20th Surugadai International Symposium &Joint Usage/
Research Program of Medical Research Institute Symposium

PROGRAM&ABSTRACT BOOK
プログラム・抄録集

20th Surugadai International Symposium

Recent Advances in Computational and Experimental Structural Biology

Mon 28 Nov 2022 13:00▶17:00 via Zoom

難治疾患共同研究拠点シンポジウム

2022年11月28日（月）

10:30▶12:00 オンライン開催

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国立大学法人 東京医科歯科大学 TOKYO MEDICAL AND DENTAL UNIVERSITY

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20th Surugaidai International Symposium & Joint Usage/ Research Program of Medical Research Institute Symposium

Monday, November 28, 2022

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Surugadai International Symposium via Zoom

“Recent Advances in Computational and Experimental Structural Biology”

難治疾患共同研究拠点シンポジウム(オンライン開催)

Welcome to the Tokyo Medical and Dental University
Medical Research Institute

Dr. Hiroshi Nishina
Director
Medical Research Institute

The Medical Research Institute of Tokyo Medical and Dental University (TMDU) is focused on tackling important issues in medical science. It is our goal to understand the basic mechanisms of pathogenesis of various intractable diseases, such as cancer and cardiovascular, neurological, metabolic and immunological disorders, and to develop measures to diagnose and treat patients with these afflictions.

To reach our goal, we employ state-of-the-art techniques in molecular biology, cellular biology and clinical science, and take advantage of insightful animal models as well as materials from patients. We are now further expanding our research approaches to include organoid formation and single cell analysis, new methods that should provide fresh basic insights into these challenging diseases and may also point towards novel therapeutic approaches. Our Institute thus enjoys a formidable arsenal of clinical and experimental resources that can be brought to bear on numerous unmet medical needs.

The Institute also plays an important role in educating medical and dental students, graduate students, and early career researchers. These individuals are of diverse backgrounds and include those studying medicine, dentistry, mammalian biology, and pharmacology. The number of young investigators in our Institute has increased significantly of late and our educational system has been attracting the attention of the medical science community.

A key objective of our Institute is to establish and maintain scientific ties with a large number of overseas universities and institutions in order to facilitate international collaborations. Our ready acceptance of many visitors from around the world, coupled with our constant sponsoring of international symposia and seminars, ensure that our Institute continues to grow its knowledge and maintain its cutting-edge position in medical research.

The unprecedented and devastating COVID-19 pandemic has affected all people on earth and, sadly, is still ongoing. As scientists working in the field of medical science, it is our responsibility to join global efforts to elucidate the pathological mechanisms of viral infection and spread, and to develop effective drugs and vaccines. We also need to develop new disciplines and research methods. To this end, it is with great pleasure that we invite world-class researchers in the field of computational and experimental structural biology to the 20th Surugadai International Symposium in 2022.

20th Surugadai International Symposium

“ Recent Advances in Computational and Experimental *Structural Biology* ”

13:00～17:00

20th Surugadai International Symposium Program

"Recent Advances in Computational and Experimental Structural Biology"

Monday, November 28, 2022 13:00-17:00 via Zoom

13:00-13:05 **Opening Remarks**

Dr. Hiroshi Nishina (Director, Medical Research Institute, TMDU)

Session1 "Computational Structural Biology"

13:05-14:55

Chair: Dr. Yuta Kochi (Medical Research Institute, TMDU)

13:05-13:35 Dr. Hafumi Nishi (Tohoku University)

Title: AlphaFold2: What It Is and What It Is Not

13:35-14:05 Dr. Mitsunori Ikeguchi (Yokohama City University)

Title: Molecular dynamics simulations of biomolecules

14:05-14:35 Dr. Daron Standley (Osaka University)

Title: Molecular mechanism of SARS-CoV-2 infection-enhancing antibodies

14:35-14:55 Dr. Nobutoshi Ito (Medical Research Institute, TMDU)

Title: Protein Data Bank: To be more reliable source

14:55-15:15 Break

Session2 "Cryo-electron microscopy"

15:15-16:55

Chair: Dr. Itoshi Nikaido (Medical Research Institute, TMDU)

15:15-15:45 Dr. Yoshinori Fujiyoshi (Cellular and Structural Physiology Laboratory, TMDU)

Title: Structural physiology and Structure-Guided Drug Development

15:45-16:15 Dr. Kayo Nozawa (Tokyo Institute of Technology)

Title: Structural and biochemical analysis of a novel structural unit of chromatin

16:15-16:55 Dr. Vinothkumar Kutti Ragunath (National Centre for Biological Sciences,India)

Title: Tools for validation of CryoEM maps and models

16:55-17:00 **Closing Remarks**

Dr. Yuta Kochi (Division Chief, Medical Research Institute, TMDU)

Session 1

“ Commputational StructralBiology ”

13:05～14:55

AlphaFold2: What It Is and What It Is Not

Dr.Hafumi Nishi

Tohoku University

Abstract

As the atomic structure of protein molecules is fundamental to understanding protein characteristics, protein structure prediction was a long-standing research challenge in structural bioinformatics. In particular, proteins without homologous structure information are classified as "difficult" targets, and the prediction accuracy of such targets had stagnated for more than a decade. However, the advent of AlphaFold2 in December 2020 suddenly changed the entire picture. Furthermore, AlphaFold2 and its precalculated database, AlphaFold Structure Database, have been accessible to the public since July 2021, which has been regarded as the beginning of "a new golden age of protein informatics."

