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Center Medical Mycology Research

ANNUAL REPORT OF MEDICAL MYCOLOGY RESEARCH CENTER, CHIBA UNIVERSITY 2024

千葉大学　真菌医学研究センター　報告

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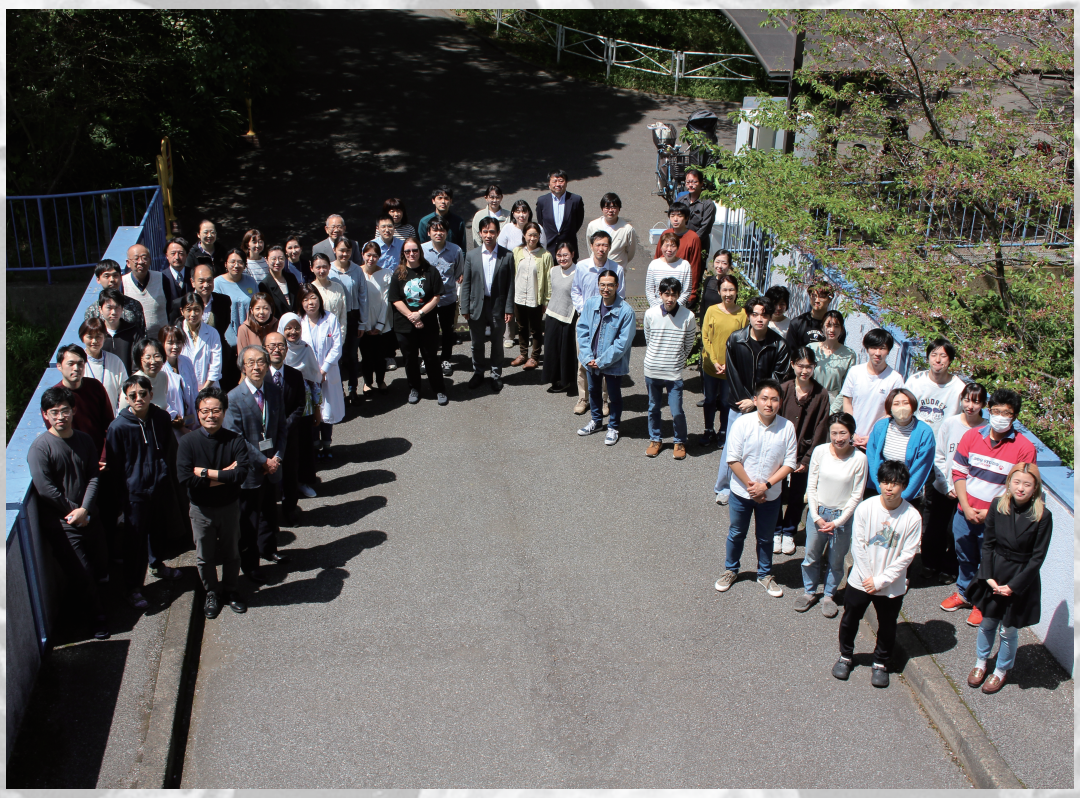

令和6年

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Preface for the FY2024 Annual Report

Japan's aging population ratio in 2023 was 29.56%, making it the second oldest society in the world. Respiratory diseases such as chronic obstructive pulmonary disease (COPD), malignant tumors, or fungal infections associated with advanced medical treatments or opportunistic infections caused by chronic diseases are major threats in an aging society. At the same time, in addition to imported fungal diseases due to economic globalization, pulmonary aspergillosis and fatal mucormycosis associated with COVID-19 and, more recently, *Candida auris* infections have also become international threats. Under these circumstances, the World Health Organization (WHO) published the WHO Fungal Pathogens List to guide research, development and public health action in October 2022 and made urgent recommendations regarding the importance of fungal infectious disease research and the need to strengthen it. Against this background, the role of the Medical Mycology Research Center, Chiba University (the Center) has expanded and diversified more than ever before.

The Center was reaccredited by the Minister of Education, Culture, Sports, Science and Technology (NEXT) in 2021 as a center for joint usage and research in the fields of infectious diseases, immunology, pathogenic microorganisms, and informatics, with a focus on pathogenic fungi. The Center also actively promotes joint usage and research, education, and medical care in close cooperation with universities, national and public research and medical institutions, and industry. In addition, as a National BioResource Project (NBRP) of MEXT, the Center conducts activities such as collection, preservation, genome information analysis, distribution, and course of pathogenic fungi and actinomycetes. In parallel with these projects, our research center is actively engaged in basic, developmental, and clinical research by independent research groups. At Chiba University Hospital, two clinical departments (Clinical Infectious Diseases and Infectious Disease Control) provide specialized outpatient services related to infectious diseases. In overseas activities, we have actively conducted international joint research with overseas fungal research centers in 2024, utilizing the framework of international collaboration established through previous international joint research.

The Center is also actively collaborating with the Graduate School of Medicine, the University Hospitals, the Faculty of Pharmaceutical Sciences, the Faculty of Science, the Research Institute of Disaster Medicine, and the Future Mucosal Vaccine Research and Development Center to further stimulate research on infectious diseases, immunity, vaccines, drug resistance, etc. within the university. As described above, in 2024, the Center continued its research activities with the pillars of "Joint Usage/Collaborative Research Center and Core Center for Bioresources," "Research on Pathogens, Infection, Immunology, and Genome Information," "Basic/Clinical Integrated Research," and "Fostering Young Researchers.

Finally, I would like to express our deepest gratitude to the members of the Governing Council of the Center, as well as to our many collaborators, and ask for your continued cooperation and guidance in our endeavors.

March 2025

Chihiro Sasakawa, PhD,
Director of the Medical Mycology Research Center, Chiba University

はじめに

我が国の2023年の高齢化比率は29.56％となり，世界で二番目の高齢社会になっています．高齢化社会では，慢性閉塞性肺疾患（COPD）等の呼吸器疾患や悪性腫瘍，あるいは先進医療や慢性疾患に起因する日和見感染症に伴う真菌感染症等が大きな脅威となっています．同時に経済のグローバル化に伴う輸入真菌症に加え，新型コロナ感染症に合併する肺アスペルギルス症や致死性のムーコル症，また近年ではカンジダ・アウリス感染症も国際的な脅威となっています．このような状況下で，2022年10月にWHO（世界保健機関）は，真菌感染症の国際的脅威と高度病原真菌の危険度分類表（WHO fungal priority pathogens list to guide research, development and public health action）を掲げ，真菌感染症研究の重要性とその強化の必要性に関して緊急提案を行いました．このような背景で千葉大学真菌医学研究センター（本センター）に求められる役割は以前にもまして拡大・多様化しています．

本センターは病原真菌を中心とする感染症・免疫・病原微生物・情報生命科学を含む領域の共同利用・共同研究拠点として，2021年度に文部科学大臣より再認定を受け，大学，国公立研究・医療機関，企業等と緊密に連携した共同利用・共同研究，教育，医療等を積極的に推進しています．また本センターでは，文部科学省のナショナルバイオリソースプロジェクト（NBRP）として，病原真菌や放線菌の収集・保存・ゲノム情報解析・分与・講習会等の活動も行なっています．さらにこれら事業と平行して，独立研究グループによる基礎研究・開発研究・臨床研究も積極的に行っています．一方附属病院では，臨床系の２分野（臨床感染症分野，感染制御分野）による感染症に関連する専門外来を開設しています．海外活動でも，これまでの国際共同研究で築かれた国際連携の枠組みを活用して，2024年度も海外の真菌研究拠点と国際共同研究が積極的に行われました．

本センターの教員は，学内においても災害治療学研究所，未来粘膜ワクチン研究開発シナジー拠点，未来粘膜ワクチン研究開発センター等に参画するとともに，感染症・免疫・ワクチン・薬剤耐性等の研究強化を目指して，医学研究院，附属病院，薬学研究院，理学研究院等と共同研究を行い，また大学院・学部の講義にも参加しています．

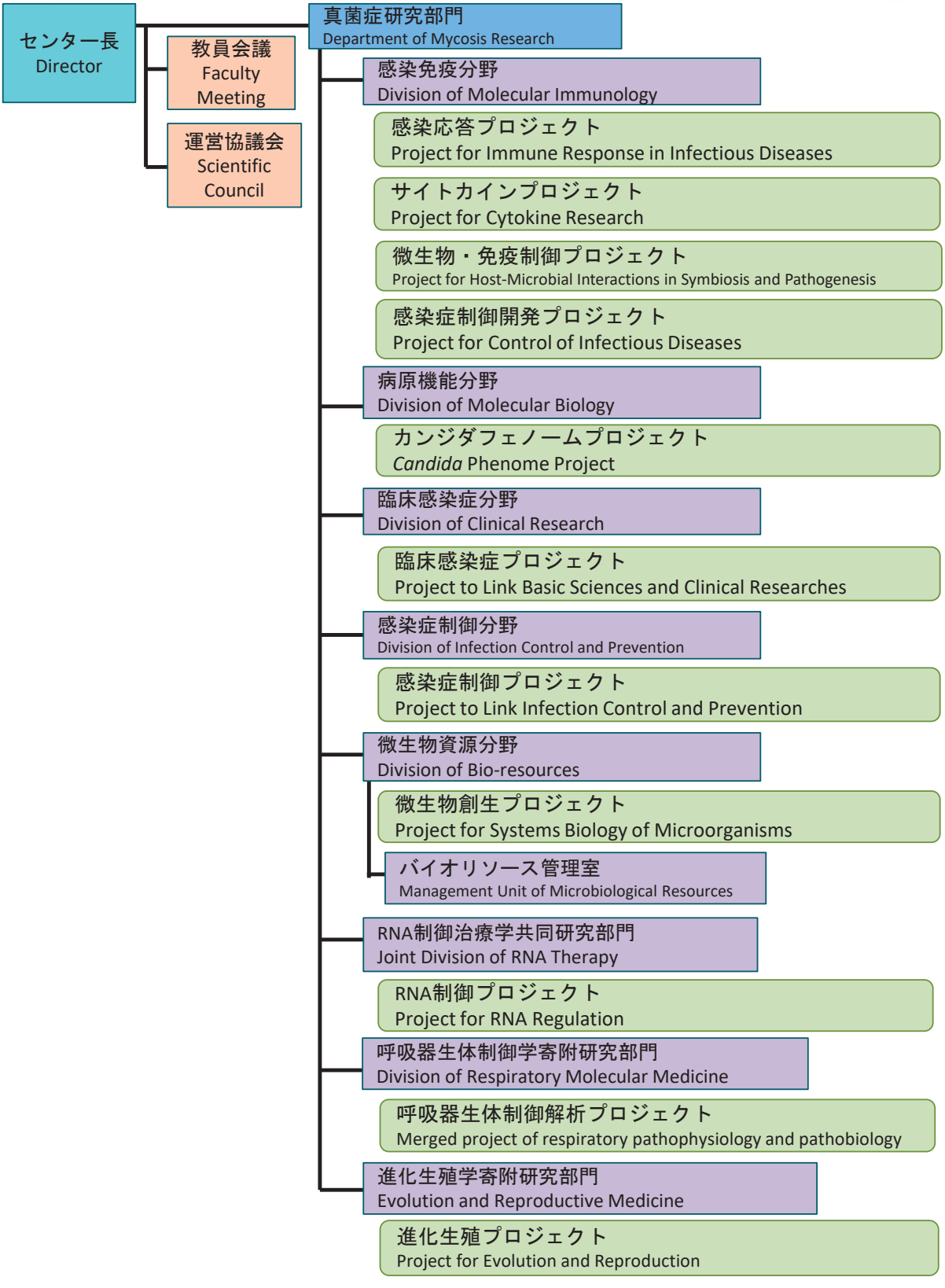
以上のように，本センターは2024年度も「共同利用・共同研究拠点及びバイオリソース中核拠点事業」，「感染症・病原体・免疫・ゲノム情報」，「基礎・臨床一体型研究」，「若手育成」を柱として研究活動を行いました．ここに本センターの運営協議会の委員の方々をはじめ，多くの共同研究者の方々にも深く感謝申し上げますとともに，引き続きご協力とご指導を賜りますようお願い申し上げます．

2025年３月

千葉大学真菌医学研究センター長

笹　川　千　尋

機構図
Organization



Project for Immune Response in Infections Diseases

米山ＰＩ（感染応答）プロジェクト

Summary（研究概要）

The innate immune system plays an essential role in self-defense against infection of various pathogens. We focus on antiviral innate immunity, especially molecular machinery for detecting viral RNA by retinoic acid-inducible gene I（RIG-I）-like receptors（RLRs）and subsequent immune responses. The results obtained from the studies will help us to establish a novel therapeutic or preventive strategy against RNA virus-induced infectious diseases.

感染に対する生体防御は，自然免疫と獲得免疫によって協調して行われている．本プロジェクトでは，ウイルス感染に応答した自然免疫誘導機構に注目し，RNAセンサー RIG-I-like受容体（RLR）によるウイルス由来非自己RNA検知の分子機構の解明と，それによって引き起こされる免疫応答シグナルの生理機能を解析することにより，ウイルス感染症に対する新たな治療戦略につながる知見を得ることを目指す．

Professor	Mitsutoshi Yoneyama	教　　授	米山　光俊
Assistant Professor	Koji Onomoto	助　　教	尾野本浩司
Research Technician	Kaho Kato（～2024.3）	技 術 職 員	加藤　香穂
Research Technician	Yuna Aoki（2024.4～）	技 術 職 員	青木　友那
Research Promotion Technician	Yukari Inada（～2024.3）	技 術 補 佐 員	稲田由圭里
Research Promotion Technician	Haruko Munakata（2024.4～）	技 術 補 佐 員	宗像　晴子

1. Regulatory mechanisms of RLR-mediated signaling via virus-inducible antiviral stress granule（avSG）formation.

Onomoto K, Sakai M, Ban M, and Yoneyama M.

Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba, 260-8673, Japan.

Stress granules（SGs）are intracellular aggregates of RNA and protein that form under various cellular stresses including viral infection and play a role in regulating RLR-mediated antiviral innate immunity during viral infection. The virus-induced SGs are referred to as antiviral SGs（avSGs）. However, the regulation of avSG formation during viral infection is not fully understood. We demonstrated that host TAR RNA-binding protein（TRBP）negatively regulates the expression of type I interferon（IFN）by inhibiting the avSG formation in response to RNA virus infection. Our results showed that overexpression of the TRBP inhibited both IFN-β promoter activity and avSG formation in response to viral infection and the transfection of viral RNA mimic, polyinosinic-polycytidylic（polyIC）. Additionally, TRBP inhibited IFN production stimulated by both short and long polyIC, indicating that TRBP regulates both RIG-I- and MDA5-mediated signaling pathways. In contrast, the cells in which TRBP gene was disrupted exhibited enhanced phosphorylation and activation of IFN regulatory factor-3（IRF-3）and elevated expression of IFN-β mRNA compared to wild-type cells. Furthermore, TRBP interacted with double-stranded RNA（dsRNA）-dependent kinase（PKR）, a key kinase for viral RNA-induced avSG formation, through its RNA-binding domains（RBDs）, and inhibited auto-phosphorylation of PKR; however, post-translational

modifications of TRBP proteins, including phosphorylation and caspase-mediated digestion, were not required. In summary, our findings reveal a novel function of TRBP as a negative regulator of RLR-mediated signaling via PKR-dependent inhibiting avSG formation.

