3rd RIKEN CLST-Karolinska Institutet-SciLifeLab Joint Symposium

Frontiers in Life Science Technologies – Decoding Health and Disease

29th September 2016
Conference room Air/Fire at Science for Life Laboratory (Gamma Building)
Tomtebodavägen 23A, Karolinska Institutet Campus in Solna, Stockholm
Welcome note

We extend a warm invitation for your participation in the third RIKEN CLST–Karolinska Institutet–SciLifeLab Joint Symposium to be held in Stockholm, Sweden.

The Joint Symposium was initiated with the objective to build a broader and closer partnership between RIKEN CLST, Karolinska Institutet, and SciLifeLab. The past symposia covered topics such as molecular imaging, molecular regulation, genomics, structural biology and drug discovery.

This year’s symposium entitled Frontiers in Life Science Technologies – Decoding Health and Disease, will focus on technologies and applications to approaching disease and health, and will consist of three sessions; Molecular imaging and disease, Single/stem cells and disease, RNA and disease. The symposium is open to the public, and active participation by scientists, young researchers, students and others interested in the field is very much welcome.
Program

9:00 – 9:30 Registration

9:30 – 9:35 Opening Remarks
Olli-Pekka Kallioniemi, Director, Science for Life Laboratory

General Chair: Kauzhiko Tanzawa (RIKEN CLST)

Session I Imaging & Disease
Chairs: Christer Halldin (Karolinska Institutet), Yasuyoshi Watanabe (RIKEN CLST)

9:35 – 9:55 Diagnostic Radiology in Oncological Clinical Trial- Experiences from a Radiology Clinical Trial Unit at Karolinska University Hospital
Chikako Suzuki, Consultant, Karolinska University Hospital Solna

9:55 – 10:15 [*FJAA-7: A novel PET tracer targeting amino acid transporter with discrimination of inflammation
Satoshi Nozaki, Research Scientist, RIKEN CLST

10:15 – 10:35 Molecular imaging of the serotonin 1B receptor in relation to major depressive disorder
Johan Lundberg, Associate Professor, Karolinska Institutet

10:35 – 10:55 Break

10:55 – 11:15 Sex difference and indifference in brain aromatase expression by clinical PET study
Kayo Takahashi, Senior Scientist, RIKEN CLST

11:15-11:35 PET-imaging of neuroinflammatory markers in CNS-disorders
Lars Farde, Professor, Karolinska Institutet

11:35 – 11:55 Pharmacokinetics study in human with PET molecular imaging
Yasuyoshi Watanabe, Director, RIKEN CLST

11:55 – 12:00 Introduction of JSPS activity
Tadaharu Tsumoto, Director, JSPS Stockholm Office

12:0 – 13:00 Lunch Break
Session II RNA & Diseases
Chairs: Juha Kere (Karolinska Institutet), Harukazu Suzuki (RIKEN CLST)

13:00 – 13:20 Activation of LTR derived non-coding RNAs in human hepatocellular carcinoma
Kousuke Hashimoto, Senior Research Scientist, RIKEN CLST

13:20 – 13:40 Next generation pathology using RNA seq in situ
Joakim Lundeberg, Professor, SciLifeLab Stockholm

13:40 – 14:00 SINEUPs: a unique class of antisense RNA enhancing protein translation
Piero Carninci, Deputy Director, RIKEN CLST

14:00 – 14:20 Gene regulation of insulin response and its dysregulation in obesity
Carsten Daub, Senior Researcher/Coordinator, Karolinska Institutet/RIKEN CLST

14:20 – 14:40 From Discovery to Functional Understanding of Long Non-Coding RNA
Jay W Shin, Unit Leader, RIKEN CLST

14:40 – 15:10 Break

Session III Single, Rare and Stem cells & Disease
Chairs: Carsten Daub (Karolinska Institutet/RIKEN CLST), Yasuhide Furuta (RIKEN CLST)