Currently, AlphaFold2 is known as the best structural prediction software. Since its release, a vast number of research papers using AlphaFold2 have been published. Although its original paper claimed that AlphaFold2 could predict various targets accurately, such as proteins binding to other molecules, it is debatable whether AlphaFold2 produces acceptable models for any targets. Furthermore, various successors of AlphaFold2 have also been developed, some of which can generate more accurate models for proteins without homologs, e.g., orphan proteins and artificially designed proteins.

In this talk, I will cover the background of the emergence of AlphaFold2 and the recent advances in structural prediction. I will begin with the history of protein structure prediction and then describe how AlphaFold2 has altered the landscape. Then, the usage of the web version of AlphaFold (ColabFold) and the interpretation of results will be briefly explained. The recent advances in related methods and databases will also be discussed. Lastly, I will demonstrate the limitations of AlphaFold and other AI-based prediction tools by providing examples of their failure.

Keywords　Protein structure prediction, AlphaFold2, ColabFold

Short biography

Hafumi Nishi has been an associate professor at Tohoku University since 2018. After receiving her Doctor of Science from Tokyo Institute of Technology in 2010, she spent two and half years at the National Center for Biotechnology Information, National Institutes of Health (NCBI, NIH) as a postdoctoral research fellow and then moved to Yokohama City University. In 2014, she began working at Tohoku University as an assistant professor.

Specialty　Bioinformatics (especially structural bioinformatics) and computational biology

Present interests　Multiomics (genomics, transcriptomics, proteomics, and structuromics)

Molecular Dynamics Simulations for Biomolecules

Dr.Mitsunori Ikeguchi

Yokohama City University

Abstract

Protein dynamics are important for functional expression. Proteins dynamically change their conformation upon ligand binding or chemical modification. Molecular dynamics simulation is a theoretical method for studying such protein dynamics in computers. Molecular dynamics simulation has been expanding its range of application with the improvement of computational power. In this talk, our recent applications of molecular dynamics simulations will be presented.

DNA methylation plays an important role in the regulation of gene expression. DNA methylation is inherited by daughter cells when cells proliferate, which is a mechanism known as DNA methylation maintenance. In the DNA methylation maintenance, two proteins, DNA (cytosine-5)-methyltransferase 1 (DNMT1) and UHRF1, are required. UHRF1 recognizes hemimethylated DNA generated just after DNA replication and recruits DNMT1 to catalyze the transfer of methyl groups to the nascent DNA. UHRF1 consists of five functional domains, of which the TTD domain has a "peptide binding cleft" that serves as a scaffold for binding of other factors. In this cleft, linker 2 and spacer, which are linker regions within the UHRF1 molecule, histone H3 with trimethylated K9, and DNA ligase 1 with trimethylated K126 are bound. Furthermore, linker 2 initially binds to the cleft and phosphorylation of S298 of UHRF1 within linker 2 weakens its binding, allowing other binding factors to bind to the cleft. Although the structure of the TTD domain of UHRF1 has been determined by X-ray crystallography, the structure after phosphorylation of S298 was not known. Therefore, we phosphorylated S298 computationally and performed molecular dynamics simulations, which revealed that phosphorylated S298 disrupts the interaction between D142 and R296, exposing the cleft and allowing other binding factors to bind.

The DNA methylation maintenance is enhanced in cancer cells, making it a target for developing anticancer drugs. Therefore, we performed in-silico screening against UHRF1 to find compounds that inhibit UHRF1. We selected candidate compounds from a virtual chemical library by docking simulation against the peptide binding cleft of UHRF1. The binding stability was then evaluated by molecular dynamics simulations. As a result, a compound with binding activity was found experimentally, and the complex structure was determined by X-ray crystallography. Furthermore, it was found that the compound was able to inhibit the binding of the original factor to UHRF1.

As described above, molecular dynamics simulations can be used complementarily with structural biology experiments to analyze protein dynamics. In this talk, I would like to discuss the potential of molecular dynamics simulation, including other applications.