2. Regulation of RLR-mediated antiviral signaling by inhibiting MAVS degradation

Suzuki Y, Onomoto K, and Yoneyama M.

Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba, 260-8673, Japan.

Type I and III IFNs are essential in the antiviral innate immune responses against diverse viral infections. In response to RNA virus infection, RIG-I-like receptors detect virus-derived non-self RNA in the cytoplasm and activate the downstream signaling via direct interaction with the mitochondrial adaptor molecule, MAVS/IPS-1. The RLR/MAVS pathway is tightly regulated by protein modifications such as ubiquitination. We demonstrated that one member of ubiquitin-specific proteases (USPs) was involved in enhancing IFN-induced signaling by deubiquitinating MAVS and thereby increasing its stabilization. The enforced expression of this USP protein increased MAVS expression in a de-ubiquitination activity-dependent manner and enhanced IFN gene expression. Conversely, deficiency of the USP significantly attenuated IFN mRNA levels. Interestingly, the USP accumulated in stress granule-like aggregates, avSG, in response to viral infection. These observations suggest that the USP might stabilize MAVS by interaction at the interface between avSGs and mitochondria.

3. Identification of natural compounds targeting viral replication.

Aoki Y, Kato K, Onomoto K, and Yoneyama M.

Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba, 260-8673, Japan.

We screened for antiviral activity against viruses, including influenza A virus (IAV) and SARS-CoV-2, using a library of more than 300 natural compounds prepared by the Faculty of Pharmaceutical Sciences, Chiba University. As a result, we found that several compounds showed significant antiviral activity against various viruses. Among them, three compounds inhibit viral replication without affecting the activity of two proteases and RNA-dependent RNA polymerase of SARS-CoV-2. This suggests that these compounds may inhibit viral proliferation via a novel molecular mechanism(s). We are conducting analyses to identify the viral or host factors these natural compounds target.

Publications

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2) Shibata K, Moriizumi H, Onomoto K, Kaneko Y, Miyakawa T, Zenno S, Tanokura M, Yoneyama M, Takahashi T, Ui-Tei K*: Caspase-mediated processing of TRBP regulates apoptosis during viral infection. ***Nucleic Acids Res***, 52(9):5209-5225, 2024.

Project for Cytokine Research

西城ＰＩ（サイトカイン）プロジェクト

Summary（研究概要）

Cytokines play a central role in maintenance of homeostasis. Because, a disease is not caused by only one problem of an organ, but caused by a systemic disorder, which is regulated by cytokines, it is important to study their functions. We aim to find new therapeutic targets for inflammatory diseases and infectious diseases by investigating the roles of cytokines in pathogenesis.

生体は，多種多様な細胞や組織が互いに時空的に作用することにより恒常性が維持される一つのシステムであり，その維持においてサイトカインは中心的な役割を担っている．多くの疾病は単に一つの臓器，組織の異常ではなく，免疫系を始めとする種々のシステムの異常であることから，これらを統合するサイトカインの役割を知ることは非常に重要である．本プロジェクトでは，感染性疾患や炎症性疾患の病態形成におけるサイトカインの役割を解明し，最終的に新たな治療薬の標的分子を見出すことを目的とする．

Associate Professor	Shinobu Saijo	准　教　授	西城　　忍
Research Assistant Professor	Fabio Seiti Yamada Yoshikawa	助　　教	ﾌｧﾋﾞｵ ｾｲﾁ ﾔﾏﾀﾞ ﾖｼｶﾜ
Research Promotion Technician	Junko Minakuchi	技術補佐員	水口　潤子

1. Dectin-1 and Dectin-2 in innate immunity against fungal infection.

Saijo S and Yoshikawa YFS

Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan

Dectin-1 and Dectin-2 are type II transmembrane proteins of the C-type lectin family with single carbohydrate recognition domains (CRDs) in their extracellular region. They are expressed mainly in dendritic cells and macrophages. Dectin-1 recognizes β-glucans with its CRD and transduces signals through its immunoreceptor tyrosine-based activation motif (ITAM)-like motif in the cytoplasmic domain, whereas Dectin-2 recognizes α-mannans and transduces its signal through association with the ITAM-containing Fc receptor γ chain. Upon ligand binding, spleen tyrosine kinase is recruited to the ITAM and activates the caspase recruitment domain family member 9 (CARD9)–nuclear factor-κB axis, resulting in the activation of various genes including those encoding pro-inflammatory cytokines. Both β-glucans and α-mannans are major cell wall components of fungi including *Candida albicans* (*C. albicans*) and *Pneumocystis carinii* (*P. carinii*). Recently, it was reported that Dectin-1 is important in protection against *P. carinii* by inducing reactive oxygen species, whereas both Dectin-1 and Dectin-2 play important roles in defense against *C. albicans* by preferentially inducing Th17 cell differentiation. In this review, we briefly revisit the structures, ligands, signal transduction and functional roles of Dectin-1 and Dectin-2 in host defense against fungal infection.

2. Dectin-1/IL-15 pathway affords protection against extrapulmonary *Aspergillus fumigatus* infection by regulating Natural Killer cell survival.

Yoshikawa YFS[1], Yabe R[1], Torigoe S[2], Yamasaki S[1, 2, 3], Saijo S[1]

[1] Division of Molecular Immunology, Medical Mycology

Research Center, Chiba University, Chiba, Japan.
[2] Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan.
[3] Laboratory of Molecular Immunology, Immunology Frontier Research Center, Osaka University, Suita, Osaka, Japan.

C-type lectin receptors (CLRs) are classical innate immunity sensors in antifungal response, mostly known for triggering inflammatory reactions that allow pathogen elimination. *Aspergillus fumigatus* (*A. fumigatus*) is an environmental mold that can cause a broad range of clinical manifestations, from mild allergic reactions to severe, life threating, invasive disease. Here we uncovered a new role of the CLR Dcir1 in response to *A. fumigatus* infection. Unlike its class counterparts, as Dectin-1, Dcir1 does not drive a prompt antifungal response, but it delays the fungal clearance as mice knockout for the receptor are able to eliminate the pathogen faster. While we observed no changes in phagocyte recruitment or inflammation induction as possible effector mechanisms enhanced by receptor deficiency, Dcir1 seems to regulate the killing function of neutrophils against *A. fumigatus* hyphae, mainly by interfering in their exocytosis/degranulation activity. Due to the constant exposure to *Aspergillus* species in the environment, Dcir1 might have persisted as a regulatory receptor to restrict neutrophil over-activation, which adds another layer of complexity in the role played by CLRs in host defense.

Publications

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Project for Host-Microbial Interactions in Symbiosis and Pathogenesis

後藤ＰＩ（微生物・免疫制御プロジェクト）

Summary（研究概要）

The gastrointestinal tract is a unique organ that is constitutively exposed by various antigens, including dietary materials, commensal bacteria, and fungi. In order to exclude pathogens and create a symbiotic environment for non-pathogenic microorganisms, intestinal epithelial cells (ECs) and immune cells contribute to establishing the homeostasis of the intestinal microenvironment. Disruption of a symbiotic relationship between host and commensals predispose to the development of pathogenic infections, inflammatory bowel diseases, and systemic disorders such as obesity and cancers. Therefore, it is important to understand the mechanism of a symbiotic and homeostatic systems regulated by intestinal ECs and immune cells. In this project, we aim to uncover the symbiotic system with commensal micro- and mycobiota. We further investigate the role of commensal microbes in the establishment of intestinal homeostasis and develop novel therapeutic approaches for the treatment of diseases such as bacterial and fungal infections caused by disruption of intestinal homeostasis.

腸管は食餌性抗原や腸内細菌・真菌など多種多様な抗原に常に曝されている特殊な組織である．これら無数の抗原に対処するため，腸管では免疫細胞と上皮細胞が相互に作用しながら病原性微生物を排除し，非病原性微生物と共存する基盤を形成することで腸管の恒常性維持に寄与している．この腸内微生物との共生関係の破綻は，炎症性腸疾患に代表される腸疾患のみならず，肥満や糖尿病などの全身性の疾患発症の素因となることから，腸内微生物との共生システムや腸管免疫細胞と上皮細胞による腸管恒常性制御システムを理解することは重要な命題である．本プロジェクトでは，宿主と腸内細菌や腸内真菌との共生機構を明らかにし，腸内微生物による腸管恒常性維持システムの解明とその破綻によって引き起こされる様々な疾患，特に細菌や真菌感染症の治療法の開発を目的としている．

Associate Professor	Yoshiyuki Goto	准　教　授	後藤　義幸
JSPS Post Doctoral Fellow	Bonita McCuaig	学振外国人特別研究員	ボニータ　マクアギ
Research Promotion Technician	Sayaka Hasegawa	技術補佐員	長谷川さや香
Research Promotion Technician	Minami Hirayama	技術補佐員	平山　　南
Research Promotion Technician	Kaho Nakamura	技術補佐員	中村　華穂

1. Commensal bacteria and host immune system regulate fungal colonization in the gut

Bonita McCuaig[1], Qiongyuan Zhang[1], Daichi Mori[1], Yoshiyuki Goto[1]

[1] Project for Host-Microbial Interactions in Symbiosis and Pathogenesis, Division of Molecular Immunology, Medical Mycology Research Center, Chiba University

Tremendous numbers of microorganisms colonize in the gut of their host. Several specific fungi, including *Saccharomyces cerevisiae* and *Candida albicans*, have been reported to reside in the human gut. Although commensal bacteria modulate gut homeostasis and dysbiosis triggers various kinds of host diseases, including infections and inflammatory bowel diseases, it is unclear how these commensal fungi colonize in the gut and regulate host physiology. In addition, *C. albicans* are also known to exert

pathogenic effects in the immunocompromised host and expand to the systemic compartments, called invasive candidiasis, one of the serious infectious diseases in the world. Importantly, colonization of *C. albicans* in the gut trigger invasive candidiasis. Therefore, it is important to identify how *C. albicans* colonize in the gut. In this study, we aim to uncover the mechanism by which commensal fungi colonize in the gut and affect the development of host diseases. We identify that commensal bacteria prevent the colonization of *C. albicans* in the gastrointestinal tract of mice. Furthermore, *C. albicans* colonizing in the gastrointestinal tracts was excluded by fecal microbiota transplantation, indicating the critical role of commensal bacteria in preventing infection by pathogenic fungi (Fig. 1). We examine the more detailed mechanism by which commensal bacteria and gut immune system regulate fungal colonization and develop novel therapeutic approaches for the treatment of infectious diseases.

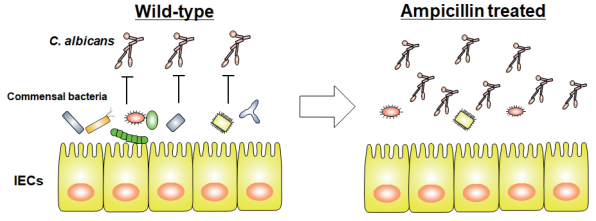

Fig 1. Commensal bacteria prevent the colonization of *C. albicans* in the gut

2. Innate and acquired immune system regulates intestinal epithelial α1, 2-fucosylation

Daichi Mori[1], Yoshiyuki Goto[1]

[1] Project for Host-Microbial Interactions in Symbiosis and Pathogenesis, Division of Molecular Immunology, Medical Mycology Research Center, Chiba University

α1, 2-fucosyl linkages located to terminal carbohydrate moiety expressed on intestinal epithelial cells are catalyzed by fucosyltransferase 2 (Fut2). Epithelial α1, 2-fucose is one of the symbiotic factors which mediate host–microbiota interaction. For example, epithelial α1, 2-fucose is utilized as a dietary carbohydrate by various symbiotic bacteria such as *Bacteroides*. Therefore, disruption of Fut2 leads to dysbiosis both in mice and humans and is predisposed to the development of inflammatory diseases such as Crohn's disease. Despite the importance of intestinal and systemic homeostasis, the molecular and cellular mechanisms of the induction of epithelial Fut2 and subsequent α1, 2-fucosylation remain unknown. We found that group 3 innate lymphoid cells (ILC3) are critical inducers of intestinal epithelial Fut2 expression and fucosylation that is mediated by the production of interleukin 22 and lymphotoxin from ILC3 in a commensal bacteria–dependent and -independent manner, respectively (Fig. 2). In addition, IL-10-producing $CD4^+$ T cells negatively regulate intestinal epithelial α1, 2-fucosylation (Fig. 2). These data unveil a novel function of innate and acquired immune cells in creating the appropriate symbiotic environment between commensal bacteria and the host through regulating the epithelial α1, 2-fucosylation.

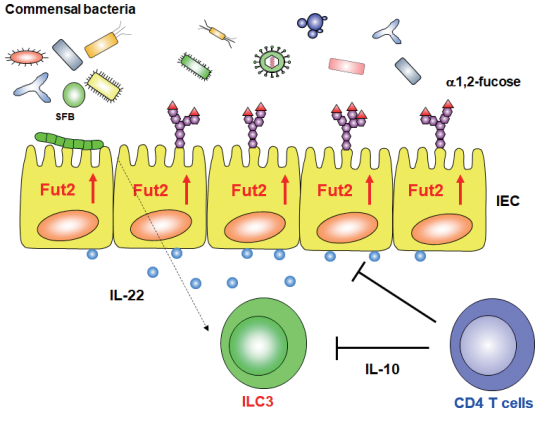

Fig 2. The inductive and regulatory mechanism of epithelial α1, 2-fucose

3. Resident microbes in the gut induce host antibody responses

Bonita McCuaig[1], Tomone Ikai[1], Momoko Kaneko[1], Yoshiyuki Goto[1]

[1] Project for Host-Microbial Interactions in Symbiosis and Pathogenesis, Division of Molecular Immunology, Medical Mycology Research Center, Chiba University

Fecal IgA levels, as well as the number of T helper 17 (Th17) cells and intraepithelial lymphocytes (IELs) in germfree mice, are dramatically reduced compared with wild-type mice, indicating that resident commensal microbes stimulate the host immune system in the gut. Although segmented filamentous bacteria (SFB) have been identified as one of the commensal bacteria capable of the induction of IgA, the mechanism of how SFB stimulates IgA induction is still unclear. In addition, the characteristics of microbes that induce IgA is not fully understood yet. This study aims to identify the microbes, especially resident bacteria and fungi, that induce IgA in the gut using next-generation sequencing techniques combined with immunological and bacteriological approaches. We also investigate whether commensal microbes stimulate antigen-specific mucosal IgA as well as systemic IgG immune responses. These studies will lead to the development of novel strategies for optimal mucosal vaccines.