15:10 – 15:30 Molecular Dynamics of Midbrain Development in Mouse, Human and Stem Cells
Sten Linnarsson, Professor, Karolinska Institutet

15:30 – 15:50 Transcriptional states of oligodendrocyte lineage cells during development and in disease
Gonçalo Castelo-Branco, Associate Professor, Karolinska Institutet

15:50 – 16:10 NG2 progenitor cells and inflammation
Yasuhisa Tamura, Senior Scientist, RIKEN CLST

16:10 – 16:30 Decoding neuronal heterogeneity in the hypothalamus by single-cell RNA sequencing and Patch-SEQ
Roman Romanov, Post-Doctoral Researcher, Karolinska Institutet

16:30 – 16:50 High-resolution characterization of drug-induced cellular response
Erik Amer, Unit Leader, RIKEN CLST

16:50 – 17:10 Transcriptional control of human Early Genome Activation
Juha Kere, Professor, Karolinska Institutet

17:10 – 17:15 Closing Remarks
Yasuyoshi Watanabe, Director, RIKEN CLST

17:15~ Social Gathering at SciLifeLab Lunch Room Gamma2
A “substantial evidence” of effectiveness is required for new cancer treatment regimens before being approved. Various surrogate indicators has been adopted to measure the drug efficacy. Objective tumor shrinkage/enlargement, for example, is one of the most commonly used as a surrogate indicator and is quantified by various imaging techniques; most commonly computed tomography (CT). A high accuracy and reproducibility is, for obvious reasons, necessary in order to achieve a meaningful evaluation of such studies. Moreover, to achieve a meaningful comparison in different judges/institutions, the common language to describe the tumor response, namely the response evaluation criteria, is essential. There are some major criteria, such as the World Health Organization criteria (WHO-criteria), the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1, which has been widely accepted in various clinical trials. Critical inabilities have been, however, pointed in those criteria, especially as the result of the recent remarkable progress in cancer treatment; the major paradigm shift from “cell kill” to “cell control” effect.

Department of Diagnostic Radiology at Karolinska University Hospital, started a dedicated radiology clinical trial September 2001. This unit now manages with more than 70 ongoing oncological clinical trials. In this session, some major practical issues regarding about current clinical trial evaluation criteria will be addressed and limitations and future perspectives will be discussed on the basis of our experience.

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[¹⁸F]AA-7: A novel PET tracer targeting amino acid transporter with discrimination of inflammation

Satoshi Nozaki

RIKEN Center for Life Science Technologies (CLST)/RIKEN Innovation Center
Research Scientist/Deputy Team Leader

[¹⁸F]FDG-PET is associated with false-positive findings due to rebound inflammation after chemo- or radio-therapy in clinical oncology. Radiolabeled amino acids including [¹¹C]methionine present higher specificity for cancer and are less influenced by inflammation than [¹⁸F]FDG. However, as [¹¹C]methionine also accumulates in normal and inflamed tissues to some extent, it is impossible to detect precisely the normal brain tissue–tumor boundary for surgical resection or radiotherapy of gliomas.

Previous studies demonstrated that high expression level of L-type amino acid transporter 1 (LAT1) is a significant factor for worse prognosis in several human cancers including glioma. LAT1 provides cancer cells with amino acids that are necessary not only for protein synthesis but also for the stimulation of tumor cell growth. Moreover, LAT1 expression yielded a significant correlation with cell proliferation (Ki-67 labeling index), the cell cycle regulator p53, and angiogenesis in cancer specimens. Hence, LAT1 is a promising target for both imaging and therapy.

The purpose of this study was to develop a novel tumor-specific PET tracer, targeting LAT1, to assess the safety, biodistribution, and dosimetric properties of the novel PET radiopharmaceutical agent, and to preliminarily evaluate its application in the diagnosis of gliomas.