Keywords

molecular dynamics, protein dynamics, molecular simulation, in-silico screening

Short biography

Education: Ph.D., Agriculture, The University of Tokyo (1994)

Employment:

1994-2001	Research Associate, The University of Tokyo
2001-2015	Associate Professor, Yokohama City University
2015-present	Professor, Yokohama City University
2018-present	Unit Leader, RIKEN

Specialty and present interests

My specialty is molecular simulation for biomolecules. My current interests are to expand applicability of molecular simulations, particularly combined with machine learning.

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Kori S, Shibahashi Y, Ekimoto T, Nishiyama A, Yoshimi S, Yamaguchi K, Nagatoishi S, Ohta M, Tsumoto K, Nakanishi M, Defossez PA, Ikeguchi M, Arita K. Structure-based screening combined with computational and biochemical analyses identified the inhibitor targeting the binding of DNA Ligase 1 to UHRF1. Bioorg Med Chem. 52:116500 (2021).

Zhang Z, Nomura N, Muramoto Y, Ekimoto T, Uemura T, Liu K, Yui M, Kono N, Aoki J, Ikeguchi M, Noda T, Iwata S, Ohto U, Shimizu T. Structure of SARS-CoV-2 membrane protein essential for virus assembly. Nat Commun. 13:4399 (2022).

Ekimoto T, Kudo T, Yamane T, Ikeguchi M. Mechanism of Vitamin D Receptor Ligand-Binding Domain Regulation Studied by gREST Simulations. J Chem Inf Model. 61:3625-3637 (2021).

Osaki K, Ekimoto T, Yamane T, Ikeguchi M. 3D-RISM-AI: A Machine Learning Approach to Predict Protein-Ligand Binding Affinity Using 3D-RISM. J Phys Chem B. 126:6148-6158 (2022).

Molecular mechanism of SARS-CoV-2 infection-enhancing antibodies

Dr.Daron M. Standley

Research Institute for Microbial Diseases, Osaka University

Abstract

One of the remarkable features of the adaptive immune system is the ability to generate antibodies that can bind to previously unknown antigens with high affinity and specificity. While this process generally prevents infection from myriad pathogens, it can sometimes result in off-target effects. One such unintended effect is the production of infection-enhancing antibodies. Recently, Arase and coworkers in Japan and Saunders and coworkers in the US simultaneously identified a group of antibodies that target an overlapping site on the SARS-CoV-2 spike protein's N-terminal domain (NTD) and enhance virus infectivity of human cells by facilitating spike protein binding to the ACE2 host entry receptor (Liu et al, Cell, 2021; Li et al, Cell 2021). Although the molecular mechanism of the observed infection enhancement has not been demonstrated conclusively, multiple lines evidence points to a model involving crosslinking of adjacent spike proteins. Structural analysis led us to hypothesize that, such crosslinking may decouple the spike's NTD from the receptor-binding domain (RBD), freeing the RBD to transition to the "up" state, where it can bind ACE2. In order to test this hypothesis, we carried out extended molecular dynamics simulations of such crosslinked spikes embedded in a biological membrane using the Fugaku supercomputer. We monitored the distances to neighboring RBDs of antibody-bound and unbound NTDs. We found that, indeed, the bound NTDs were significantly more separated from neighboring RBDs than unbound NTDs, supporting our hypothesis. We further examined the distribution of antibodies that were highly similar to known enhancing antibodies in the B cell receptor (BCR) repertoires of healthy donors and COVID-19 patients. We found that the distributions of sequence identities were similar in the two donor groups. However, when we expressed the enhancing-like BCRs as antibodies and tested their binding to the known enhancing site on the spike NTD, we found that the frequency of true binders was roughly 100 times lower in healthy donors. Furthermore, when we assessed the ACE2 binding enhancement of the NTD binders, we found that many of the COVID-19 patient-derived, but none of the healthy donor-derived, antibodies exhibited the enhancing effect (Ismanto et al, bioRxiv 2022.07.09.499414). Taken together, these results suggest that enhancing antibodies are far more frequent in COVID-19 patients than in healthy donors, but that a reservoir of potential enhancing antibodies exists in healthy donors that could potentially mature to actual enhancing antibodies upon infection.

Keywords COVID-19, SARS-CoV-2, Infection enhancing antibodies, Antibody repertoire, InterClone

Short biography

Professor Standley received his PhD in Chemistry from Columbia University in 1998. He then joined Schrodinger,Inc. where he worked as a scientific software developer for 5 years.

In 2003 he moved to the institute for Protein Research Osaka University as a Senior Scientist. He joined the Immunology Frontier Research Institute (IFReC) as a Principal Investigator in 2008 and after a two-year cross appointment at Kyoto University's Institute for Virus Research, became a professor full time at the Research Institute for Microbial Diseases in 2016.