Publications

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3) Kurihara S, Suzuki K, Yokota M, Ito T, Hayashi Y, Kikuchi R, Kageyama T, Meguro K, Tanaka S, Iwata A, Goto Y, Suto A, Nakajima H. Eosinophils Contribute to Oral Tolerance via Induction of RORγt-Positive Antigen-Presenting Cells and RORγt-Positive Regulatory T Cells. ***Biomolecules***. 14: 89, 2024

Project for Control of Infectious Diseases

高屋ＰＩ（感染症制御開発）プロジェクト

Summary（研究概要）

Excessive antibiotic exposures let bacteria be in a dormant state, allowing bacteria to survive in harsh environments. This phenomenon called "persisters" also causes the emergence of drug-resistant bacteria and intractable bacterial infections such as persistent bacterial infections. In this project, we aim to elucidate the molecular mechanism of persister control through research on developing systemic infections and persistent infections and to create new compounds that can control dormant cells. In this year, using compounds discovered from our unique natural product library, we investigated the molecular mechanisms that control the virulence factors of methicillin-resistant *Staphylococcus aureus*, as well as methods for analyzing the antibacterial activity and fluorescence of compounds in Gram-negative bacteria.

細菌感染症で用いられる抗菌薬を細菌に曝露すると休眠状態となり，過酷な環境でも生存することができる．この現象は薬剤耐性菌出現や細菌持続感染などの難治性細菌感染症の原因ともなる．本プロジェクトでは，病原細菌の全身感染症発症と持続感染機構研究を通して休眠制御の分子機構を解明し，休眠細胞を制御できる新たな化合物の創出を目指している．本年度は独自の天然物ライブラリーから見出した化合物を用いて，メチシリン耐性黄色ブドウ球菌の病原制御因子を制御する分子機構や化合物の抗菌活性と蛍光性を利用した細菌での作用解析法について検討した．

Associate Professor　Akiko Takaya
Research Promotion Technician　Yuriko Nomura

准　教　授　高屋　明子
技術補佐員　野村祐理子

1. CMM230 as a Potent Species-Specific Quorum Sensing Inhibitor Against Methicillin-Resistant Staphylococcus aureus

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Methicillin-resistant *Staphylococcus aureus*（MRSA）is a major human pathogen responsible for significant morbidity and mortality, with its virulence regulated by the agr quorum sensing（QS）system. QS inhibitors targeting *agr* represent a promising therapeutic strategy for treating staphylococcal infections. Pentacyclic triterpenes, such as betulin and betulinic acid, have been shown to inhibit QS-regulated virulence factor production in *Pseudomonas aeruginosa*, but their ability to inhibit *agr*-QS in *S. aureus* remains unexplored. In this study, we evaluated the agr-QS inhibitory activity of 14 structurally diverse pentacyclic triterpenes from our compound library. RNA was extracted from bacterial cultures incubated for 8 hours with each compound, and RT-qPCR analysis revealed that six compounds suppressed *RNAIII* expression by 97–99.9% compared to cultures treated with DMSO, with CMM230 showing the most potent effect. When the toxin activity was examined, hemolysis was reduced to 70% compared to the DMSO control in cultures

treated with CMM230. Notably, this inhibitory effect persisted when CMM230 was added after 8 hours of culture, demonstrating its potential to suppress *RNAIII* expression during the late exponential growth phase. Comparative analysis of QS inhibition revealed that QS inhibition was specific to MRSA and not observed in *P. aeruginosa*. These findings emphasize the potential of pentacyclic triterpenes as species-specific and structure-dependent QS inhibitors, offering a novel therapeutic approach to combat MRSA infections and address the global challenge of antibiotic resistance.

2. Dual-Function Coumarins: A Fluorescent and Antibacterial Platform for Overcoming Gram-Negative Bacterial Barriers

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The increasing prevalence of drug-resistant Gram-negative bacteria, including *Salmonella* Typhimurium and other enterobacteria, represents a pressing challenge in modern medicine, necessitating the development of novel antibiotics. A key barrier to effective treatment is the outer membrane of Gram-negative bacteria, which limits the intracellular uptake of antibacterial compounds. Structural modifications that enhance compound penetration through the outer membrane are, therefore, crucial for developing new therapeutic strategies. Fluorescent molecules can be beneficial for monitoring bacterial uptake, but compounds combining strong fluorescence with antibacterial activity remain scarce. In this study, we investigated the potential of 7-hydroxycoumarin derivatives as antibacterial agents and fluorescent probes for Gram-negative bacteria. Among the tested compounds, 5-geranyloxy-7-hydroxycoumarin (1) exhibited growth-inhibitory activity against an *S.* Typhimurium strain lacking the outer membrane protein TolC. Flow cytometry analysis revealed intracellular fluorescence signals in TolC-deficient strains within 3 hours of treatment with compound 1, indicating its efficient cellular uptake. In contrast, compound 1 displayed no antibacterial activity or detectable fluorescence uptake in wild-type strains, highlighting the critical role of the outer membrane in modulating compound efficacy. To explore the structure-activity relationship, we synthesized several derivatives by modifying the geranyl side chain at the 5-position. These derivatives demonstrated varying levels of uptake and antibacterial activity in TolC-deficient strains, depending on the side chain structure. Notably, some derivatives accumulated in wild-type strains over time, providing insights into structural features that enhance outer membrane permeability. These findings suggest that 7-hydroxycoumarin derivatives, with their dual antibacterial and fluorescent properties, offer a promising platform for studying Gram-negative outer membrane permeability and developing innovative antibacterial agents.

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Candida phenome project
知花ＰＩ（カンジダフェノーム）プロジェクト

Summary（研究概要）

The pathogenic yeasts *Candida glabrata* and *Candida auris* have garnered significant attention in recent years as critical challenges in infectious disease treatment driven by the increasing prevalence of drug-resistant strains. Particularly, *C. auris* has been reported to cause outbreaks in healthcare settings worldwide making its control an urgent global priority. To address these issues our laboratory has developed a comprehensive gene-editing platform for *C. glabrata* which has been utilized to facilitate the identification and functional analysis of genes involved in pathogenicity and to advance the development of novel antifungal drugs. In year 2024 we successfully established CRISPR-Cas9 systems for four pathogenic *Candida* species further advancing fungal genetic modification technologies. Additionally, we conducted a comparative study on the pathogenicity of *C. auris* and *C. glabrata* focusing on the antifungal efficacy against *C. auris* following macrophage phagocytosis. This research provided new insights into the adaptive mechanisms within macrophages that influence drug resistance and pathogenicity. In the realm of education two graduate students were welcomed into the laboratory where they received guidance to foster the development of young researchers. On the funding side external support was obtained through multiple grants including one KAKENHI Grant-in-Aid for Scientific Research（B）two for Scientific Research（C）one for Exploratory Research one for a research fellow one AMED project and three grants from private foundations. These resources were effectively utilized to elucidate the functions of previously unknown genes associated with pathogenicity. Furthermore, research efforts included the structural and pathogenic analyses of microorganisms leveraging electron microscopy for technological development collaborative studies technical training and research support. As results, our laboratory produced three first-author papers one co-authored paper and filed one patent application contributing to a foundational understanding that supports the development of innovative treatments for infectious diseases.

病原性酵母カンジダ・グラブラータおよびカンジダ・アウリスは，近年，薬剤耐性菌の増加に伴い感染症治療の重要な課題として注目されている．特にカンジダ・アウリスは，世界各地で医療現場におけるアウトブレイクが報告されており，その対策が国際的に喫緊の課題とされている．これらの問題に対処すべく，当研究室で構築したカンジダ・グラブラータの全遺伝子改変株プラットフォームを活用し，新規抗真菌薬の開発および病原性に関与する遺伝子の特定とその機能解析を進めてきた．

令和６年には，病原性カンジダ，４種に対するCRISPR-Cas9系の構築にも成功し，真菌遺伝子改変技術のさらなる発展に貢献した．加えて，カンジダ・アウリスとカンジダ・グラブラータの病原性を比較し，特にマクロファージによる貪食後のカンジダ・アウリスに対する抗真菌薬の薬効を評価した．この研究により，マクロファージ内での適応メカニズムが薬剤耐性と病原性に及ぼす影響について新たな知見を得た．

教育面では大学院生２名を受け入れ，研究指導を通じて若手研究者の育成を進めた．外部資金は，科研費基盤研究Ｂ（１件），基盤研究Ｃ（２件），萌芽研究（１件），特別研究員（１名），AMED（１件），民間助成金（３件）を獲得している．これらの研究資金を活用し，病原性に関与する未知の遺伝子群の機能解明を進めた．また，微生物の構造的特徴と病態形成に関する解析について電子顕微鏡を用いた技術開発や共同研究，技術者教育，研究支援を通じて展開した．

これらの成果として，研究室主体の論文３報，共著論文１報，特許１件が受理され，感染症治療に新

たな基盤を提供した.

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CRISPR-Cas9 RNP-mediated deletion of *ERG25* in non-albicans *Candida* species including *Candida auris*

Michiyo Okamoto, Kaname Sasamoto, Azusa Takahashi-Nakaguchi, Zhao Fujiang, Masashi Yamaguchi and Hiroji Chibana

The infections caused by non-*albicans Candida* (NAC) species, such as *Candida glabrata* and *Candida tropicalis*, increased recently. *Candida auris* has also emerged as a multidrug-resistant species, posing a serious global health threat. To treat the increasing number of drug-resistant fungi, it is essential to advance basic research such as genetic manipulation techniques for NAC species to continuously develop antifungal agents with new modes of action. The advancements using the CRISPR-Cas9 system have improved genetic analysis of NAC species. The RNP-based system, where the Cas9-gRNA complex is assembled *in vitro* and introduced into cells, simplifies genetic modifications by avoiding the need for species-specific plasmids. Previous research identified *ERG25* gene, encoding C-4 sterol methyl oxidase, as a promising antifungal target in *C. glabrata*. This study demonstrated that deleting *ERG25* homologue in *C. glabrata* and *C. auris* using the RNP-based CRISPR-Cas9 system. The deletion of *ERG25* in *C. auris* and *C. glabrata* indicated that Erg25 is essential for survival within the host in these pathogenic yeasts. We also have successfully deleted the *ERG25* alleles in *C. tropicalis* and *C. parapsilosis*, demonstrating the effectiveness of using both the CRISPR-Cas9 and Cre-loxP systems in these species for the first time.

Sandwich freezing and freeze substitution of Arabidopsis plant tissues for electron microscopy.

Masashi Yamaguchi, Mayuko Sato, Azusa Takahashi-Nakaguchi, Michiyo Okamoto, Kiminori Toyooka, Hiroji Chibana

Sandwich freezing is a method of rapid freezing by sandwiching specimens between two copper disks and it has been used for observing exquisite close-to-native ultrastructure of living yeast and bacteria. Recently this method has been found to be useful for preserving cell images of glutaraldehyde-fixed cultured cells as well as animal and human tissues. In the present study this method was applied to observe the fine structure of living Arabidopsis plant tissues and was found to achieve excellent ultrastructural preservation of cells and tissues. This is the first report of applying the sandwich freezing method to observe plant tissues.

Fungicidal Efficacy of Amphotericin B and Micafungin Against Candida auris Within Macrophage

Fujiang Zhao, Azusa Takahashi-Nakaguchi, Michiyo Okamoto, Kaname Sasamoto, Masashi Yamaguchi, and Hiroji Chibana

Candida auris poses a significant therapeutic challenge due to its resistance to azoles echinocandins and amphotericin B (AMPH-B). While *C. auris* strains are known to exhibit high survival rates within macrophages the susceptibility of phagocytosed cells to antifungal agents remains unclear. To

address this, we evaluated the fungicidal effects of AMPH-B and micafungin (MCFG) on *C. auris* strains from four distinct clades within macrophages. Our results suggested that both AMPH-B and MCFG retain fungicidal activity against the *C. auris* strains after being phagocytosed by macrophages providing insights into the intracellular activity of these antifungal agents.

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特許

2024年受理　特許第747165号　被験物質の標的遺伝子の同定方法

Project of Clinical Investigation

渡邉ＰＩ（臨床感染症）プロジェクト

Summary（研究概要）

We have been doing basic and clinical research primarily on fungal infections while examing patients in the Specialty Clinic for Fungal Infections at the University Hospital. Working as the Reference Center for fungal diseases, we were designated as an Advanced Progressive Laboratory by the Japanese Society for Infectious Diseases and Japanese Society for Clinical Microbiology and take consulting services on fungal diseases from all over the country（ca. 400 cases in 2024）. Concerning research activities, we are investigating various aspects of systemc mycoses with many universities, hospitals, and medical institutions such as NIID. The main research topics are:

the mechanisms and the epidemiology of antifungal resistance of *Candida* sp., *Aspergillus* sp. and *Scedosporium* spp.

the development of their diagnostic methods and new treatment strategy.

The SATREPS project between Sao Paulo State University of Campinas, Brazil（UNICAMP）and MMRC had been finished in 2022, but we still continue a collaborative study with UNICAMP including acceptance of Brazilian syudents.

我が国における「真菌症リファレンスセンター」（輸入真菌症を含む）として一般施設では実施困難な菌種同定, MIC測定, 血清診断（輸入真菌症, スエヒロタケなどを含む), 検体からのPCR検査などの特殊検査を受け入れるとともに, 並行して診療サポートも行なっており, 日本感染症学会, 臨床微生物学会から先進的感染症検査が実施可能な施設として「先進的感染症検査施設」に指定されている. 2024年の全国の医療機関からの依頼件数は400件あまりに達した. この診療サポートにより全国の医療機関によるネットワークが形成され, 菌株を含めた検体や貴重な臨床情報の収集と研究に役立つとともに, 多くの共同研究を生む母体ともなっている. 診療活動としては, 全国から寄せられる真菌症のコンサルテーションに対応する一方で, 附属病院に真菌症専門外来を設け, 全国からの患者の診療を行なうなど精力的に臨床活動を行っている. 研究面では国立感染症研究所をはじめ帯広畜産大, 東京理科大, NHO東京病院など国内のさまざまな研究機関, 医療施設と協力して臨床・基礎研究を行っており, 難治性真菌症の感染機構や診断・治療法の開発研究を進めている. 中でもカンジダ症, アスペルギルス症およびスケドスポリウム症の原因菌について, 耐性株の疫学と耐性機構や感染機構, 診断法や新たな治療戦略についての研究を進めている.

2016年から開始したブラジル・カンピーナス大学感染症内科とのSATREPS（地球規模課題対応国際科学技術協力プログラム）は2022年に事業終了したが, その後もブラジルから留学生を受け入れる等, 積極的に研究交流を継続している.