In a preclinical study, we successfully developed a novel ¹⁸F-labelled LAT1-specific PET probe [¹⁸F]AA-7 by cell-based screening. [¹⁸F]AA-7 highly accumulated in LAT1 positive tumors compared to [¹¹C]methionine. Moreover, although [¹⁸F]FDG also highly accumulated in inflamed regions, noticeable accumulation of [¹⁸F]AA-7 was not observed in the two mice models of inflammation. In a clinical study, PET imaging of [¹⁸F]AA-7 resulted in extremely clear images in patients with suspected glioblastoma.

[¹⁸F]AA-7 may be useful as a novel PET tracer for LAT1-positive tumor imaging, which has low accumulation in inflamed tissues. It may be suitable for PET imaging in patients with glioblastoma, and for early-phase monitoring of cancer therapy outcomes.
Molecular imaging of the serotonin 1B receptor in relation to major depressive disorder

Johan Lundberg
Karolinska Institutet
Associate Professor

The serotoninergic neurons in the primate brain are distributed from the brainstem and throughout basal ganglia, limbic system and neocortex. Its function, modulated by transport proteins and some 14 G-protein coupled receptors, is implicated in cognition, personality and major psychiatric disorders such as major depressive disorder (MDD) and schizophrenia. Selective PET radioligands have been developed for a handful of these targets, enabling in vivo quantification in the monkey and human brain.

To allow in vivo studies of 5-HT1B binding in primates, a selective ligand, AZ10419369, has been labeled with 11-C at the KI PET center. Following successful characterization and validation of [11C]AZ10419369 for quantification of 5-HT1B binding and displacement in the monkey and human brain1,2 the focus has shifted to applied studies.

Globally, Major Depressive Disorder (MDD) is among the top five causes of disability. Present treatment options typically have a remission rate of 30%. Valid biomarkers to predict treatment response are currently not available. There is thus a need for both biomarkers to indicate the most effective treatment for individual patients and for novel treatment options.

Preclinical and human post-mortem data indicate the 5-HT1B receptor to be a putative biomarker and treatment target for MDD. For example, using [11C]AZ10419369 PET in rhesus monkey, Yamanaka et al have shown that [11C]AZ10419369 binding is increased following infusion with the antidepressant ketamine in an AMPA dependent manner3. Also, an association between central 5-HT1B expression and the protein p11 has been described in non-human systems. P11, being available in peripheral blood, constitutes a putative proxy marker for central 5-HT1B binding.

Using [3H]AZ10419369 autoradiography (ARG) and post mortem tissue of control subjects and patients with MDD and other CNS disorders we have examined the sub regional distribution of 5-HT1B binding as well as the diagnostic specificity of post mortem binding quantification in the anterior cingulate cortex. Furthermore we have examined the in vivo 5-HT1B binding in brain regions implicated in the pathophysiology of MDD, in control subjects and unmedicated MDD patients using PET and [11C]AZ10419369. The patients were then reexamined following cognitive behavioral treatment. Relation between change in MDD sensitive cognitive performance and 5-HT1B binding was specifically examined. Finally the relation between change in central 5-HT1B binding and change in peripheral p11 concentration was investigated.

Sex difference and indifference in brain aromatase expression by clinical PET study

Kayo Takahashi
RIKEN Center for Life Science Technologies (CLST)
Senior Scientist