Specialty and present interests

Bioinformatics immunology

References

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Protein Data Bank: To be more reliable source

Dr.Nobutoshi Ito

Tokyo Medical and Dental University

Abstract

Rapid progress of artificial intelligence (AI) in recent years has been making a big impact on the structural biology and the impact will continue to increase in the future. On the other hand, machine learning generally requires a large amount of experimental data, whose quality influences the result from AI significantly.

Unlike genome sequencing, where data are discrete, most methods used to determine protein 3D structure contain "interpretation of data" by the investigator and are, therefore, relatively prone to errors. Unfortunately, history has proven it and quite a few erroneous structures were published in the past. To minimize such risk, the solid validation of protein structure is essential and various methods have been proposed.

Protein Data Bank (PDB) has been the main source of 3D protein structures since it was established at the Brookhaven National Institute in 1971. It is now maintained by joint effort of wwPDB, whose members are Research Collaboratory for Structural Bioinformatics (RCSB), PDB Europe (PDBe), PDB Japan (PDBj), Biological Magnetic Resonance Data Bank (BMRB) and Electron Microscopy Data Bank (EMDB).

PDB has been and will be continuing to improve its data both in quality and format. Various task forces have been assigned to achieve this purpose and useful recommendations were made. PDB now offers various tools and result of structure validation to both the depositors of structures and the users of the archive.

Keywords　Protein Data Bank, Structure Validation, X-ray crystallography

Short biography:

1986	B. Eng from University of Tokyo
1991	Ph D from University of Leeds
1991–1992	Research Fellow, University of Leeds
1992–1994	Scientific Staff, MRC Laboratory of Molecular Biology
1994–1996	Research Fellow, University of Leeds
1996–2001	Senior Research Fellow, Biomolecule Engineering Research Institute
2001–2003	Research Fellow, Protein Data Bank Japan
2003–	Professor, Tokyo Medical and Dental University

Specialty and present interests　X-ray crystallography

References

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Session 2

“ Cryo-electron Microscopy ”

15:15～16:55

Structural physiology and Structure-Guided Drug Development

Dr.Yoshinori Fujiyoshi

TMDU Advanced Research Institute, Tokyo Medical and Dental University

Abstract

Elucidation of brain functions and other biological functions requires molecular-level structural analyses of research targets in different functional states. To understand the dynamic function of a protein in detail, at least some of its structures must therefore be analysed in different states. Conformational changes of proteins will however destroy the crystals or deteriorate their crystallinity. These obstacles might deter researchers from attempting structural studies of their targets of interest. In late years, the field of structural biology, nevertheless, underwent dramatic changes due to advancements in single particle analysis based on cryo-electron microscopy (cryo-EM). The number of analysed structures is dramatically increasing in recent years. By this method and our cryo-EM system, we could analyse structure of innexin gap junction channel in short period, two months, although we could not analyse the structure by crystallography for long years. Therefore, single particle analyses utilizing cryo-EM could serve scientists in wider research field. For example, this method enables us to challenge to develop a new strategy named as SGDD: Structure-Guided Drug Development. Many lead compounds as well as target proteins have to be thrown into garbage owing to adverse effects and/or weaker bindings. Based on the structural information of the target protein to which the ligand binds, we will be able to reduce side effects without changing the drug's efficacy by freely modifying the area of the ligand molecule unrelated to the pharmacological action. Structural information is also useful for improving ligand binding activity. To put this another way, effective modification based on structural information can rescue target proteins from garbage bin, and I have named this strategy Drug Rescuing, which is one of the typical strategies of SGDD. Therefore, high resolution structures of the ligand-membrane protein complexes, which are important drug targets, are crucial for the improvement of drug candidates. The recent speed of high-resolution structural analyses of membrane proteins is almost an order or more of magnitude faster than that achieved a decade ago. Therefore, SGDD including drug rescuing technique might be a powerful way for effective drug development. A talk in this symposium will present examples of structural physiology studies of water channels and also studies of SGDDs about them as representative examples.

Keywords Cryo-electron microscopy, membrane proteins, Structural physiology, Structure-Guided Drug Development, Drug Rescuing

Short biography:

1980-1987 Institute for Chemical research, Kyoto University. Research Assistant (1980-1984); Instructor (1985-1987)

1987-1994 Protein Engineering Research Institute. Senior Research Scientist (1987-1988); Research Director (1988-1994)

1994-1996 International Institute for Advanced Research, Matsushita Electric Industrial Co., Ltd. Research Director

1996-2012 Structural physiology, Department of Biophysics, Graduate School of Science, Kyoto University. Professor

2012-2019 Graduate School of Pharmaceutical Sciences, Nagoya University (2012-2017), Cellular and Structural Physiology Institute (CeSPI), Nagoya University. Professor; Director (2012-2013)

2017- CeSPIA Inc. Director

2019- TMDU Advanced Research Institute, Tokyo Medical and Dental University. Distinguished Professor

