Analytical Tools for studying Brain Physiology and Disease

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Selenocysteine-specific mass spectrometry reveals tissue-distinct selenoproteomes and new selenoproteins

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Selenoproteins, defined by the presence of selenocysteines (Sec), play important roles in a wide-range of biological processes. All known selenoproteins are marked by the presence of selenocysteine insertion sequence (SECIS) at their mRNA. The lack of an effective analytical method has hindered our ability to explore the selenoproteome and new selenoproteins beyond SECIS. Here, we develop a Sec-specific mass spectrometry-based technique, termed “SecMS”, that allows the systematic profiling of selenoproteomes by selective alkylation of Sec. Using SecMS, we quantitatively characterized the age- and stress-regulated selenoproteomes for 9 tissues from mice of different ages and mammalian cells, demonstrating tissue-specific selenoproteomes and an age-dependent decline in specific selenoproteins in brains and hearts. We established an integrated platform using SecMS and SECIS-independent selenoprotein (SIS) database and further identified 5 new selenoprotein candidates. We propose that application of this integrated platform provides an effective strategy to explore the selenoproteome independent of SECIS.

Repression of human and mouse brain inflammaging transcriptome by broad gene body histone hyperacetylation

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Brain inflammaging is a major hallmark for aging-related neurodegenerative diseases. Here, by profiling H3K27ac and gene expression patterns in human and mouse brains, we found that age-related upregulated (Age-Up) and downregulated (Age-Down) genes have distinct H3K27ac patterns. Although both groups show promoter H3K27ac, the Age-Up genes, enriched for inflammation-related functions, are additionally marked by broad H3K27ac distribution over

their gene bodies, progressively reduced during aging. Age-related gene expression changes can be predicted by gene body H3K27ac level. Contrary to the presumed transcription activation function of promoter H3K27ac, we found broad gene body hyper H3K27ac suppresses overexpression of inflammaging genes. Altogether, our findings revealed opposite regulations by H3K27ac on Age-Up and Age-Down genes and a novel mode of broad gene body H3K27ac in repressing transcription.

Why monkeys are excellent animals for human brain diseases study - evidence from the disease modeling perspective

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It is well known that the ineffectiveness of rodents models in the development of treatments for several human brain diseases. Many researchers speculate that this issue could be dealt with by using monkeys for their phylogenetic affinity to human, their dependence on social relationships, their ability to engage in complex cognitive processes and their similarities in central nervous system (CNS) functions to human beings compared to the rodents. But to prove this hypothesis, two evidences have to be obtained:
1) The existence of naturally developed brain disease monkeys similar to the human patients. 2 The feasibility of inducing those disease models on normal monkeys so that they can be produced in large quantity to fulfil the demands from both disease study and drug screening. In the lecture, evidences of the two aspects will be presented and we believe that monkeys are excellent animals to develop human brain diseases models.

Metformin induces IKKa dependent chaperone-mediated autophagy and reduces the cytotoxicity of Aβ in Alzheimer's disease

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Metformin is well known as a drug for Diabetes. Recently there is growing evidence that metformin is beneficial to Alzheimer's disease, however the mechanism is not clear. We will provide a model suggesting that metformin could induce IKKa dependent chaperone-mediated autophagy and reduce the cytotoxicity of Aβ. This finding will suggest a new insight for the mechanism of metformin regulating Alzheimer's disease.

Psychiatric disorder biomarker discovery using animal models

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To identify biosignatures for psychiatric disorders and the antidepressant drug response we are using sensitive, high-throughput proteomics and metabolomics platforms that provide a rich source of data for in silico pathway analyses. Quantitative mass spectrometry is used to compare protein expression levels in mouse brains and periphery. This information is complemented with metabolomics data for in silico delineation of affected pathways in specific brain regions. Our ultimate goal is to complement imprecise DSM-based clinical parameters with molecular biosignatures in order to improve patient diagnosis, stratification and treatment. We have analyzed mouse models that represent defined endophenotypes characteristic for human psychiatric disorders including posttraumatic stress disorder, anxiety, and schizophrenia. Drugs that target the monoaminergic (SSRI) and glutamatergic (ketamine) systems are also studied in mice with the goal to delineate mechanisms relevant for the therapeutic response and novel drug targets. Biomarkers predicting a priori whether an individual patient will respond to the treatment of choice and an early distinction of responders and non-responders during antidepressant therapy can have a significant impact for realizing strategic treatment.