Aromatase is an enzyme converting androgens to estrogens. Several reports suggested its relevance to Alzheimer’s disease, Parkinson’s disease, and autistic spectrum disorder. Animal experiments also showed the involvements of aromatase to aggressive or depressive behaviors. To investigate the association between aromatase and human emotions/characters, one of the most favorable techniques is Positron Emission Tomography (PET), which allows quantitative analysis of the accumulated compounds in tissues. To quantify aromatase expression level in the brain, we developed novel PET tracers for aromatase, $^{11}$C-cetrozole and its analogs, $^{11}$C-meta-cetrozole, $^{11}$C-nitro-cetrozole, and $^{11}$C-iso-cetrozole. Each analog has a difference from cetrozole in position of $^{11}$C-labeling, nitrogen group instead of cyano group, the position of nitrogen in triazole, respectively. $^{11}$C-Cetrozole and 3 analogs were evaluated by PET imaging in nonhuman primates and inhibition rate for aromatase. Then, $^{11}$C-cetrozole and the most advanced analog (iso-cetrozole) were used for clinical PET study in healthy human subjects. In the case of rhesus monkeys, $^{11}$C-iso-cetrozole was superior to $^{11}$C-cetrozole, but it was not the case in humans because of the difference of metabolism. Using $^{11}$C-cetrozole, PET scans were performed with 21 healthy human subjects (10 females and 11 males). All subjects were asked to answer the questionnaire to assess their aggression and characters (Buss-Perry Aggression Questionnaire, and Temperament and Character Inventory). High accumulation of $^{11}$C-cetrozole was observed in the thalamus, medulla, amygdala, and hypothalamus. Aggression score was correlated with the binding potential of $^{11}$C-cetrozole (aromatase level) in the female amygdala. The score of cooperativeness was correlated with the binding potential in the thalamus of both females and males. These results indicate that some traits may relate to sex hormonal system in the brain in a sex-dependent manner and the other traits may relate in common way between sexes.
PET-imaging of neuroinflammatory markers in CNS-disorders

Lars Farde
Karolinska Institutet
Professor

The immune surveillance system in brain is thought to play a role in the pathogenesis of a number of neurological and psychiatric disorders. The translocator protein (TSPO) is predominantly expressed by microglia cells, the resident macrophages in brain, and may serve as a disease biomarker. For that purpose, radioligands such as [11C]PBR28 have been developed for PET imaging of TSPO-binding in the human brain.

At the RIKEN-KI symposium 2014, we presented our initial experience with [11C]PBR28. We have subsequently examined whether there is an interchange of substance or information between immune cells in brain and the periphery at physiological conditions (1). We included a total of 32 healthy individuals of which 26 individuals were examined twice with varying time intervals. When comparing TSPO binding in brain and blood cells, there was a strong positive correlation both at baseline and when analyzing change between two PET examinations. These results indicate an association between immunological cells in blood and brain via intact BBB.

Myeloperoxidase is a reactive oxygen generating enzyme expressed by microglia. The novel AstraZeneca compound AZD3241 is a selective and irreversible inhibitor of myeloperoxidase. The hypothesized mechanism of action of AZD3241 involves reduction of oxidative stress leading to reduction of sustained neuroinflammation. The purpose of a phase 2a randomized placebo controlled multicentre PET study was to examine the effect of 8 weeks treatment with AZD3241 on microglia in patients with Parkinson’s disease (2). In the AZD3241 treatment group (n = 18) the total distribution volume of 11C-PBR28 binding to TSPO was significantly reduced compared to baseline both at 4 and 8 weeks. The reduction of 11C-PBR28 binding to TSPO in the brain of patients with Parkinson’s disease after treatment with AZD3241 supports the hypothesis that inhibition of myeloperoxidase has an effect on microglia. To our knowledge this is the first study demonstrating a drug effect on TSPO binding in the living human brain.