Awards: Keio Medical Science Prize, Honorary Doctor of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, Minister for Science and Technology Policy Award of the Industry-Academia-Government Collaboration Contribution, The Yamazaki-Teiichi Prize (2005), Shimadzu Prize, Medal with Purple Ribbon (2006), Japan Academy Prize (2008), Christian B. Anfinsen Award (2010), Fujihara Award (2016), The Order of the Sacred Treasure, Gold and Silver Star (2021)

Specialty and present interests

Structural physiology of channels, Structure-guided drug development

References

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- Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. T. Gonen, et al. Nature, 438, 633-638 (2005).
- Crystal structure of a Claudin provides insight into the architecture of tight junctions. H. Suzuki, et al. Science, 344, 304-307 (2014).
- [Review] Development of the field of structural physiology. Y. Fujiyoshi. Proc., Jpn Acad., Ser. B, 91, 447-468 (2015).

Structural and biochemical analysis of a novel structural unit of chromatin

Dr.Kayo Nozawa

School of Life Science and Technology, Tokyo Institute of Technology

Abstract

In eukaryotes, genomic DNA is packaged into chromatin, and its basic unit is the nucleosome core particle, in which two histone H2A-H2B and H3-H4 dimers form the histone octamer together with an approximately 145 base-pair DNA fragment. The histones can be post-translationally modified, expressed as various isoforms, and even form atypical subnucleosomal unit. These subnucleosomes have different histone contents and DNA wrapping manner, contributing to the complexity to the genomic regulations via chromatin structure. Here we report cryo-EM structures of a unique subnuclesome, called H3-H4 octasome, at 3.6 Å and 3.9 Å, in closed and open conformations. The structural analysis revealed that a 145 bp DNA and a dimer of H3-H4 tetramers form a stable nucleosome core similar to the canonical nucleosome even in the absence of H2A and H2B. In the H3-H4 octasome structures, approximately 60 base-pair DNA segments are symmetrically wrapped around two H3-H4 tetramers, connected by the unique H4-H4 interface at the dyad axis location. Interestingly, the H3-H4 octasome lacks common foothold known as the acidic patch that is utilized as a major binding site for nucleosome binding proteins, such as histone modification enzymes and nucleosome remodelers. In addition, the core of H3-H4 octasome consists of shorter 120 bp DNA which resembles the archaeal nucleosome. A flexible H3-H4 octasome, even in its closed or compact form, had longer maximum inter-disk distance compared to that of the canonical nucleosome. Structure-based crosslinking experiments in Saccharomyces cerevisiae suggested that detectable amounts of the H3-H4 octasome exist in cellular chromatin. The present H3-H4 octasome structures revealed the atomic resolution picture of a unique subnucleosome existing in cell. It is anticipated that H3-H4 octasome plays an important role in epigenetic gene regulation, as key determinants for functional chromatin architecture.

Keywords Cryo-electron microscopy, RNA polymerase II, Mediator, Chromatin structure, Nucleosome

Short biography

EDUCATION

Bachelor of Science, March, 2007

School of Science and Engineering, TEIKYO UNIVERSITY,

Supervisor: Professor Takeyuki Wakabayashi

Master of Science, March, 2009

Graduate School of Bioscience and Biotechnology, TOKYO INSTITUTE OF TECHNOLOGY, Supervisor: Professor Osamu Nureki

Doctor of Science, March, 2012

Graduate School of Science, THE UNIVERSITY OF TOKYO

Supervisor: Professor Osamu Nureki

ACADEMIC CAREER

Postdoctoral fellow, April-August, 2012

Graduate School of Science, THE UNIVERSITY OF TOKYO

Host Supervisor: Professor Osamu Nureki

Postdoctoral fellow, September, 2012-July, 2014

Ludwig-Maximilians-Universität München

Host Supervisor: Professor Patrick Cramer

Postdoctoral fellow, August, 2014-December, 2017

Max Planck Institute for Biophysical Chemistry

Host Supervisor: Professor Patrick Cramer

Project Research Assistant Professor, January-March, 2018

Faculty of Science and Engineering, WASEDA UNIVERSITY

Host Supervisor: Professor Hitoshi Kurumizaka

Research Assistant Professor, April, 2018-March, 2022

Institute for Quantitative Biosciences, THE UNIVERSITY OF TOKYO

Host Supervisor: Professor Hitoshi Kurumizaka

Associate Professor, April, 2022-Now

School of Life Science and Technology, TOKYO INSTITUTE OF TECHNOLOGY

Specialty and present interests

Cryo-electron microscopy, Genome structure, Transcriptional regulation

References

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3. K, Nozawa., TR, Schneider., P, Cramer.
   Core Mediator structure at 3.4 Å extends transcription initiation complex model
   Nature., 545, 248–251 (2017).