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1. Insights into *Aspergillus fumigatus* colonization in cystic fibrosis and cross-transmission between patients and hospital environments

Pontes L, Giordano ALPL, Reichert-Lima F, Beraquet CAG, Pigolli GL, Arai T, Ribeiro JD, Gonçalves AC, Watanabe A, Gustavo Henrique Goldman GH, Maria Luiza Moretti ML, Angélica Zaninelli Schreiber AZ.

Background: Approximately 60% of individuals with cystic fibrosis (CF) are affected by *Aspergillus fumigatus* infection. This condition is correlated with a decline in lung function and is identified as an independent risk factor contributing to hospital admissions among CF patients. This study investigates the dynamic interplay of A. fumigatus within the context of CF patients, tracing its evolution over time, with a specific emphasis on colonization dynamics.
Methods: An analysis was conducted on 83 sequential *A. fumigatus* isolates derived from sputum samples of six patients receiving care at a renowned CF hospital in Brazil. Employing microsatellite genotyping techniques, alongside an investigation into *cyp51A* gene mutations, this research sheds light on the genetic variations, colonization, and resistance of *A. fumigatus* within the CF respiratory environment.
Results: Our research findings indicate that CF patients can harbor *A. fumigatus* strains from the same clonal complexes for prolonged periods. Additionally, we identified that clinical isolates have the potential to spread among patients in the same healthcare facility, evidencing hospital contamination. Two patients who underwent long-term Itraconazole treatment did not show phenotypic resistance. However, one of these patients exhibited mutations in the *cyp51A* gene, indicating the need to monitor resistance to azoles in these patients colonized for long periods by *A. fumigatus*. We also observed co-colonization or co-infection involving multiple genotypes in all patients over time.
Conclusion: This comprehensive examination offers valuable insights into the pathogenesis of *A. fumigatus* infections in CF patients, potentially shaping future therapeutic strategies and management approaches. This enhanced understanding contributes to our knowledge of *A. fumigatus* impact on disease progression in individuals with cystic fibrosis. Additionally, the study provides evidence of cross-contamination among patients undergoing treatment at the same hospital.

2. Genetic mutations in FKS1 gene associated with acquired echinocandin resistance in *Candida parapsilosis* complex

Khalifa HO, Watanabe A, Kamei K.

Candida parapsilosis complex has recently received special attention due to naturally occurring FKS1 polymorphism associated with high minimal inhibitory concentrations for echinocandin and the increase of clonal outbreaks of strains resistant to commonly used antifungals such as fluconazole. Despite the previous fact, little is known about the genetic mechanism associated with echinocandin resistance. Therefore, the present study was designed to investigate the mechanism of acquired echinocandin resistance in C. parapsilosis complex strains. A total of 15 clinical *C. parapsilosis* complex isolates were sub-cultured for 30 days at a low concentration of micafungin at ½ the lowest MIC value of the tested isolates (0.12 μg/ml). After culturing, all the isolates were checked phenotypically for antifungal resistance and genotypically for echinocandin resistance by checking FKS1 gene hot spot one (HS1) and HS2 mutations. In vitro induction of echinocandin resistance confirmed the rapid development of resistance at low concentration micafungin, with no difference among *C. parapsilosis*, *C. metapsilosis*, and *C. orthopsilosis* in the resistance development. For the first time we identified different FKS1 HS1 and or HS2 mutations responsible for echinocandin resistance such as R658S and L1376F in C. parapsilosis, S656X, R658X, R658T,

W1370X, X1371I, V1371X, and R1373X (corresponding to their location in *C. parapsilosis*) in *C. metapsilosis*, and L648F and R1366H in *C. orthopsilosis*. Our results are of significant concern, since the rapid development of resistance may occur clinically after short-term exposure to antifungals as recently described in other fungal species with the potential of untreatable infections.

3. Fungal chitin-binding glycoprotein induces Dectin-2-mediated allergic airway inflammation synergistically with chitin

Muraosa Y, Hino Y, Takatsuka S, Watanabe A, Sakaida F, Saijo S, Miyazaki Y, Yamasaki S, Kamei K.

Although chitin in fungal cell walls is associated with allergic airway inflammation, the precise mechanism underlying this association has yet to be elucidated. Here, we investigated the involvement of fungal chitin-binding protein and chitin in allergic airway inflammation. Recombinant *Aspergillus fumigatus* LdpA (rLdpA) expressed in *Pichia pastoris* was shown to be an O-linked glycoprotein containing terminal α-mannose residues recognized by the host C-type lectin receptor, Dectin-2. Chitin particles were shown to induce acute neutrophilic airway inflammation mediated release of interleukin-1α (IL-1α) associated with cell death. Furthermore, rLdpA-Dectin-2 interaction was shown to promote phagocytosis of rLdpA-chitin complex and activation of mouse bone marrow-derived dendritic cells (BMDCs). Moreover, we showed that rLdpA potently induced T helper 2 (Th2)-driven allergic airway inflammation synergistically with chitin, and Dectin-2 deficiency attenuated the rLdpA-chitin complex-induced immune response in vivo. In addition, we showed that serum LdpA-specific immunoglobulin levels were elevated in patients with pulmonary aspergillosis.

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Project for Infection Control and Prevention

石和田PI（感染症制御）プロジェクト

Summary（研究概要）

Our research focuses on epidemiology and pathogenesis of *Haemophilus influenzae Streptococcus pneumoniae* and *Streptococcus agalactiae*. The pathogenic analysis of *Staphylococcus aureus* and the rapid diagnosis of BCG infection are also our research theme. We organize several clinical researches for the development of diagnostic and therapeutic methods for intractable respiratory infectious diseases and also care for patients in the clinic of the University Hospital.

インフルエンザ菌，肺炎球菌，B群レンサ球菌の病原性解析ならびに各感染症の疫学調査を継続的に行っている．結合型ワクチン導入後，新しく問題となっているワクチン非含有型株による病原因子の解析を行い，新たな予防法の開発を目指す．BCG感染症の迅速診断，黄色ブドウ球菌の病原性解析も行っている．また，難治性呼吸器感染症の診断，治療法開発のための臨床研究を実施している．同時に，附属病院における診療活動及び学内外でのコンサルテーションを行っている．

Professor	Naruhiko Ishiwada	教授	石和田稔彦
Assistant Professor	Noriko Takeuchi	特任助教	竹内　典子
Research Technician	Misako Ohkusu	技術職員	大楠美佐子
Adjunct Research Technician	Mihoko Ohata	非常勤技術職員	大畑美穂子
Visiting Professor	Katsuhiko Kamei	特任教授	亀井　克彦
Visiting Lecturer	Akio Toh-E	特別協力研究員	東江　昭夫

1. Cost-effectiveness analysis of maternal respiratory syncytial virus vaccine in protecting infants from RSV infection in Japan

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Introduction: Respiratory syncytial virus（RSV）is one of the major causes of respiratory tract infections among children. Until recently, the monoclonal antibody palivizumab was the only RSV prophylaxis available in Japan. In 2024, the bivalent RSV prefusion F protein-based（RSVpreF）vaccine was approved for the prevention of RSV infection in infants by active immunization of pregnant women. In this study, we assessed the cost-effectiveness of a combined strategy of RSVpreF vaccine and palivizumab in Japanese setting.

Methods: Using a Markov model, we evaluated prevented cases and deaths of medically attended RSV infections from birth to age 11 months for each of the three healthcare settings: inpatient（hospitalization）, emergency department visits, and outpatient visits. Incremental cost-effectiveness ratios（ICERs）were calculated from economic outcomes（intervention costs, medication costs, and productivity losses）and quality-adjusted life years（QALYs）. Further, we calculated the maximum price of RSVpreF vaccine within

which the program would be cost-effective.

Results: In comparison with the current prophylaxis (palivizumab alone), a combined prophylaxis of year-round RSVpreF vaccination of pregnant women and palivizumab prescription for premature infants born in < 32 weeks gestational age (wGA) and all infants with high risk prevented 14,382 medically attended cases of RSV (hospitalization, 7,490 cases; emergency department, 2,239 cases; outpatient, 4,653 cases) and 7 deaths, respectively. From a healthcare payer perspective, when the price of RSVpreF vaccine was equal to or less than ¥23,948 (US $182), a combination prophylaxis was cost-effective under the ICER threshold of ¥5 million per QALY. The other combination prophylaxis of year-round RSVpreF vaccination and palivizumab prescription of premature born in < 32 wGA regardless of risk in infants was a dominant strategy (more effective and less costly).

Conclusion: A combined prophylaxis of year-round RSVpreF vaccine and palivizumab could be a cost-effective strategy to protect neonates throughout the infant stage (< 1 years old) in Japan.

2. Prospective hospital-based cohort studies of Respiratory Syncytial Virus (RSV) infections in infants under one year during and after the SARS-CoV-2 pandemic in Japan

Nagasawa K[1], Ohata M[2], Igarashi A[3], Arashiro T[4], Ogawa T[2], Ohkusu M[2], Takeuchi N[2], Shizuno K[5], Kurihara E[1,5], Yoshida M[5], Kodama T[5], Abe K[5,6], Hoshino T[7], Arii J[8], Takeshita K[8], Hishiki H[9], Ota S[9], Takahashi Y[10], Omata Y[10], Nakazawa T[10], Someya T[11], Ishiwada N[2]

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Objectives: In Japan, population-based epidemiological data on respiratory syncytial virus (RSV) infections are limited. To elucidate the epidemiology of RSV before the introduction of new prophylactic drugs, we conducted a population-based study during and after the SARS-CoV-2 pandemic.

Methods: This study was performed in four hospitals in Chiba City and three hospitals in Ichihara City. Clinical information and residual samples from RSV rapid antigen tests of infants under one year old were collected. Samples from patients with lower respiratory tract infections (LRTI) were analyzed using the FilmArray Respiratory 2.1 panels.

Results: A total of 1,200 infants underwent the RSV rapid antigen test, with 497 diagnosed with LRTI. Although five samples could not be stored, 252 out of 492 (51.2%) were positive for RSV. Among the RSV PCR-positive infants, 63 (25.0%) had underlying diseases, compared to 100 out of 240 (41.7%) RSV PCR-negative infants ($P < 0.05$). In Chiba City, the annual incidence of hospitalization per 1,000 children was 12.7 in 2021, 4.4 in 2022, and 9.2 in 2023.

Conclusions: During and after the SARS-CoV-2 pandemic, most hospitalized infants with RSV-LRTI did not have underlying diseases. Widespread use of prophylaxis in infants without underlying disease is desirable.

3. Comparative genomic and morphological analyses of capsular and capsular-deficient pneumococcal strains simultaneously isolated from a patient with invasive pneumococcal disease

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Introduction: To understand the in-vivo dynamics in pneumococci, investigation into the carriage in patients with invasive pneumococcal disease (IPD) is extremely important.
Methods: To clarify genomic and morphological differences between pneumococcal strains simultaneously isolated from different sites in a patient with IPD, we conducted comparative analyses of two strains. A capsular strain isolated from the blood and a non-capsular strain isolated from the sputum of a patient with IPD were used.
Results: The strain isolated from blood was serotype 24B with capsule. The strain isolated from sputum with capsular type 24 genes was non-encapsulated, and genomic analysis revealed an insertion region in the *wcxK* gene. Its biofilm-forming capacity was higher than that of the capsular strain, as was that of the *pspK*-positive true non-encapsulated strain. Furthermore, observing the microbe using transmission electron microscopy revealed that the strain isolated from sputum lacked a capsule, like the *pspK*-positive true non-encapsulated strain.
Conclusions: Our analysis of the two strains isolated from the blood and sputum of a patient with IPD showed one possible in-vivo morphological change in Streptococcus pneumoniae.

4. Analysis of toxin-producing and antiseptic resistance genes of methicillin-resistant *Staphylococcus aureus* isolated from patients in a neonatal intensive care unit

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Background: Polymerase chain reaction–based open-reading frame typing (POT) is used to investigate nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). We analyzed the relationship between POT types, nosocomial infections, and toxin-producing and antiseptic resistance genes of MRSA isolated from patients in neonatal intensive care units.
Methods: Forty-four strains were typed by POT method. We identified toxin genes *lukpvSF*, *tst*, *eta* and *etb*, and antiseptic resistance genes *qacA/B* and *smr*.
Results: Fourteen POT types were identified; 43 strains were considered community-acquired MRSA. Twenty-eight strains were nosocomial. Eleven strains were positive for toxin-producing genes (9 for tst and 2 for *lukpvSF*) and classified into 6 POT types. Six strains were positive for antiseptic resistance genes (*qacA/B*) and classified into 2 POT types. Overall, 11 MRSA isolates were positive for toxin-producing or antiseptic resistance genes (6 nosocomial, 5 non-nosocomial). Strains with POT types 106-9-80 (2 strains) and 106-221-120 (4 strains) were positive for *tst* and *qacA/B*, equally divided between nosocomial and non-nosocomial.
Discussion: Some POT types are prone to nosocomial infections. However, no clear association between toxin-producing or antiseptic resistance genes and nosocomial infections was observed.
Conclusions: Factors other than toxin production and resistance to disinfectants may be related to nosocomial infection.

5. Effectiveness of isavuconazole in invasive cerebral aspergillosis during hematopoietic stem cell transplantation in a pediatric patient with myelodysplastic syndrome: A case report

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Pediatric myelodysplasia syndrome is often characterized by hypoplastic bone marrow morphology and predisposition to infection. Invasive aspergillosis during hematopoietic stem cell transplantation poses a significant threat and often requires voriconazole (VRCZ) therapy. However, difficulties in achieving appropriate VRCZ blood levels due to drug interactions have prompted the exploration of alternative treatments, such as isavuconazole (ISCZ). We present the case of a 4-year-old boy with myelodysplasia syndrome who developed multiple abscesses, including a brain abscess caused by *Aspergillus fumigatus*, and was successfully treated with ISCZ. Despite initial treatment with liposomal amphotericin B and VRCZ, the patient's condition deteriorated. Transitioning to ISCZ treatment resulted in significant clinical improvement, resolution of the abscesses, and reduced antigen levels. Although ISCZ induced hepatic enzyme elevation, supportive care improved without discontinuation of treatment. This case highlights the potential of ISCZ in cases of pediatric invasive aspergillosis where traditional therapies fail, underscoring the need for further research and formulation development to optimize its use in this population. As more cases accumulate, ISCZ may become a promising option for treating severe invasive aspergillosis in pediatric patients undergoing hematopoietic stem cell transplantation.

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Project for Systems Biology of Microorganisms

高橋ＰＩ（微生物創生）プロジェクト

Summary（研究概要）

Our research areas are Bioinformatics and Molecular Biology. We aim at unravelling the molecular mechanisms underlying pathogenicity and drug resistance in pathogenic fungi by integrating bioinformatics with molecular biology. Our Bioinformatics approach aims to deeply and clearly understand massive biological experiment data, e. g., sequence data by next generation sequencers.