In vivo molecular imaging has become a key technology for medical innovation, i.e., integrated live science, patho-physiological science, molecular evidence-based diagnosis, innovative drug development, and preemptive medicine. Rapid progress in life science brought the stage up to the living objects or subjects, even functioning human beings. The invention of a variety of imaging techniques including Positron Emission Tomography (PET) accelerated the paradigm shift in life science from patho-physiological science in disease model animals to that realized in patients. Especially, the molecular imaging techniques could promote the bridging between findings in gene-manipulated animals and those in healthy volunteers and patients. When the biomarkers for early detection of signs toward diseases and also those for surrogate end-point were established and their changes could be followed by molecular imaging, it could be quite beneficial for preemptive medicine and evaluation of therapeutic outcome. For example, beta-amyloid imaging and pancreatic beta-cell mass imaging have been highlighted for prediction of Alzheimer’s disease and diabetes mellitus, respectively. Such types of valuable biomarkers related to the cause or influencing factors are also really important to develop the new drugs. In this sense, mutual collaboration among the research consortia in biomarker exploration, in gene manipulation, and in molecular imaging would be really important. In this context, in 2005, our Center in RIKEN was selected as the key hub center of the Molecular Imaging Research Program under MEXT, Japanese Government, for development of All-Japan research network to further promote mutual international and multi-disciplinary collaboration on in vivo molecular imaging. To realize the molecular probing concept, we have so far developed a variety of novel chemical methods for labelling the low and higher molecular weight compounds with due positron emitters, such as C-11, F-18, Ga-68, and Cu-64, and the number of different types of the molecular probes to be used in PET study is more than 265 so far developed in our Center and the repertoire being increasing every day. Direct application of this molecular probing concept is to pharmacokinetics and efficient DDS development. The concept, outline of our activities, focusing on PK/PD studies with efficient application of molecular imaging will be presented.
Hepatocellular carcinoma (HCC) accounts for 70-85% of the total liver cancers. The major risk factors of HCC are chronic HBV and HCV infections and alcohol. The development of HCC is a heterogeneous multistep process associated with genetic alteration and dysregulation of gene expression. We used CAGE to map transcription start sites across various types of human HCCs with emphasis on ncRNAs distant from protein-coding genes. We found that retroviral LTR promoters, expressed in healthy tissues such as testis and placenta but not liver, are widely activated in liver tumors. Despite HCC heterogeneity, a subset of LTR-derived ncRNAs were more than 10-fold up-regulated in the vast majority of samples. HCCs with a high LTR activity mostly had a viral etiology, were less differentiated and showed higher risk of recurrence. Globally, CAGE enabled us to build a mammalian promoter map for HCC, which uncovers a new layer of complexity in HCC genomics.
Histological analysis with different staining techniques first established the basic structural organization of healthy organs and the changes that take place in common pathologies more than a century ago. Yet these histological techniques are limited by low throughput, being restricted to the analysis of single or few markers in individual tissue sections. With the introduction of next generation sequencing more global and quantitative measurements can be achieved. In this presentation a new approach will be demonstrated that combines histology and RNA sequencing denoted Spatial Transcriptomics. This allows for visualization and quantitative analysis of the transcriptome with spatial resolution in individual tissue sections. This presentation will demonstrate some key applications with applications in diseased tissue.
SINEUPs: a unique class of antisense RNA enhancing protein translation

Piero Carninci
RIKEN Center for Life Science Technologies (CLST)
Deputy Director

High throughput transcriptome studies have identified that the majority of genome is transcribed into long non-coding RNAs (lncRNAs). An important subset of lncRNA is constituted by antisense RNAs, which generally known to suppress the expression of the sense genes.

We have identified a class of non-coding antisense RNAs, which have surprising property to up-regulate protein translation of the sense RNA that they overlap. Enhancement of protein translation is mediated by SINE elements (SINEB2 in the mouse versions), which share common ancestor with tRNAs. The specificity of action is mediated by the region antisense to the 5'UTRs of the target mRNAs. We renamed these RNAs “SINEUPs”, because they contain a SINE elements that can up-regulate translation.

We are extensively characterizing natural SINEUPs and the functional domain of this interesting new class of RNAs, identifying several novel mouse and human natural SINEUPs and mapping the functional domain of these lncRNAs. Although the active SINE elements differ in sequences, they show common function. Importantly for future gene therapeutics applications, artificial SINEUPs can also be designed to regions around the 5'UTR of target RNAs. Although mostly used SINEUPs originate from the mouse, they are active in other mammalian and lower vertebrates, suggesting that specie-specific SINEs have function that goes across species borders, probably mediated by specific structures.