Tools for validation of CryoEM maps and models

Dr.Vinothkumar Kutti Ragunath

National Centre for Biological Sciences, TIFR, Bangalore, India

Abstract

During the last few years, there has been enormous technical progress in the structure determination of biological macromolecules by electron cryomicroscopy (cryoEM) both as single molecules as well as molecules within the cells and has now become a popular technique. The resolutions obtained by cryoEM are very often 3.5 Å or better, allowing for de novo model building, modelling of solvent/ions as well as small molecule ligands. A number of tools developed for X-ray crystallography have now been adapted to be used in cryoEM and these have become invaluable in the process of model building on maps derived from cryoEM. In this talk, I will summarize the tools that are available for validating the cryoEM maps and models, and how they can aid researchers in assessing the quality of the models.

Keywords CryoEM, heterogeneity, local resolution, model refinement, validation

Short biography

Dr. Vinothkumar Kutti Ragunath is a biochemist and structural biologist, currently a faculty at the National Centre for Biological Sciences, Bangalore, India and also directs the National CryoEM facility. Vinoth was trained first at Madurai Kamaraj University, Madurai and moved to Max-Planck Institute of Biophysics, Frankfurt for his PhD. His postdoctoral training was at MRC Laboratory of Molecular Biology, Cambridge with 2017 Nobel Prize winner in Chemistry, Dr. Richard Henderson.

Vinoth and his colleagues in the lab are interested in understanding how things move across the membranes and how communication occurs. Many of these proteins reside in the membrane and mutations in these proteins are often the cause for many disease conditions in humans. Naturally, the proteins in the membrane are also targets for pathogens to entry into the cell and any fundamental understanding how these proteins function can be used in disease conditions as well as pathogen entry. His lab uses electron cryo microscopy and X-ray crystallography as the major techniques to understand the functioning of the proteins. Apart from membrane proteins, various macromolecular complexes in the cell and development and application of cryoEM as a technique is also a major interest in his lab.

Specialty and present interests Macromolecular Complexes; Membrane proteins; CryoEM

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20th Surugadai International Symposium

Organized by the Medical Research Institute, Tokyo Medical and Dental University

Organizing Committee: Itoshi Nikaido, Nobutoshi Ito, Tetsushi Furukawa, Yuta Kochi

難治疾患共同研究拠点シンポジウム

10:30～12:00

東京医科歯科大学

難治疾患研究所

2022 年度　難治疾患共同研究拠点シンポジウム

プログラム

2022 年 11 月 28 日（月）

10：30　–　12：00（オンライン開催）

10:30-10：35　開会の辞　仁 科 博 史 所長（東京医科歯科大学）

10:35-10：55　長 井　淳　先生（理化学研究所）

「神経-グリア活動の全脳プロービング」

10:55-11：15　佐 瀬 美和子 先生（自治医科大学）

「ヒト舌癌オルガノイドバイオバンクの構築」

11:15-11：35　中 村 由 和　先生（東京理科大学）

「上皮性制御におけるホスファチジルイノシトール 4,5-二リン酸の役割の解明」

11:35-11：55　加 藤 忠 史　先生（順天堂大学）

「双極性障害の神経生物学」

11:55-12：00　閉会の辞　樗 木 俊 聡 部門長（東京医科歯科大学）

10:35-10:55

神経–グリア活動の全脳プロービング

理化学研究所・脳神経科学研究センター　チームリーダー　長井淳

慢性的なストレス暴露はうつ病など精神疾患発症の要因になりうるが、この細胞メカニズムについては不明な点が多い。脳にはニューロンと同等の数のグリア細胞が存在する。グリアの一種アストロサイトは、ストレスに反応するニューロン活動に応答する一方で、大うつ病患者では細胞数の減少や機能分子の発現低下を呈することが報告されている。しかし、今日に至るまで、「ストレス性精神病態とアストロサイトの間に直接的な因果関係があるのか」という根本的な問いに対する答えは得られていない。 申請者は、アストロサイト活動を可視化・活性化・不活性化する遺伝学ツールを開発し、アストロサイトが精神病態様のマウス行動を制御することを明らかにしてきた（Nagai et al., Cell 2019; Neuron 2020; Neuron 2021)。そこで、ストレスに反応するアストロサイトおよびニューロンを全脳で捉え、解析し、操作するための遺伝子学ツールを作出した。これにより、特定の行動/状態下（例えばストレスと受けた状態）に反応するニューロン/アストロサイトの①脳内マップ・数の定量化、②分子特性、③行動における因果的機能（例えば、ストレスによるうつ様行動を増悪させるか、改善させるか）の検証が可能になる。本研究の展望はアストロサイトに根差した行動・精神状態を制御するメカニズムの一端を解明し、これまでにない治療標的を提案することである。双極性障害は（軽）躁状態とうつ状態を繰り返す疾患である。リチウム、抗てんかん薬、非定型抗精神病薬および認知行動療法などの心理社会的治療が有効であるが、治療薬の作用機序については不明な点が多い。遺伝要因の関与が明らかであり、ゲノムワイド関連研究、エクソーム解析研究から、細胞内カルシウムシグナリング関連遺伝子の関与が示唆される。