我々は病原真菌の感染機序と薬剤耐性について研究を展開している．宿主との相互作用を含めた感染機序，並びに薬剤耐性機構について分子レベルでの解明を目指している．バイオインフォマティクスを駆使したアプローチも取り入れて，生命現象を俯瞰的に捉えて真に重要な因子の探索も展開している．

Associate Professor	Hiroki Takahashi	准　教　授	高橋　弘喜
Research Assistant Professor	Jun-ichi Ishihara（～2024.3）	特　任　助　教	石原　潤一
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1. Investigation of the relationships between heterogeneity against environmental stresses and pathogenicity in pathogenic fungi *Aspergillus fumigatus*

Saho Shibata, Momotaka Uchida, Xiaohui He, Yu Lu, Hiroki Takahashi

Stress responses and pathogenicity have been extensively studied in *Aspergillus fumigatus*, the main causative pathogen of life-threatening aspergillosis. The heterogeneity in this pathogen has recently attracted increasing attention. In this project, we used more than 100 clinically isolated strains to investigate several properties relevant to the pathogenicity of *A. fumigatus*, namely, hypoxia growth, adaptation to nutrients such as copper, mimicking human lung. We compared these strains in whole genome level and tried to uncover genomic variations. In addition, we conducted comparative transcriptome analysis to uncover the genes underpin the heterogeneity.

2. Development for genome analysis tools and bioinformatic analysis for collaborative projects.

Xiaohui He, Momotaka Uchida, Hiroki Takahashi

Since NGS development, genome and omics data are rapidly accumulating. We collaborate with several researchers to analyze their own genome and omics data, and give the overview of the data by using multivariate, statistical and machine-learning analysis.

Publications

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Management of Unit of Microbiological Resources

バイオリソース管理室

Summary（研究概要）

We are developing a system for preservation, management and distribution of pathogenic fungi and actinomycetes. We support the base of research and education of mycoses and their pathogens in order to supply reliable strains that are added new information.

病原真菌・放線菌の「保存・管理・提供」体制を整備し，最新情報が付加された信頼できる菌株の提供を通じて，真菌症ならびにその原因菌の研究・教育の基盤を支援している．

Associate Professor	Takashi Yaguchi	准　教　授	矢口　貴志
Assistant Professor	Sayaka Ban	助　　教	伴　さやか
Research Technician	Junko Ito	技 術 職 員	伊藤　純子
Post Doctoral Fellow	Isato Yoshioka	特任研究員	吉岡　育哲
Research Promotion Technician	Akiko Kota	技術補佐員	甲田　暁子
Research Promotion Technician	Yu Uehara	技術補佐員	上原　ゆう
Research Promotion Technician	Kuniko Shimamura	技術補佐員	島村具仁子

1. Generation of citric acid-hyperproducers independent of methanol effect by high-level expression of cexA encoding citrate exporter in *Aspergillus tubingensis*.

Yoshioka I[1,2], Kirimura K[2,3].

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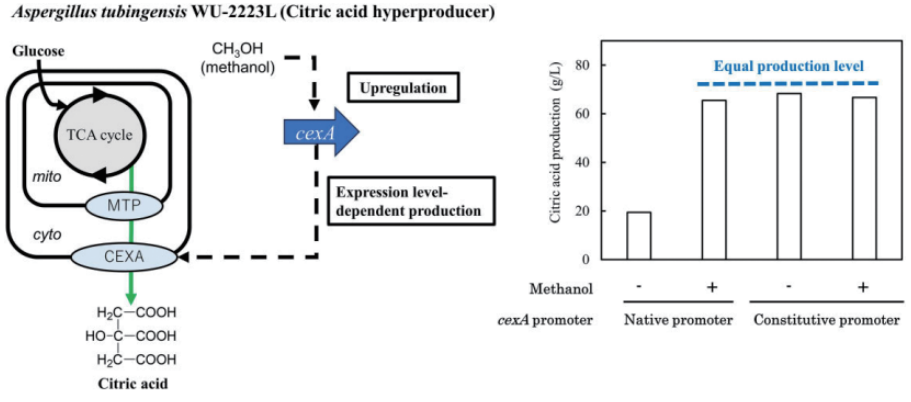
Methanol reportedly stimulates citric acid (CA) production by *Aspergillus niger* and *A. tubingensis*; however, the underlying mechanisms remain unclear. Here, we elucidated the



Methanol amplifies citric acid production via the cexA gene. Engineered strains LhC-1 and LhC-2 demonstrate methanol-independent CA hyperproduction.

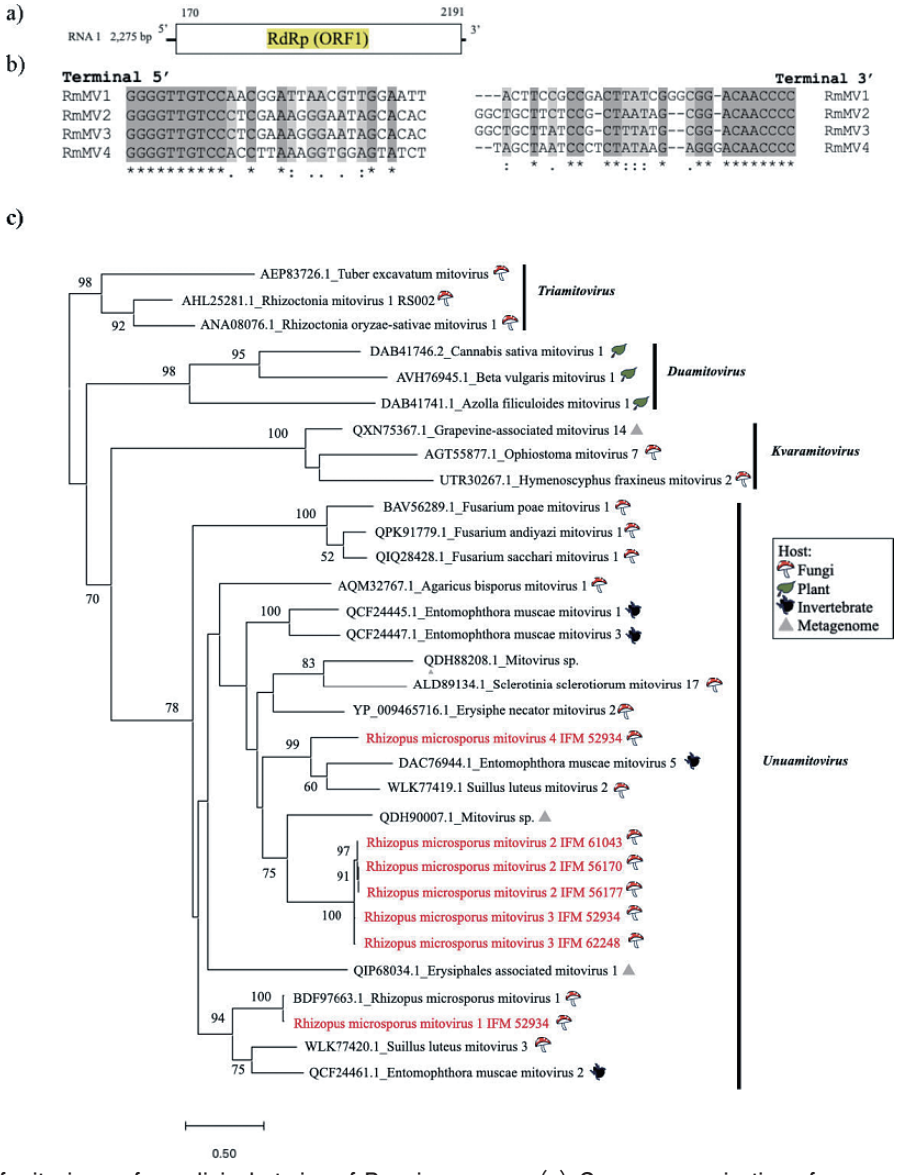
molecular functions of the citrate exporter gene cexA in relation to CA production by *A. tubingensis* WU-2223L. Methanol addition to the medium containing glucose as a carbon source markedly increased CA production by strain WU-2223L by 3.38-fold, resulting in a maximum yield of 65.5 g/L, with enhanced cexA expression. Conversely, the cexA-complementing strain with the constitutive expression promoter Ptef1 (strain LhC-1) produced 68.3 or 66.7 g/L of CA when cultivated without or with methanol, respectively. Additionally, strain LhC-2 harboring two copies of the cexA expression cassette produced 80.7 g/L of CA without methanol addition. Overall, we showed that cexA is a target gene for methanol in CA hyperproduction by *A. tubingensis* WU-2223L. Based on these findings, methanol-independent CA-hyperproducing strains, LhC-1 and LhC-2, were successfully generated.

2. New lineages of RNA viruses from clinical isolates of *Rhizopus microsporus* revealed by fragmented and primer-ligated dsRNA sequencing (FLDS) analysis.

Sa'diyah W[1,2], Zhao Y[1], Chiba Y[1], Kondo H[2], Suzuki N[2], Ban S[3], Yaguchi T[3], Urayama S[1,4], Hagiwara D[1,4]

[1] Department of Life and Environmental Sciences, Laboratory of Fungal Interaction and Molecular Biology (Donated by IFO), University of Tsukuba, Ibaraki, Japan
[2] Institute of Plant Science and Resources, Okayama University, Okayama, Japan
[3] Medical Mycology Research Center, Chiba University, Chiba, Japan
[4] Microbiology Research Center for Sustainability (MiCS), University of Tsukuba, Ibaraki, Japan

Rhizopus microsporus is a species in the order Mucorales that is known to cause mucormycosis, but it is poorly understood as a host of viruses. Here, we examined 25 clinical strains of *R. microsporus* for viral infection with a conventional doublestranded RNA (dsRNA) assay using agarose gel electrophoresis (AGE) and the recently established fragmented and primer-ligated dsRNA sequencing (FLDS) protocol. By AGE, five virus-infected strains were detected. Then, full-length genomic sequences of 12 novel RNA viruses were revealed by FLDS, which were related to the families Mitoviridae, Narnaviridae, and Endornaviridae, ill-defined groups of single-stranded RNA (ssRNA) viruses with similarity to the established families Virgaviridae and Phasmaviridae, and the proposed family "Ambiguiviridae." All the characterized viruses, except a potential phasmavirid with a negative-sense RNA genome, had positive-sense RNA genomes. One virus belonged to a previously established species within the family Mitoviridae, whereas the other 11 viruses represented new species or even new genera. These results show that the fungal pathogen *R. microsporus* harbors diverse RNA viruses and extend our understanding of the diversity of RNA viruses in the fungal order Mucorales, division Mucoromycota. Identifying RNA viruses from clinical isolates of *R. microsporus* may expand the repertoire of natural therapeutic agents for mucormycosis in the future.



Characterization of mitoviruses from clinical strains of R. microsporus. (a) Genome organization of a representative Rhizopus microsporus mitovirus (RmMV1); predicted ORF is indicated by the white box. (b) Comparison of the 5′ and 3′ termini of the RmMV1–4 genomes (represented as DNA sequence). (c) Phylogenetic tree of RdRPs of RmMV1–4 variants and selected mitovirids computed with RAxML and the PROTGAMMALG model, with 1,000 bootstrap replicates. Red font indicates mycoviruses found in this study. The scale bar shows the number of substitutions per site.

3. Activation of secondary metabolism and protease activity mechanisms in the black koji mold *Aspergillus luchuensis* through coculture with animal cells.

Asano Y[1], Saito S[1], Ujie Y[1], Iwata C, Yaguchi T[2], A. Arai M[1].

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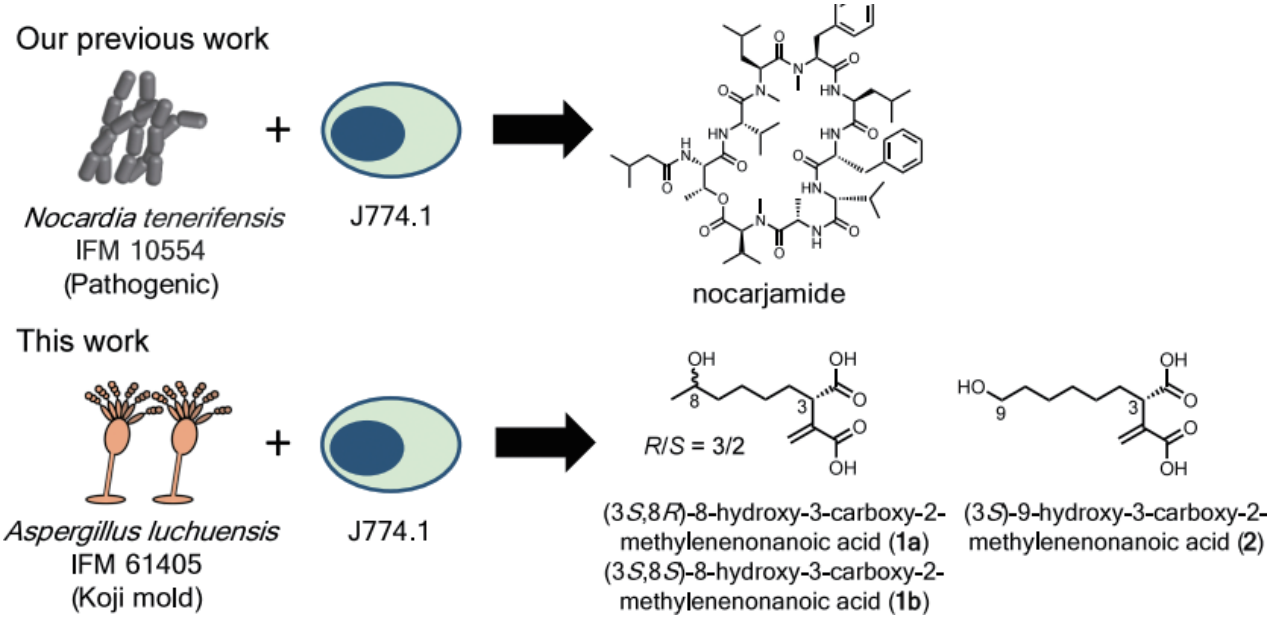
[2] Medical Mycology Research Center, Chiba University, Chiba, Japan

The activation of secondary metabolism plays a pivotal role in the discovery of novel natural products. We recently developed a coculture method involving actinomycetes and mouse macrophage-like cells to stimulate the production of



Coculture of microorganisms with animal cells (J774.1 mouse macrophage cells).

bioactive compounds. A black koji mold, *Aspergillus luchuensis* IFM 61405, markedly enhanced the production of (3S, 8R)-8-hydroxy-3-carboxy-2-methylenenonanoic acid (1a), (3S, 8S)-8-hydroxy-3-carboxy-2-methylenenonanoic acid (1b), and (3S)-9-hydroxy-3-carboxy-2-methylenenonanoic acid (2) when coincubated with J774. 1 mouse macrophage cells. The production of 1 and 2 increased by at least 3. 5-fold and 2. 7-fold, respectively, compared to monoculture after 7 days. A mechanistic investigation revealed that a protease from strain IFM 61405 plays a key role in enhancing the production of 1 and 2. This enhancement was not replicated in *A. niger* IFM 59706, a nonkoji mold, despite the presence of biosynthetic genes for 1 and 2 in *A. niger* IFM 59706. Furthermore, the addition of protease inhibitors suppressed the production of 1 and 2, suggesting that proteins secreted from animal cells, likely degraded by proteases secreted by strain IFM 61405, serve as precursors for 1 and 2. The results show that the strategy of coculturing koji mold with animal cells has the potential to enhance the production of natural products.