For the flexibility of their synthesis, SINEUPs can be used broadly to address many purposes, which range from increasing protein expression in the laboratory, industrial production of recombinant protein and future therapies of haploinsufficiencies.
Gene regulation of insulin response and its dysregulation in obesity

Carsten Daub
Karolinska Institutet/RIKEN Center for Life Science Technologies (CLST),
Senior Researcher/Coordinator

Human white adipose tissue responds to insulin by taking up blood glucose. In obese individuals, this glucose uptake is impaired and can lead to type 2 diabetes. However, up to 30% of obese individuals display normal glucose levels and respond well to insulin. We performed Cap Analysis Gene Expression (CAGE) transcriptome profiling of obese insulin resistant and insulin sensitive individuals as well as in lean controls both at fasting level and at high blood sugar level (hyperglycemia). We identified the insulin response mediating genes as well as they key regulation events driving these responses and their dys-regulation in obese individuals including transcription factors and enhancers.
From Discovery to Functional Understanding of Long Non-Coding RNA

Jay W. Shin
RIKEN Center for Life Science Technologies (CLST)
Unit Leader

In the 6th edition of FANTOM, we aim to broadly classify long non-coding RNA by large-scale perturbation and infer molecular phenotype using CAGE. As a pilot study, we established a systematic workflow to knockdown 300 IncRNAs in human dermal fibroblasts followed by deep CAGE sequencing. The functional genomics analyses revealed diverse groups of molecular phenotypes – including chromatin remodeling, cell migration – and in some cases, no apparent changes in the transcriptome. Further integrating newly identified molecular phenotypes to RNA classes; we could reveal localization-, conservation-, and strand-dependency to many novel IncRNAs. In this presentation, I will highlight both global and specific characteristics of IncRNAs and demonstrate functional relevance in both health and disease. The combined “wet” and “dry” approach to interrogate IncRNA sets the basis for the next phase of FANTOM6, allowing the investigation of thousands of IncRNAs in cellular contexts.
Molecular Dynamics of Midbrain Development in Mouse, Human and Stem Cells

Sten Linnarsson
Karolinska Institutet
Professor

The ventral midbrain is of major interest for the development of therapies for Parkinson’s disease. We have used single-cell RNA-seq to explore the development of the ventral midbrain in mouse and human, and compared it to the adult mouse. We found 25 molecularly distinct human cell types, including five subtypes of radial glia-like cells and four progenitors. In the mouse, two mature embryonic dopaminergic neuron subtypes were found to diversify into five adult classes during postnatal development. Cell types and gene expression were generally conserved across species, but with clear differences in cell proliferation, developmental timing and dopaminergic neuron development. We next used these findings as a reference to rigorously assess dopaminergic neurons derived in vitro from human pluripotent stem cells, at a single-cell level. We show that in vitro cells mimic the in vivo developmental program, including progenitors, neuroblasts and multiple types of mature neurons. Our study showcases the power of single-cell RNA-seq to reveal developmental processes both in vivo and in vitro.
Oligodendrocytes are glial cells that mediate myelination of neurons, a process that allows efficient electrical impulse transmission in the central nervous system. An autoimmune response against myelin triggers demyelination in multiple sclerosis (MS). Oligodendrocyte precursor cells (OPCs) can initially differentiate and promote remyelination in MS, but this process eventually fails in progressive MS. OPCs undergo through several states during development and disease, that ultimately define their potential to differentiate and myelinate. In order to clearly define distinct transcriptional/epigenetic states of OPCs and other oligodendrocyte lineage cells during development, we have performed single-cell and bulk RNA sequencing of cells of the oligodendrocyte lineage from mouse brain. We identified several cell states/populations, representing unique stages during the process of differentiation, myelination and final stages of maturation. We have further identified non-coding RNAs that are uniquely expressed in these distinct populations of the oligodendrocyte lineage. We are currently investigating if these non-coding RNAs play important roles in OPC differentiation and myelination.