我々は、双極性障害の重症型である双極型統合失調感情障害に関して不一致な一卵性双生児より作成したiPS 細胞から脳オルガノイドを作成し、二階堂研との共同研究により、Quartz-Seq2 を用いて、GABA 神経細胞への分化促進を見出した。一方、他のグループの研究では、双極性障害患者の iPS 細胞由来神経細胞で、過剰興奮性が報告されている。

【略歴】

2013.4　日本学術振興会(JSPS)特別研究員 DC1
2015.9　早稲田大学大学院生命医科学科修了、博士（理学）
2015.10 JSPS 特別研究員 PD
2016.4　UCLA 医学部ポスドク、JSPS 海外特別研究員
2018.3　上原記念生命科学財団ポストドクトラルフェロー
2020.11 理化学研究所脳神経科学研究センター チームリーダー(PI)

10:55-11:15

ヒト舌癌オルガノイドバイオバンクの構築

自治医科大学歯科口腔外科学講座　病院助教　佐瀬美和子

舌癌は、口腔癌の約半数を占め、その特徴は、早期ステージでも潜在的リンパ節転移が存在し、しばしば後発転移を来たす。また、根治的治療後の再発率は高く、再発例は治療抵抗性であり、初回治療時の診断・治療が重要である。しかし、舌癌特異的な治療法はなく、また治療で使用可能な抗腫瘍薬は限られており、その治療効果や副作用は個人差が大きい。現時点では、予後予測に有用なバイオマーカーがないため、治療抵抗性の評価は不可能である。従来、前臨床ヒト癌モデルとして用いられてきた癌細胞株は、患者ごとの癌の特徴（多様性、組織構造、遺伝子変異）を反映しておらず、長期の樹立期間を要し、樹立効率も低い。そのため、癌細胞株では各患者の癌の性質や機能の解析が困難である。そこでこの問題点を解決するため、患者ごとの癌の特徴を in vitro で再現可能なヒト癌オルガノイドに着目した。癌オルガノイドは癌細胞株とは異なり、ヒト癌組織と同様の特徴を保持している。これまでに、大腸癌、肝癌、乳癌などの様々な癌組織からヒト癌オルガノイドバイオバンクが構築され、その有用性が示されている。しかし、扁平上皮癌からのヒト癌オルガノイドの樹立は難しく、舌癌に特化したオルガノイドバイオバンクは報告されていない。そこで本研究では、患者ごとの舌癌の性状や薬剤感受性、放射線感受性を解析可能な新規モデルの確立を目的とし、各患者のヒト舌癌オルガノイドを樹立し、それらを集約した“舌癌オルガノイドバイオバンク”を構築した。方法は、新規の舌癌患者の手術検体から、舌癌組織および正常舌組織を採取した。酵素処理により細胞を分散し、単離した細胞をマトリゲルに包埋し、増殖因子を含む培養液を用いて三次元培養を行った。培養方法を詳細に比較検討することで、培養条件を最適化し、正常舌オルガノイド（34 症例由来）、舌癌オルガノイド（28 症例由来）を樹立した。

【略歴】

2006 年　広島大学歯学部歯学科卒業
2008 年　自治医科大学病院臨床研修修了
2008 年～2017 年 自治医科大学附属病院臨床助教
2017 年　自治医科大学大学院医学研究科入学
　　東京医科歯科大学難治疾患研究所生体防御学分野(研究指導委託)
2019 年～2021 年 日本学術振興会・特別研究員 DC2
2021 年　自治医科大学大学院医学研究科修了
2021 年～自治医科大学附属病院病院助教

11:15-11:35

上皮性制御におけるホスファチジルイノシトール 4,5-二リン酸の役割の解明

東京理科大学理工学部応用生物科学科　准教授　中村由和

上皮細胞が上皮細胞特有の性質を失うことは癌や線維症をはじめとした様々な疾患の発症や悪化に関与する。上皮細胞が上皮細胞特有の性質を維持する仕組みに関して、タンパク質の重要性は明らかにされてきたが、リン脂質が果たす役割についての知見は乏しい。