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Project for RNA Regulation

原口ＰＩ（RNA制御）プロジェクト

Summary（研究概要）

Gene regulatory networks determine not only cellular specificity of development, differentiation, and proliferation but also cellular response or competency to viruses, bacteria, and mycetes. In this project, we concentrate on miRNA which suppresses expression of many genes at the post-transcriptional level to develop basic research of new therapeutic strategies for human diseases such as cancer.

遺伝子発現の制御ネットワークは，その細胞の発生，分化，増殖に関する特異性はもちろん，真菌・細菌・ウイルス等の寄生体に対する宿主の応答性やcompetencyをも規定している．本プロジェクトでは，多数の遺伝子群の発現をpost-transcriptionalレベルで一括して負に制御するmiRNAの制御法の開発を行い，がんなどのヒト疾患の制圧への基盤研究を展開する．

Research Associate Professor	Takeshi Haraguchi	特任准教授	原口　健
Research Assistant Professor	Kazuyoshi Kobayashi	特任助教	小林　和善
Research Promotion Technician	Noriko Sakurai	技術補佐員	桜井　典子
Research Promotion Technician	Naomi Aikawa	技術補佐員	相川　尚美
Visiting Professor	Hideo Iba	客員教授	伊庭　英夫

1. Development of drug delivery system（DDS）for Super-S-TuD to establish RNA medicine for cancer therapy.

Takeshi Haraguchi, Kazuyoshi Kobayashi and Hideo Iba

Joint Division of RNA Therapy, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan

We previously developed the RNA decoy suppressing specific miRNA activity very efficiently, which was designated TuD（Tough Decoy）and expressed from viral vectors. S-TuD（Synthetic TuD）, which mimics the unique secondary structure of TuD was also developed as RNA medicine. It has been further improved as Super-S-TuD, which showed 3-7 folds enhancement in its specific activity of the target miRNA inhibition. For the efficient delivery of systemically administrated Super-S-TuD into tumor tissues is the major challenge at present. We previously established basic formulation for Lipid nanoparticle（LNP）preparation using COATSOME-X（developed by NOF）and Super-S-TuD 141/200c（suppresses the entire miR-200 family）encapsulated by such LNPs was shown to suppress the formed tumors efficiently when intravenously administrated into nude mice bearing tumors formed by a human tumor cell line.

For innovative therapy for broad spectrum of tumors, we now target miR-21, which is expressed in almost all the epithelial tumors at very high levels and has been shown to be strong causative of cancer through inhibition of many important tumor suppressor genes simultaneously. Since miR-21 is one of the most abundant miRNA species in cancer cells, both high dosage of Super-S-TuD21（targeting miR-21）and efficient DDS would be required. However, high dosage of Super-S-TuD encapsulated by COATSOME-X was toxic to nude mice. We therefore used COATSOME-Y instead, which showed very effective intracellular delivery and much lower toxicity in mice. We optimized the method of preparing LNP composed of COATSOME-Y, helper lipids and PEGylated lipids and established the formulation of LNP encapsulating Super-S-TuD21. This LNP encapsulating Super-S-TuD21 is about 30nm and can fully suppress miR-21

activity in cancer cell lines at the dosage of 300nM (Nucleic acids Conc.). Such LNP showed high retentivity in blood and good pharmacokinetics with specific accumulation of LNP into tumor tissues, when administrated into tail vain of tumor bearing mice.

For pharmaceutical applications, it is important to preserve LNP from the time of manufacturing to the time of use without loss of its biological activity. Therefore, we investigated the cryopreservation method of Super-S-TuD encapsulated LNP using various buffer solutions as solvents for freezing LNP. Particle properties of LNP that were frozen, stored at -80°C and thawed were evaluated together with their biological activity. Although there was only a slight increase in particle size, there was no loss in the recovery rate and encapsulation rate. Using this method, we can any time supply LNP with the same properties.

Merged project of respiratory pathophysiology and pathobiology

巽浩一郎・磯野史朗・並木隆雄（呼吸器生体制御解析）プロジェクト

Summary（研究概要）

When we consider overcoming intractable infections encountered in clinical respiratory medicine, we should take morphologically / functionally impaired biological structure and functions in hosts into consideration other than pathogens that cause infection. To control intractable respiratory diseases including intractable respiratory infections, elucidation of respiratory pathobiological control mechanisms could be essential in regard with treatment strategy aimed for recovery and regeneration from lung injury.

Three major topics have been set up since this merged project of respiratory pathophysiology and pathobiology was started. 1) search for new treatment seeds based on the combining deep clinical phenotyping and omics analysis. 2) search for mechanisms of disordered respiratory control of breathing during sleep, and search for neurotransmitters and neuromodulators associated with disordered respiratory control of breathing. 3) search for mechanistic functions to overcome respiratory infection.

呼吸器臨床で遭遇する真菌を含む難治性感染症は，感染を生じる病原体pathogenの問題以外に，生体構造が形態的／機能的に障害を受けているhostに発症することが問題となる．難治性呼吸器感染症を含む難治性呼吸器病態の制御には，呼吸器生体制御機構の解明，その障害からの回復／再生を目指した治療戦略が必要になる．

呼吸器生体制御解析プロジェクトの立ち上げ以来，３つの主な研究テーマ（Research Focus）を挙げており，呼吸器領域全体を対象として基礎的／臨床的研究を施行することにより，幅広い視点から呼吸器生体制御に関する知見を得る必要がある．

１）難治性呼吸器疾患に対する新規治療戦略の探索

２）睡眠調節障害の病態の解明と神経伝達物質／神経修飾物質の観点からの新規治療法の開発

３）生体制御の観点からの呼吸器感染症の病態解明

Research Professor	Koichiro Tatsumi	特任教授	巽　浩一郎
Research Professor	Shiro Isono	特任教授	磯野　史朗
Research Professor	Takao Namiki	特任教授	並木　隆雄
Research Associate Professor	Yoko Irukayama	特任講師	入鹿山容子
Research Assistant Professor	Keiko Yamamoto	特任助教	山本　慶子

1. Functional roles of CD26/DPP4 in bleomycin-induced pulmonary hypertension associated with interstitial lung disease. Int J Mol Sci. 2024;25(2):748.

Okaya T, Kawasaki T, Sato S, Koyanagi Y, Tatsumi K, Hatano R, Ohnuma K, Morimoto C, Kasuya Y, Hasegawa Y, Ohara O, Suzuki T.

Pulmonary hypertension (PH) with interstitial lung diseases (ILDs) often causes intractable conditions. CD26/Dipeptidyl peptidase-4 (DPP4) is expressed in lung constituent cells and may be related to the pathogenesis of various respiratory diseases. We aimed to clarify the functional roles of CD26/DPP4 in PH-ILD, paying particular attention to vascular smooth muscle cells (SMCs). Dpp4 knockout (Dpp4KO) and wild type (WT) mice were administered

bleomycin (BLM) intraperitoneally to establish a PH-ILD model. The BLM-induced increase in the right ventricular systolic pressure and the right ventricular hypertrophy observed in WT mice were attenuated in Dpp4KO mice. The BLM-induced vascular muscularization in small pulmonary vessels in Dpp4KO mice was milder than that in WT mice. The viability of TGFβ-stimulated human pulmonary artery SMCs (hPASMCs) was lowered due to the DPP4 knockdown with small interfering RNA. According to the results of the transcriptome analysis, upregulated genes in hPASMCs with TGFβ treatment were related to pulmonary vascular SMC proliferation via the Notch, PI3K-Akt, and NFκB signaling pathways. Additionally, DPP4 knockdown in hPASMCs inhibited the pathways upregulated by TGFβ treatment. These results suggest that genetic deficiency of Dpp4 protects against BLM-induced PH-ILD by alleviating vascular remodeling, potentially through the exertion of an antiproliferative effect via inhibition of the TGFβ-related pathways in PASMCs.

2. Eliglustat exerts anti-fibrotic effects by activating SREBP2 in TGF-β1-treated myofibroblasts derived from patients with idiopathic pulmonary fibrosis. Eur J Pharmacol. 2024;966:176366.

Kurumiya E, Iwata M, Kasuya Y, Tatsumi K, Honda T, Murayama T, Nakamura H.

Idiopathic pulmonary fibrosis (IPF) is a progressive chronic lung disease. Myofibroblasts play a critical role in fibrosis. These cells produce the extracellular matrix (ECM), which contributes to tissue regeneration; however, excess ECM production can cause fibrosis. Transforming growth factor-β (TGF-β)/Smad signaling induces ECM production by myofibroblasts; therefore, the inhibition of TGF-β/Smad signaling may be an effective strategy for IPF treatment. We recently reported that miglustat, an inhibitor of glucosylceramide synthase (GCS), ameliorates pulmonary fibrosis by inhibiting the nuclear translocation of Smad2/3. In the present study, we examined the anti-fibrotic effects of another GCS inhibitor, eliglustat, a clinically approved drug for treating Gaucher disease type 1, in myofibroblasts derived from patient with IPF (IPF-MyoFs). We found that eliglustat exerted anti-fibrotic effects independent of GCS inhibition, and inhibited TGF-β1-induced expression of α-smooth muscle actin, a marker of fibrosis, without suppressing the phosphorylation and nuclear translocation of Smad2/3. RNA sequencing analysis of eliglustat-treated human lung fibroblasts identified sterol regulatory element-binding protein 2 (SREBP2) activation. Transient overexpression of SREBP2 attenuated the TGF-β1-induced increase in the expression of Smad target genes in IPF-MyoFs, and SREBP2 knockdown nullified the inhibitory effect of eliglustat on TGF-β1-induced expression of α-SMA. These results suggested that eliglustat exerts its anti-fibrotic effects through SREBP2 activation. The findings of this study may contribute to the development of novel therapeutic strategies for IPF treatment.

3. Functional roles of CD26/DPP4 in lipopolysaccharide-induced lung injury. Am J Physiol Lung Cell Mol Physiol. 2024;326(5):L562-L573.

Sato S, Kawasaki T, Hatano R, Koyanagi Y, Takahashi Y, Ohnuma K, Morimoto C, Dudek SM, Tatsumi K, Suzuki T.

Acute respiratory distress syndrome (ARDS) is characterized by dysregulated inflammation and increased permeability of lung microvascular cells. CD26/dipeptidyl peptidase-4 (DPP4) is a type II membrane protein that is expressed in several cell types and mediates multiple pleiotropic effects. We previously reported that DPP4 inhibition by sitagliptin attenuates lipopolysaccharide (LPS)-induced lung injury in mice. The current study characterized the functional role of CD26/DPP4 expression in LPS-induced lung injury in mice, isolated alveolar macrophages, and cultured lung endothelial cells. In LPS-induced lung injury, inflammatory responses [bronchoalveolar lavage fluid (BALF) neutrophil numbers and several proinflammatory cytokine levels] were attenuated in Dpp4 knockout (Dpp4 KO) mice. However, multiple assays of alveolar capillary permeability were similar between the Dpp4 KO and wild-type mice. TNF-α and IL-6 production was suppressed in alveolar macrophages isolated from Dpp4 KO mice. In

contrast, in cultured mouse lung microvascular endothelial cells (MLMVECs), reduction in CD26/DPP4 expression by siRNA resulted in greater ICAM-1 and IL-6 expression after LPS stimulation. Moreover, the LPS-induced vascular monolayer permeability in vitro was higher in MLMVECs treated with Dpp4 siRNA, suggesting that CD26/DPP4 plays a protective role in endothelial barrier function. In summary, this study demonstrated that genetic deficiency of Dpp4 attenuates inflammatory responses but not permeability in LPS-induced lung injury in mice, potentially through differential functional roles of CD26/DPP4 expression in resident cellular components of the lung. CD26/DPP4 may be a potential therapeutic target for ARDS and warrants further exploration to precisely identify the multiple functional effects of CD26/DPP4 in ARDS pathophysiology.

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Project for Evolution and Reproduction

生水ＰＩ（進化生殖）プロジェクト

Summary（研究概要）

Reproduction is fundamental to living organisms. Over the course of evolution, reproductive strategies have shifted—from producing numerous offspring with low survival rates to having fewer offspring and nurturing them to increase their chances of survival. In mammals, the number of eggs produced at one time has drastically decreased, from millions in fish to a single egg in humans. However, humans generate nearly 7 million oocytes during fetal development and support the growth of over 30 follicles per cycle in adulthood. This suggests that humans have acquired a specialized mechanism to limit the number of offspring, which may contribute to the reduction of the oocyte reserve with age. If this mechanism can be overridden, it may be possible to preserve the oocyte reserve and enhance female fertility. Our research aims to explore this process, with the hope of developing new strategies for infertility treatment.

生殖は生物の本質に関わる機能であり，生物進化にともない生殖戦略は大きく変化してきた．低コストで多くの子孫を作る戦略から，少ない子孫を生みコストをかけて育てる方向への進化である．哺乳類においても，一度に生む卵子数は魚類の数百万からヒトの１個にまで漸減した．しかし，ヒト胎児は700万個に迫る数の卵子を有しており，月経周期当たり30個以上の卵胞が発育することなどから，進化の過程で積極的に子供の数を減らす特別な機序を獲得してきたと考えられる．この産子数減少は，加齢に伴う卵子減少にも作用している可能性がある．われわれは，この機序を明らかにすることで，不妊症治療にあらたな展開をもたらすことが出来ると考えて研究をおこなっている．

Professor	Makio Shozu	特任教授	生水真紀夫
Professor	Hisao Osada	特任教授	長田　久夫

1. Symbiosis with Commensal Candida During Pregnancy.

Vaginal candidiasis is a condition characterized by itching, vaginal discharge, and, often, vulvar skin lesions. Although no clear benefit has been reported for commensal *Candida*, we are interested in its potential advantages, given that *Candida* commensalism has been shown to provide immunological benefits in the intestines. Vaginal *Candida* is most commonly found in women of reproductive age, particularly during pregnancy. It is detected in the vagina of 10-40% or more of asymptomatic pregnant women.