Stem/progenitor cells are immature cells with the capacity for self-renewal and differentiation. There are two types of stem/progenitor cells in the adult brain. One of them is glial fibrillary acidic protein (GFAP)-expressing special astrocytes that are present in only two regions: subventricular zone of the lateral ventricles and subgranular layer of the hippocampal dentate gyrus. The other is NG2-expressing progenitor cells that are ubiquitously distributed throughout the gray and white matter in the adult brain. GFAP-expressing stem/progenitor cells produce mainly new neurons whereas NG2 progenitor cells give rise to a few of mature oligodendrocytes and a lot of themselves throughout life. Recently, it has been reported that NG2 progenitor cells could rapidly proliferate and migrate to maintain their cellular density in response to loss of the cells following brain injury. NG2 progenitor cells are consistently renewed under the physiological and pathological conditions. These suggested that NG2 progenitor cells might be essential for maintaining brain environment, although new roles of the progenitor cells without cell genesis have not been elucidated.

We produced transgenic rats expressing herpes simplex virus thymidine kinase (HSVtk), known as a suicide gene, under control of the NG2 promoter gene to investigate the new roles of NG2 progenitor cells. Almost all NG2 cells expressed HSVtk in the brain of transgenic rats. When ganciclovir (GCV), an antiviral drug, was infused continuously into the lateral ventricle of the transgenic rats, the proliferating NG2 cells disappeared in surrounding area of the lateral ventricle. GCV-treated transgenic rats also showed degeneration of nerve fibers and neuronal cell death in these regions as well as enlarged lateral ventricle along with a decrease in the number of NG2 cells. Concomitantly with these phenomena, a lot of activated CD11b-expressing microglia were observed in these regions. Almost all the activated microglia in the periventricular area were immunopositive for inducible nitric oxide synthase (iNOS), indicating that these cells were pro-inflammatory M1 microglia, but not anti-inflammatory M2-type cells. Moreover, ablation of NG2 progenitor cells led to increased expression of pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α and decreased expression of anti-inflammatory cytokines: IL-4, IL-13and TGF-β.

These findings suggested that NG2 progenitor cells maintain brain function by regulating the immune system in the brain.
Decoding neuronal heterogeneity in the hypothalamus by single-cell RNA sequencing and Patch-SEQ

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Karolinska Institutet
Post-doctoral researcher

The hypothalamus undoubtedly contains the highest diversity of neurons in the brain, which use dependently co-release neurotransmitters and neuropeptides. Investigators have hitherto relied on candidate protein-based tools to correlate behavioral, endocrine and gender traits with hypothalamic neuron identity. We first used ‘Fluidigm C1’ and then ‘Drop-seq’ single-cell RNA-sequencing to generate a comprehensive catalogue of neuronal identities in the midline column of hypothalamic nuclei. We distinguish 62 neuronal subclasses, many of which cluster uniquely through novel identity marks (transcription factors, neuropeptides, receptors and their combinatorial co-existence). We also reveal neuronal subtypes whose molecular make-up allows for either the coincident use or the rapid switching of at least two classical non-peptide neurotransmitters. In contrast to many glutamatergic neuronal clusters, both GABA and dopamine neurons contain at least one neuropeptide for probable co-release. We then applied this knowledge to anatomically map and functionally interrogate intra-hypothalamic synaptic circuits that regulate bodily metabolism. Here, we will discuss the predictive power of our transcriptomics-based data emphasizing the expression of neuropeptides (>50 analysed) and their receptors in delineating neurochemically-segregated intra-hypothalamic connectivity maps. We will exemplify the usefulness of a multiparametric approach relying on circuit mapping, transgenic reporters, Ca²⁺ imaging and optical imaging in intact tissues by defining a neureomedin S (NmS)-Nmur2 axis between glutamatergic suprachiasmatic nucleus neurons and periventricular dopamine cells. We show that our pathway mapping can successfully resolve the conundrum of which neurons integrate dopamine output into the circadian circuitry. Lastly, we describe ‘Patch-seq’ that combines ex vivo electrophysiology with single-cell RNA sequencing to reveal molecular underpinnings of neuronal excitability. Overall, a combined optogenetics-electrophysiology-single-cell RNA-seq platform is presented for the in situ molecular profiling of neurons entraining specific ensembles of synaptically-wired neuroendocrine output cells. Thus, we are confident to reveal the intrinsic heterogeneity of hypothalamic neurons and the hierarchy of neuronal circuits regulating body metabolism.