我々は、マウス皮膚組織やヒト細胞株を用いた解析により、上皮細胞では間葉細胞と比較して、形質膜に存在する微量リン脂質ホスファチジルイノシトール 4,5-二リン酸 [PI(4,5)P2]の量が多いことを明らかにした。さらに、上皮細胞の形質膜にPI(4,5)P2 代謝酵素を発現させ、形質膜PI(4,5)P2 量を減少させた際には、強固な細胞間接着やコンパクトな細胞形態などの上皮細胞の特徴が失われ、間葉細胞に特徴的な性質の一部が観察されることが明らかになった。また、形質膜のPI(4,5)P2 量が上皮細胞と比べて少ない骨肉腫細胞において、PI(4,5)P2 合成酵素の過剰発現により、形質膜PI(4,5)P2 量を増加させたところ、細胞間接着形成が促進され、骨肉腫細胞の移動能、浸潤能が低下することも明らかになった。続いて、PI(4,5)P2 が上皮細胞特有の性質を制御する分子機構について検討したところ、PI(4,5)P2 はPI(4,5)P2 結合タンパク質Par3 を形質膜へ集積させ、細胞間接着タンパク質の形質膜への輸送を促すことにより、上皮細胞特有の性質を制御することが示唆された。

以上の結果より、形質膜リン脂質PI(4,5)P2 が上皮細胞特有の性質の維持や獲得に関わることが強く示唆された。

【略歴】

2000 年　東京大学薬学部　卒業
2003 年　東京薬科大学生命科学部分子生命科学科　助手
2007 年　カリフォルニア大学サンディエゴ校　博士研究員
2009 年　東京薬科大学生命科学部分子生命科学科　助教
2010 年　東京薬科大学生命科学部分子生命科学科　講師
2018 年　東京薬科大学生命科学部生命医科学科　准教授
2019 年　東京理科大学理工学部応用生物科学科　准教授
2020 年　東京理科大学生命医科学研究所　准教授（兼任）

11:35-11:55

双極性障害の神経生物学

順天堂大学医学部精神医学講座／大学院医学研究科精神・行動科学
主任教授　加藤忠史

双極性障害は（軽）躁状態とうつ状態を繰り返す疾患である。リチウム、抗てんかん薬、非定型抗精神病薬および認知行動療法などの心理社会的治療が有効であるが、治療薬の作用機序については不明な点が多い。遺伝要因の関与が明らかであり、ゲノムワイド関連研究、エクソーム解析研究から、細胞内カルシウムシグナリング関連遺伝子の関与が示唆される。

我々は、双極性障害の重症型である双極型統合失調感情障害に関して不一致な一卵性双生児より作成したiPS 細胞から脳オルガノイドを作成し、二階堂研との共同研究により、Quartz-Seq2 を用いて、GABA 神経細胞への分化促進を見出した。一方、他のグループの研究では、双極性障害患者の iPS 細胞由来神経細胞で、過剰興奮性が報告されている。

双極性障害を高頻度に伴うミトコンドリア病である慢性進行性外眼筋麻痺（CPEO）の原因遺伝子 ANT1 の遺伝子改変マウスの研究では、縫線核のセロトニンニューロンの過剰興奮が示された。CPEO の原因遺伝子 POLG（ミトコンドリア DNA 合成酵素）の神経特異的変異マウスは、反復性うつ状態を示し、視床室傍核に最も多くミトコンドリア DNA 変異が蓄積していることが分かった。マウスにおいて、視床室傍核の過剰興奮が、同様の反復性低活動エピソードを引き起こすことから、この表現型には視床室傍核の過剰興奮が関与している可能性が考えられた。視床室傍核は、セロトニンニューロンからの強い投射を受け、前部帯状回、島皮質、扁桃体、側坐核などに投射する。特に、ネガティブな情動に関わる扁桃体と、ポジティブな情動に関わる側坐核に側枝を送っていることが注目される。

これらの研究から、視床室傍核が双極性障害の有力な候補脳部位と考えられた。現在我々は、Quartz-Seq2 を用いて、マウスの視床室傍核にどのような細胞種があるのかの検討を進めている。また、ヒト死後脳においても、視床室傍核細胞が存在するかどうか、single nucleus RNA シーケンスにより検討を進めている。

これまでの研究から、視床室傍核をめぐる神経回路の過剰興奮性により情動／認知バランスが乱れることが、双極性障害の病態に関与していると想像される。そして、抗てんかん薬は、情動関連神経回路の過剰興奮を改善させることで、双極性障害に奏効するのかも知れない。

【略歴】

1988 年東京大学医学部卒業。同附属病院にて臨床研修。
1989 年滋賀医科大学附属病院精神科助手。
1994 年同大学にて博士（医学）取得。
1995～1996 年文部省在外研究員としてアイオワ大学精神科にて研究に従事。
1997 年東京大学医学部精神神経科助手、1999 年同講師。
2001 年理化学研究所脳科学総合研究センター（2018 年より脳神経科学研究センター）精神疾患動態研究チーム チームリーダー
2020 年 4 月より順天堂大学医学部精神医学講座／大学院医学研究科精神・行動科学　主任教授