In Japan, universal screening for vaginal infections, including *Candida*, is conducted during early pregnancy. We analyze screening data to investigate the incidence of asymptomatic commensal *Candida*, its association with bacterial vaginosis, and its potential contribution to vaginal discharge or other symptoms. Additionally, we examine the possible effects of vaginal *Candida* on pregnancy outcomes.

2. A Female Specific Sdverse Effect of the COVID-19 Vaccines.

Messenger RNA（mRNA）COVID-19 vaccines are effective in preventing severe disease. However, after their implementation, real-world surveys identified several rare but severe adverse events unique to mRNA vaccines, including pericarditis in young males, cerebral venous sinus thrombosis, and Guillain-Barré syndrome. Gender differences exist in both the incidence of adverse effects and the incidence of

COVID-19 itself. Sex-dependent variations in the endocrine environment, such as differences in estrogen and androgen levels, as well as immune responses, may contribute to these disparities.

We focus on vulvar ulcers as a previously unrecognized adverse effect of COVID-19 vaccines. By analyzing publicly available data from the Vaccine Adverse Event Reporting System (VAERS) and the COVID Data Tracker, we identified a strong association between vulvar ulcers and vaccine use. These findings support the idea that acute vulvar ulcers are a rare but distinct adverse event of the vaccine, particularly in young women.

3. Novel therapy for Autoantibody-associated infertility

Autoantibodies may impact reproductive function. Antiphospholipid autoantibodies are known to cause recurrent pregnancy loss, while antinuclear autoantibodies contribute to oocyte loss in the ovary, leading to premature ovarian insufficiency. However, there are many types of antinuclear autoantibodies, and their pathognomonic significance remains largely unknown.

Recently, we screened infertile patients for antinuclear antibodies to determine which specific types are associated with fertility outcomes. We identified a titer-dependent association between anti-centromere antibodies and embryonic developmental failure during IVF/ICSI. Anti-centromere antibodies, which are characteristic of scleroderma, have been previously suggested to be linked to decreased fertility in scleroderma patients. However, infertile patients with these antibodies showed no immunologic signs or symptoms, suggesting a pathogenic mechanism distinct from scleroderma.

To further investigate this, we developed an *in vitro* disease model using mouse oocytes and found that anti-centromere antibodies directly enter the oocyte and disrupt chromosome segregation during meiosis. Furthermore, we are developing a novel therapy to target anti-centromere antibodies, with the aim of increasing the rate of good-quality blastocyst formation and improving live birth outcomes.

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Ministry of Education, Culture, Sports, Science and Technology National BioResource Project "Pathogenic Microorganisms"

文部科学省　ナショナルバイオリソースプロジェクト「病原微生物」

In FY2002, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) implemented the National BioResource Project (NBRP) to construct the framework for systematic collection, preservation, and distribution of bioresources, with a focus on those that required strategic development by the national government. After the reviewing the NBRP every five years, in FY2022, the fifth phase has stared.

Chiba University's Medical Mycology Research Center (MMRC) is the "NBRP Center" for pathogenic microorganism, and this project is carried out by MMRC (pathogenic fungi/actinomycetes) and Nagasaki University's Institute of Tropical Medicine (pathogenic protozoa). Working together, they cooperate in various efforts to support education and research pertaining to infectious diseases and pathogens. Specifically, they are developing a system for collection, preservation, and distribution of pathogenic microorganisms, and they supply reliable strains of pathogenic microorganisms that are backed by high-level information. Furthermore, in order to utilize the data for quality control of stored strains, we are collaborating with the RIKEN BioResource Center and the Center for Conservation of Microbial Genetic Resources, Gifu University to maintain MALDI-TOF MS data.

The project aims to establish a reliable and sufficient at the collection to deal with infectious diseases carried by any pathogenic microorganisms.

文部科学省では2002年度からナショナルバイオリソースプロジェクト（NBRP）を開始し，国が戦略的に整備することが重要なものについて体系的に収集，保存，提供などを行うための体制を整備してきた．その後５年ごとの見直しを行い，2022年度より第５期が開始された．

NBRP病原微生物中核機関である千葉大学真菌医学研究センター（病原真菌・放線菌）と長崎大学熱帯医学研究所（病原性原虫）は，相互の機関の連携を図り，これらの病原微生物株の収集・保存・提供体制を整備して，高度情報を賦与した信頼できる病原微生物株として提供し，感染症と病原体の教育・研究をする人々を支援している．さらに，保存株の品質管理に活用するため，理化学研究所バイオリソースセンター，岐阜大学微生物遺伝資源保存センターと連携し，MALDI-TOF MSのレファレンスライブラリー整備を行っている．

本プロジェクトは，今後いかなる感染症が発生しても対応できる病原微生物コレクションを目指している．

TABLE 1. Results for the fourth quarter of NBRP (strains).

Number of strains	FY2020	FY2021	FY2022	FY2023	FY2024*
Collection	886	837	702	688	235
Preservation	25, 785	26, 591	24, 689	25, 322	25, 884
Provision	222	1, 319	846	1, 161	928

*: to 31th Aug., 2024

FIG. 1. Exhibition at the Annual Meeting of the Molecular Biology Society of Japan and the Japanese Society for Clinical Mycology.

International Collaborative Research Program for Tackling the NTDs (Neglected Tropical Diseases) Challenges in African Countries

"Research on the diagnostics of early or latent eumycetoma: Search for new biomarkers, POC diagnostics, and development of a clinical epidemiology platform"

アフリカにおける顧みられない熱帯病（NTDs）対策のための国際共同研究プログラム

「早期・潜在性真菌腫診断に関する研究：バイオマーカーの探索・POC診断と臨床疫学プラットフォームの開発」

This research program is led by Prof. Satoshi Kaneko, Institute of Tropical Medicine, Nagasaki University, in collaboration with the Institute of Transformative Bio-Molecules, Nagoya University, Tokai National Higher Education and Research System, the Medical Mycology Research Center, Chiba University, the Graduate School of Human Development and Environment, Kobe University, and the Mycetoma Research Center, University of Khartoum. The goals of the project are as follows:

(1) Identification of metabolites detected in mycetoma patients that can be used as a guide for early diagnosis and completion of treatment, and development of diagnostic tools targeting the identified metabolites

(2) Development and evaluation of a rapid PCR diagnostic method using the LAMP (Loop-Mediated Isothermal Amplification) method that can be performed at rural medical facilities with limited facilities.

(3) Establishment of a technique for measuring environmental DNA from soil to determine the geographic distribution of mycetoma-causing fungi for diagnosis and prevention measures, and development of a system for measuring geographic distribution.

The Center will be responsible for (2). Sharing mycetoma-causing fungi and their information with the University of Khartoum, designing LAMP primers and creating a prototype LAMP diagnostic kit with the support of Eiken Chemical Co, Ltd. Furthermore, guidelines will be developed for implementation at medical institutions in areas where facilities are not available.

本研究プログラムは，長崎大学熱帯医学研究所　金子聰先生がプロジェクトリーダーとなり，名古屋大学 トランスフォーマティブ生命分子研究所，千葉大学 真菌医学研究センター，神戸大学大学院 人間発達環境学研究科，ハルツーム大学 マイセトーマ研究センターが協力し推進する．その目標は以下の通りである．

(1) 早期診断・治療終了の目安となるマイセトーマ患者から検出される代謝物の特定と特定された代謝物を標的とした診断ツール開発に向けての検討

(2) LAMP（Loop-Mediated Isothermal Amplification）法を用いた設備の整わない地方の医療施設において実施可能な迅速PCR診断法の開発と評価

(3) 診断並びに予防対策に向けてのマイセトーマ原因真菌の地理的分布を把握するための土壌から環境DNA測定技術の確立と地理分布測定に向けての仕組みの開発

当センターは，(2) を担当する．ハルツーム大学とマイセトーマ原因真菌とその情報を共有し，LAMP法プライマーの設計と栄研化学（株）の支援によるLAMP診断キットのプロトタイプを作成する．さらに，設備の整わない地域の医療機関での実施に向けたガイドラインを作成する．

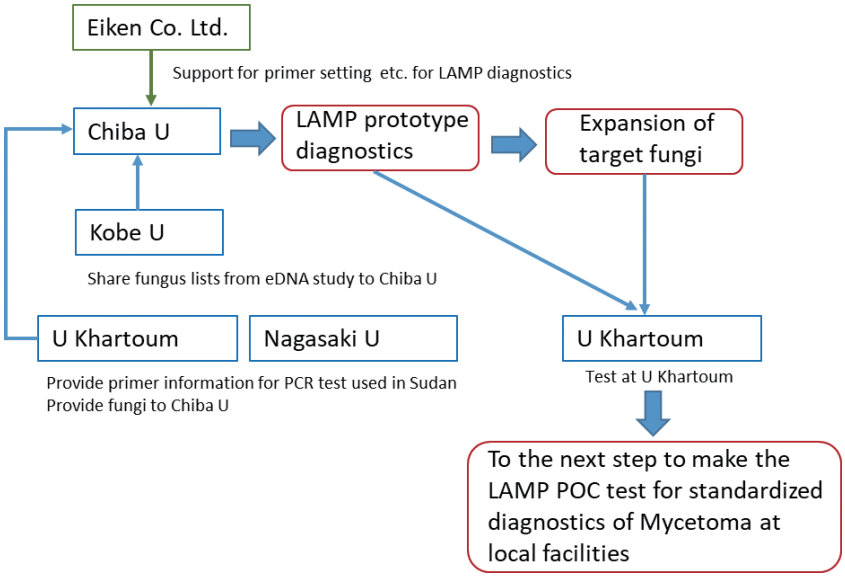

FIG. 1. Finding POC diagnosis using LAMP method of mycetoma infection.

FIG. 2. The cooperative institutions of Mycetoma Research Center, University of Khartoum and Symptoms of mycetoma.

Generating research infrastructure and novel technologies for anti-infective drug and vaccine discovery, AMED-CREST

"Study of the molecular mechanism of persistent infection and identifying novel privileged molecular structures for the next-generation antibacterial drug discovery"

日本医療研究開発機構革新的先端研究開発支援事業

感染症創薬に向けた研究基盤の構築と新規モダリティ等の技術基盤の創出：「難治性感染症制御に資する細菌持続感染機構解明と次世代型抗感染症化合物の創出」

Bacteria that endure harsh environmental changes, such as antibiotic treatments and host immune responses, acquire genetic mutations while adapting to their surroundings. This adaptation enables them to survive and multiply, leading to persistent infections that are difficult to treat. Among these bacteria are "persisters" that withstand antibiotic exposure and pathogens responsible for long-term persistent infections within hosts. However, the mechanisms behind these adaptations remain unclear.

To address this gap, we conducted research aimed at developing innovative treatments for infectious diseases. Our approach involved analyzing the molecular mechanisms that allow bacteria to survive environmental changes and identifying compounds that target bacterial proteins acting as regulatory factors. We obtained the following results:

1. According to the analysis of characteristics and DNA methylation in methicillin-resistant *Staphylococcus aureus* (MRSA) strains responsible for an outbreak in a hospital, we clarified how changes in the DNA methylation rate of specific genes lead to reversible alterations in the expression of the Agr system. This system controls virulence factors, contributing to the long-term establishment of the strain within the hospital.
2. We identified several novel compounds, including Physalin H, as inhibitors of the Agr system in MRSA. We determined the structural components that enhance inhibitory activity through structure-activity relationship studies.

抗菌薬投与や宿主免疫応答等の過酷な環境変化に耐えて生存する細菌は，環境に適応し生存・増殖を繰り返す間に遺伝子変異を獲得し，難治性感染症の原因となる．環境に適応する細菌には，抗菌薬曝露でも生き残る"パーシスター"や，宿主内で長期間持続感染する病原菌があるが，これらの適応機構は未だ明らかではない．そこで，細菌が環境変化に耐えて生存する分子機構を解析し，制御因子となる細菌蛋白質を標的とした化合物を同定・有効性を検証することで，革新的な感染症治療薬を創成することを目的に研究を進めている．

これまでに次のような結果を得た．

（1）院内でアウトブレイクを引き起こした多剤耐性黄色ブドウ球菌（MRSA）の系統の性状およびDNAメチル化解析から，特定遺伝子のDNAメチル化率の変化が病原因子制御を担うAgrシステムの可逆的な発現変化を起こし，院内に定着する機構を明らかにした．

（2）MRAのAgrシステムを阻害する化合物としてPhysalin Hなど数種の化合物を新たに同定し，構造活性相関研究から阻害活性向上に関わる構造を見出した．

Japan Agency for Medical Research and Development（AMED）

Japan Initiative for World-leading Vaccine Research and Development Centers
Chiba University “Synergy Institute for Futuristic Mucosal Vaccine Research and Development”（cSIMVa）

AMEDワクチン開発のための世界トップレベル研究開発拠点の形成事業

ワクチン開発のための世界トップレベル研究開発拠点群 千葉シナジーキャンパス
（千葉大学 未来粘膜ワクチン研究開発シナジー拠点）

As uncovered by the recent COVID-19 pandemic, research on infectious diseases and the development of vaccines in Japan lagged behind Western countries. In addition to infectious disease research during normal times, AMED will continue to support research and development using cutting-edge approaches over the long term to equip for future pandemics. In fiscal 2022, AMED launched the “Japan Initiative for World-leading Vaccine Research and Development Centers.”

Currently, most developed vaccines are injection-type and induced blood IgG antibodies alone that cannot effectively prevent the invasion of pathogens on mucosal surfaces. As one of the synergy institutes, Chiba University will develop and implementation of mucosal vaccines that are expected to both prevent infection and avoid exacerbation of diseases based on the understanding of the mechanism of infection by pathogens at the mucosal sites such as respiratory and intestinal tracts and the host mucosal immune system. In addition, we will promote the commercial licensing of mucosal vaccines and mucosal adjuvants developed through this research. We aim to implement and market mucosal vaccines as a new vaccine modality.

今般の新型コロナウイルスによるパンデミックで顕在化したように，我が国における感染症研究やワクチン開発は欧米諸外国に比して後塵を拝している状況にある．AMEDでは，今後のパンデミックに備えるため，平時から感染症研究に加え，最先端アプローチによる研究開発を長期継続的に支援する「ワクチン開発のための世界トップレベル研究開発拠点の形成事業」を2022年度から開始した．

現在，開発されているワクチンのほとんどが注射型のワクチンであり，ワクチン接種によって誘導される血中IgG抗体だけでは粘膜面における病原体の侵入は効果的に防げていない．この課題に対し，千葉大学は本事業におけるシナジー拠点の一つとして，呼吸器や腸管などの粘膜面における感染性病原体の感染機序および宿主粘膜免疫システムの理解を基盤とした，病原体の感染阻止と重症化回避の両側面が期待できる粘膜ワクチンの開発と実装化を目的として研究に取り組む．さらに，本研究を通して開発された粘膜ワクチンや粘膜アジュバントの企業導出を進め，新規ワクチンモダリティーとしての粘膜ワクチンの実用化と市場展開の実現を目指す．

Research Institute of Disaster Medicine

災害治療学研究所

In October 2021, Chiba University established the Research Institute of Disaster Medicine, which aims to protect the health and safety of the people, the environment, and social activities against threats such as natural disasters and pandemics. The Institute brings together researchers from diverse backgrounds from the departments of Chiba University to promote interdisciplinary research and to conduct co-creative research and development and social implementation through collaboration between industry, academia, and government.