Pericytes and myeloid cells in brain tumors share markers and drive angiogenesis but maintain distinct phenotypes

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We investigated neoplastic vascularization of the adult brain in a transgenic lineage-tracing model. We found that our model specifically traces the vast majority of newly generated intratumoral pericytes and a population of myeloid cells. These myeloid cells can express pericyte markers, are of non-hematopoietic origin and remain distant from blood-vessels. The newly generated pericytes are maintained at the vasculature, proliferatively expand and show aberrant morphological features. Together, myeloid cells and pericytes have a strong impact on glioma angiogenesis.

Nanoliter-scale oil-air-droplet chip-based single cell proteomic analysis

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Single cell proteomic analysis provides crucial information on cellular heterogeneity in biological systems. Herein, we describe a nanoliter-scale oil-air-droplet (OAD) chip for achieving multistep complex sample pretreatment and injection for single cell proteomic analysis in the shotgun mode. By using miniaturized stationary droplet microreaction and manipulation techniques, our system allows all sample pretreatment and injection procedures to be performed in a nanoliter-scale droplet with minimum sample loss and a high sample injection efficiency (>99%), thus substantially increasing the analytical sensitivity for single cell samples. We applied the present system in the proteomic analysis of 100 ± 10, 50 ± 5, 10, and 1 HeLa cell(s), and protein IDs of 1360, 612, 192, and 51 were identified, respectively. The OAD chip-based system was further applied in single mouse oocyte analysis, with 355 protein IDs identified at the single oocyte level, which demonstrated its special advantages of high enrichment of sequence coverage, hydrophobic proteins, and enzymatic digestion efficiency over the traditional in-tube system.

Identification of human functional cell groups of the oculomotor system allows the post-mortem analysis of clinical cases with eye movement disorders on the cellular level

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Eye movement abnormalities often provide diagnostic clues in patients, since their premotor network is well-known. With combined tract-tracing and immunocytochemistry in monkey the histochemical signature of functional cell groups of the oculomotor system can be determined. This information is then used to identify homologue cell groups in human brainstem sections, such as premotor burst and omnipause neurons in the pontine and mesencephalic reticular formation, which are essentiell for saccade generation. Here we demonstrate in a post-mortem study of a clinical case with '*saccadic palsy following cardiac surgery*', that the specialized extracellular matrix ensheathing omnipause and burst neurons is damaged thereby providing a possible cause for their malfunction.

Nanopore sequencing and its applications in error-free DNA lesion spotting

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Recent developments in nanopore sequencing are redefining new concepts in personalized medicine and precision medicine. Nowadays, a commercial Minion sequencer could sequence

DNA in a flash-drive sized device, which is inspiring new discoveries of its applications in different fields of researches. Unlike traditional sequencing methods, nanopore sequencing discriminates DNA bases according to differences in their chemical or physical properties, which is highly suitable for epigenetic sequencing applications. We have demonstrated the detection of some uncommon DNA base modifications using nanopore sequencing methods. The results show impressive discrimination between modified and canonical DNA bases with ~0% error rate in single molecule resolution. This desired performance leads to further investigations of its clinical uses.

Activatable molecular probes for multimodality imaging

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Precise measurement of biomolecules in living systems with molecular imaging probes is highly important in unraveling the functional roles of different biomolecules in biological processes. Recent progress have been actively made on the development of a myriad of molecular imaging probes featured with different imaging modalities, including optical imaging, magnetic resonance imaging (MRI), nuclear imaging, and photoacoustic imaging, allowing for non-invasive detection of various enzyme activities *in vivo* with high sensitivity and high spatial resolution. Among these imaging probes, activatable probes whose imaging signals can be specifically switched from “off” to “on” state upon interaction with a biological of interest are particularly attractive owing to the improved sensitivity and specificity. Here I will briefly discuss the general strategy for the design of activatable probes, and give some examples that my lab has been doing to detect brain tumors or Amyloid Beta Species in living mice.