analysis of KLB synthesis and maturation pathways and its interactions with the target, bacterial ribosome, will be presented to illustrate the excellent potential to serve as a starting point for rational development of new bioactive compounds.

Advanced Drug Delivery Systems for Medical Innovation

Hidefumi Mukai

RIKEN Center for Life Science Technologies (CLST)

Unit Leader

Agonists, antagonists, and other related molecules are one of the most beneficial things in science but only as raw stones. To polish them and make jewelry, that is for practical drug use, the technology that allows to deliver them to the desired areas and not to the undesired areas is essential; that is drug delivery system (DDS). Currently, we have some workable DDSs, such as liposomes and antibody-drug-conjugates; unfortunately, they are not perfect at all. Nevertheless, the DDS research has recently plateaued. That is probably due to diversity loss, which is a big problem for future pharmaceutical sciences.

Because medical innovation is highly dependent on advancements in technology, it would be a good idea to focus on current hot topics in technology to address future medicine. Synthetic biology is one of them; it will bring a much more developed medicine, where the necessary amount of drugs is produced where and when needed in our bodies. That means a significant change in thinking, from drug delivery systems to drug production systems.

One way to achieve this is the “therapeutic bacterial machine” approach that is functionalized by the assembly of certain building blocks. To realize that, we are addressing the challenges especially concerning the expansion of bacterial species and functional assembly. We demonstrated that *Brevibacillus choshinensis* has preferable characteristics as an effective and safe provider of anticancer protein in the body for bacterial cancer therapy, which is hoped to have a high degree of usability as a delivery system of protein pharmaceuticals from the viewpoints of loading capacity and cost effectiveness. In addition, we are currently developing the CTL-mimic bacterial machine where the function of cancer-selective infection and toxin secretion is assembled. Intravenously injected *Escherichia coli* was found to survive and grow selectively in the tumor; it was successfully infected and located in cytoplasm of cancer cells through cancer targeting peptide display.

We are hoping that the “therapeutic bacterial machine” approach will escape the problems of current drug therapies and lead to medical innovation in future.

Peptide-based Antitumor Technology Using Tumor-homing CPPs

Eisaku Kondo
Niigata University
Professor

Recently, functional peptides are gaining much attention in nanomedicine for their in vivo utility as non-invasive biologics. Among these, cell-penetrating peptides (CPPs) are one of the useful biotools as a molecular carrier for in vivo delivery systems. The TAT peptide is a representative CPP which has been preferentially used for molecular transduction into cells of diverse origins. However, this activity is nonselective between neoplastic and non-neoplastic cells. Here we report the artificial cell-penetrating peptides isolated from the random peptide library using mRNA display technology that shows highly-shifted incorporation into human tumor cells according to their specific lineage. In this session, as an example of these tumor-lineage homing CPPs, we demonstrate the performance of the pancreatic ductal adenocarcinoma (PDAC)-homing peptide (Pancreatic cancer cell-penetrating peptide; PCPP) which we developed and analyzed its distribution in tumor-bearing mouse in vivo.

Peptides also have another utility as a functional regulatory peptide in vivo, which is able to regulate specific cellular activity like hormonal peptides such as Insulin, Oxytocin, somatostatin, and so on. We focused on developing the novel antitumor peptide to suppress tumor cell growth via the specific interaction to the tumor accelerator. Here let us briefly mention about one of those peptides which we have obtained in our studies. Thus, we would demonstrate diverse aspect of peptides as a biotool. Our final aim is to contribute next-step technology to the cancer therapeutics through our peptide research.