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High-resolution characterization of drug-induced cellular response

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. The major resources currently available are the Connectivity Map (CMAP) and the Library of Integrated Cellular Signatures (LINCS). Using the drug response expression profiling approach, there are many cases of successful drug repositioning, especially for cancer drugs, as well as novel insights into MOA.

Although useful, the existing resources lack in several important aspects, regarding the resolution they can achieve and which kinds of cell types can be profiled. Firstly, the response measure is biased by only considering a fixed set of genes to profile. This may leave out genes that are very specific to the pathways involved, and in addition, RNAs not commonly present on microarrays such as long non-coding RNAs (lncRNAs) and enhancer RNAs (eRNAs). By using a sequencing based method it is possible to get an unbiased response measure, and also obtain specific response profiles at different genomic elements such as enhancers and promoters. Secondly, studies of drug response in bulk cell culture do not address the heterogeneity in the drug response. Several studies have shown that cells do not react to drug treatment in a uniform way, including at the transcriptional level. If done at the single cell level, it is possible to get a more precise characterization of the response, and to identify genes and pathways that enable or disable an efficient response, by using advanced gene network reverse-engineering approaches, which require multiple expression profiles to work properly. Thirdly, existing resources are mainly done in cancer cell lines, which are not always good models for in vivo situations. In particular, rare cell types are impossible to profile using bulk cell approaches.

In this project, we measure the transcriptional drug response at promoters and enhancers using C1 CAGE, a newly developed method for doing CAGE in single cells. CAGE is the only validated technology that robustly detects expression at enhancers and promoters in a single experiment at genome wide scale. By using C1 CAGE, we can address the shortcomings of currently used methods outlined above, and in addition to achieving unbiased expression measurements with higher genomic resolution, we are also able to assess population response heterogeneity as well as profile rare cell types.
Transcriptional control of human Early Genome Activation

Juha Kere
Karolinska Institutet, Department of Biosciences and Nutrition, CIMED and Science for Life Laboratory
Professor

The earliest stages of human development involving days before embryo implantation remain poorly charted. After fertilization of the egg cell, the embryonal development starts with its individual transcriptome activation (Embryo Genome Activation, EGA) accompanied by the degradation of maternal transcripts, to be followed later with new waves of transcriptional activation. Due to their nature, these steps are especially amenable to transcriptomic analysis, but pose also challenges such as ≈30-fold changes in cellular mRNA content. In order to understand these critical steps, we performed single-cell transcriptome sequencing of over 340 cells, including oocytes, zygotes and single blastomeres from 4-cell and 8-cell embryos, obtained by informed consent as donations after in vitro fertilization treatments. The total content of mRNA molecules remained essentially unchanged between oocytes and zygotes.

Comparison of the transcriptomes of oocytes and 4-cell stage blastomeres identified the first 32 embryonally transcribed genes, including previously uncharacterized transcripts and promoters, as well as the significant reduction of thousands of maternal transcripts. At the 8-cell stage, 129 additional genes were upregulated compared to the 4-cell stage. 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. We cloned and confirmed the genomic structures of seven new regulatory genes expressed only during EGA. Functional analysis of one of these, LEUTX, suggests its central role for EGA.

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.