Faculty members of the MMRC have joined this Institute as members of the Division of Pandemic and Post-disaster Infectious Diseases in collaboration with the Department of Infectious Diseases of the Chiba University Hospital. We will conduct basic and clinical research on various infectious diseases, such as severe respiratory disorders caused by SARS-CoV-2 infection, complex infectious diseases caused by immune suppression, and respiratory infectious diseases caused by stress and dust inhalation associated with natural disasters.

Prof. Goto's lab of the Division of Molecular Immunology at MMRC is managing the Biosafety level 3 (BSL3) facility of the institute. In addition, an animal BSL3 facility (ABSL3) will be established in the institute by the end of 2025.

千葉大学では，2021年10月に自然災害やパンデミックなどによる社会的脅威に対して，国民の健康・安全および社会の環境・活動性を守ることができる「災害レジリエントな社会」を構築することを目標に，千葉大学が有する多様な部局から多彩なバックグラウンドを有する研究者が集結し，学際的研究の推進と，産学官が連動した共創的な研究開発と社会実装を目指して，災害治療学研究所を設立しました．

真菌医学研究所の教員も本研究所に参画し，「災害感染症部門」のメンバーとして附属病院の感染制御部と連携し，新型コロナウイルス感染症に伴う重篤な呼吸器障害，免疫低下に起因する複合感染症や自然災害に伴うストレス・塵埃吸入等に起因する呼吸器感染症等の多様な感染症に関する基礎・臨床一体型研究を推進しています．

2023年に設置された新研究棟では，感染免疫分野の後藤研究室がバイオセーフティレベル３（BSL3）実験施設を管理しながら，研究活動を行っています．また2025年度中には，同施設内にABSL3施設が設置される予定です．

URL: https://www. ridm. chiba-u. jp/
https://www. ridm. chiba-u. jp/en/index. html

The training course of pathogenic fungi

真菌医学研究センター病原真菌講習会

We annually held the training course of pathogenic fungi to learn knowledge and technique in order to treat pathogenic fungi and actinomycetes and the number of participants is 10. Every year, a number of application is over the participant and the course has been in a great demand. But due to the COVID-19, the course was cancelled in FY2020 and FY2021. From FY2022, the course content was reviewed and the number of participants was limited to 8.

Practice/Lectures: Pathogenic yeasts, pathogenic Aspergillus, causative agents of dermatological mycoses, imported and emerging pathogenic fungi, pathogenic zygomycetes, pathogenic actinomycetes, pathogenicp rotozoan, drug susceptibility testing methods, MALDI-TOF MS rapid identification methods, strain preservation methods, infectious disease methods, etc.

病原真菌講習会は，病原真菌・放線菌の基本的取り扱いの知識と技術を習得するために，本センターが実習を中心に，年１回定員10名で開催していた．例年，定員大きく超える応募があり，大変好評を得ていたが，2020, 21年度はコロナ禍の影響で講習会は中止となった．2022年度より，実施期間を３日に短縮，参加者を８名に限定するなど感染防止措置を万全にする代わりに，外部講師の招聘，講習内容の見直しを実施した．

実習・講義内容：病原性酵母，病原性アスペルギルス，皮膚科領域真菌症原因菌，輸入および新興病原真菌，病原性接合菌，病原性放線菌，薬剤感受性試験法，MALDI-TOF MS迅速同定法，菌株保存法，感染症法など

FIG 1. Scenes from the training course of pathogenic fungi.

miRaX Therapeutics K. K.

ミラックスセラピューティクス株式会社

MiRaX Therapeutics K. K., established in May 2020, is a drug discovery venture company originated from Chiba University and the University of Tokyo. Our main targets are "Development of nucleic acid drugs using miRNA inhibition technology" and "Development of novel NF-κB inhibitors".

1. Development of nucleic acid drugs using miRNA inhibition technology

The miRNA inhibition technology developed by the founders is based upon RNA decoy with unique secondary structure and has already been licensed out as a research reagent in many countries. It is now highly evaluated for its strong and long-lasting inhibitory effects. Our mission is to apply this technology to pharmaceuticals and create nucleic acid medicine for several diseases including liver fibrosis.

2. Development of NF-κB inhibitors

Since the transcription factor NF-κB is constitutively activated in inflammatory diseases and cancers, it is a promising therapeutic target. Since currently available NF-κB inhibitors affects several signal transduction pathways simultaneously, their biological effects are broad and not specific. To develop specific inhibitor for NF-κB, we focus on d4 family proteins (DPF1, DPF2, DPF3a/b) which are crucial for NF-κB transactivation as adaptor proteins connecting NF-κB and SWI/SNF complexes. We identified compounds that bind to these adaptor proteins, and are in the process of verifying the inhibitory activity on NF-κB.

当社は，2020年５月に設立された千葉大学・東京大学発の創薬ベンチャー企業です．主な事業は「miRNA阻害技術を活用した核酸医薬品開発」と「新規NF-κB阻害薬の開発」です．

1.　miRNA阻害技術を活用した核酸医薬品開発

創業者らが開発したmiRNA阻害技術は，独特の２次構造をもったRNAデコイであって，すでに研究用試薬として世界各国で販売されております．これまでに阻害効果の強さや持続の長さで，高い評価を得ています．この技術をDDS技術と組み合わせ，MASHを含む種々の対象疾患に対する核酸医薬品の開発を行っています．

2.　NF-κB阻害薬の開発

転写因子NF-κBは多くの炎症疾患やがんなどで構成的に活性化されているため，その活性化に至る経路は，これらの治療の有望な標的となると考えられます．しかし既存の多くのNF-κB阻害剤は，多くのシグナル伝達経路を同時に抑制することから，その効果は広範囲に及び非特異的です．そこで我々はNF-κBとSWI/SNF複合体をつなぐアダプタータンパク質として転写活性化を担うd4ファミリータンパク質（DPF1, DPF2, DPF3a/b）に着目しました．これまでに，低分子化合物のスクリーニングを行い，これらのアダプタータンパク質に結合する化合物の同定に成功しており，これらの化合物のNF-κBの阻害活性能の検証を進めています．

HP: https://www. mirax-t. co. jp

Electron microscope facility

電子顕微鏡施設

Transmission electron microscope (JEOL JEM-1400) and scanning electron microscope (Hitachi S-3400N) are installed on the 1st floor in A building, and used for ultrastructural research of cells and tissues. An ultramicrotome, rapid-freezing device, critical point drying equipment, carbon coater is also installed, and all procedure from fixation of biological samples to observation with electron microscopes can be carried out.

Original research is promoted, and joint research with not only laboratory in the center but also outside the center such as faculty of medicine in Chiba university is conducted. Electron microscopy training courses are opened several times a year and people not only in Japan but also from abroad participate. Consultation on electron microscope observation is accepted at any time by contacting with yama@faculty. chiba-u. jp (Yamaguchi).

センターA棟1階には，透過電子顕微鏡（日本電子JEM-1400）と走査電子顕微鏡（日立S-3400N）が設置されていて，細胞，組織の微細構造研究に用いられている．超薄切片作製装置（ウルトラミクロトーム），急速凍結装置，臨界点乾燥装置，凍結乾燥装置，イオンスパッタ装置，カーボンコーター等を揃え，現在千葉大学にて唯一生物試料の超微形態観察を行うことができる施設となっている．

独自の研究を推進するとともに，センター内だけでなく，千葉大医学部など多くの研究室と共同研究を実施している．また，日本顕微鏡学会の電顕技術講習会を毎年複数回実施しており，日本国内だけでなく，海外からも参加者を迎えている．電子顕微鏡観察に関するご相談は，随時受け付けておりますので，ご興味のある方は，メールyama@faculty. chiba-u. jp，またはお電話で（043-222-7171　内線5964　山口），ご連絡ください．

FIG. 1. Transmission electron microscope JEM-1400.

FIG. 2. Scanning electron microscope H-3400N.

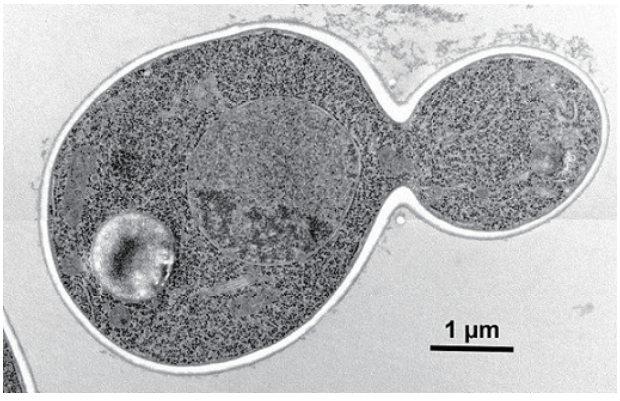

FIG. 3. Ultrathin section of *Saccharomyces cerevisiae*.

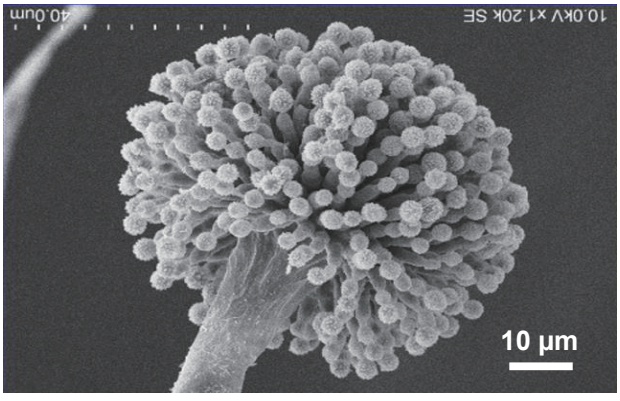

FIG. 4. Spores of *Aspergillus*.

2023 Fiscal Year Cooperative Research Program Report

令和５年度 共同利用・共同研究報告書

研究課題　’23－01

Analysis of Sequence-Based Identification and Antifungal Susceptibility of Aspergillus from Clinical Respiratory specimens

Junko Suzuki, Keita Takeda
（Center for Respiratory Diseases, National Hospital Organization, Tokyo National Hospital）
Akira Watanabe, Katsuhiko Kamei
（Medical Mycology Research Center, Chiba University）

Aspergillus 呼吸器検体臨床分離株の菌種同定・薬剤感受性の検討

鈴木純子
（国立病院機構東京病院呼吸器センター呼吸器内科医長）
武田啓太
（国立病院機構東京病院呼吸器センター呼吸器内科医員）
渡辺　哲
（千葉大学真菌医学研究センター准教授）
亀井克彦
（千葉大学真菌医学研究センター教授）

研究成果

2013年の研究開始から2024年３月までの間に，国立病院機構東京病院の呼吸器疾患患者の下気道検体から検出された*Aspergillus*または担子菌の菌種同定・感受性を千葉大学真菌医学研究センターにて行い，これまで計482件同定している．当院慢性肺アスペルギルス症患者呼吸器検体から検出された*Aspergillus*の菌種同定薬剤感受性を検討し，*A. fumigatus*陽性257株について，*A. fumigatus*のアゾール耐性株は20株，7.8％であった．

23年度は2022年までに提出し，同定・感受性検査を行った*A. fumigatus*259株についてITCZ＞4μg/ml, VRCZ＞4μg/mlをアゾール耐性と定義し，耐性が確認された20症例21株について，菌の遺伝子解析結果とともに，診療録から検出患者の臨床所見，治療経過，予後を後方視的に検討した．耐性株が検出されたのは慢性肺アスペルギルス症16例，ABPA疑い２例，アスペルギルス膿胸１例，コロニゼーション疑い例１例であった．耐性菌検出例経過としては，外科的治療を行ったのは慢性肺アスペルギルス症と膿胸例の計８例で，うち７例は抗真菌薬の内服終了し再発なし．１例は慢性肺アスペルギルス症が進行した状態で手術を行っており，術後１年以上の経過で２型呼吸不全の進行で死亡した．非手術例の12例のうち慢性肺アスペルギルス症８例の転帰は死亡が３例，悪化が４例で手術例に比較し不良であった．耐性薬剤はアゾール治療歴のある患者で，未使用のアゾール薬に対して耐性を認めた例も多かった他，アゾール使用歴がなく耐性を認めた症例を2020年以降３例認め，いずれもITCZ感受性，VRCZ耐性であった．アゾール耐性機序は日本ではこれまでアゾール使用歴を有する例の報告が主であったが３例は環境由来の可能性もあり今後の結果に注意が必要である．耐性原因遺伝子の検討ではCyp51A遺伝子変異を12例に，hmg1遺伝子変異を４例に認め，hmg1遺伝子変異例はITCZ／VRCZ両者に耐性であった．今回の耐性例で経時的に*A. fumigatus*が検出されている症例は５例あり，菌株の遺伝子学的検討から，３例は同一菌株，２例は異なる株の検出であった．

研究課題　’23－02

Analysis of a protein with unknown function that reduces azole susceptibility of *Aspergillus fumigatus*

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Aspergillus fumigatus におけるアゾール感受性低下をもたらす機能未知タンパク質の機能解析

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研究成果

渡辺，豊留らはRttAを*Aspergillus fumigatus*においてアゾール感受性に関与する因子として2021年に報告した．我々はこのタンパク質をコードする領域について見直した結果，本遺伝子は今まで見逃されていたN末端領域を持つことを明らかとした．このN末端領域も含む形で*rttA*遺伝子破壊株を令和４年度の共同利用研究において取得し，*rttA*遺伝子破壊株では親株であるA1159株に比べてボリコナゾールへの薬剤感受性が高まることを明らかとした．令和５年度の本研究では，*gpdA*遺伝子のプロモーターを*rttA*遺伝子上流に配置した*rttA*高発現株を取得することに成功した．*rttA*高発現株では，*rttA*遺伝子破壊株で得られた結果とは逆に，A1159株に比べてボリコナゾールへの薬剤感受性が低下したことを明らかにした．このことから，*rttA*はボリコナゾールへの薬剤感受性に寄与する因子であることがあらためて示された．また，これらの株からRNAを調製してRNA-Seq解析を行い，発現比較解析を行った．その結果，*rttA*高発現株において，A1159株および*rttA*遺伝子破壊株よりも２倍以上発現上昇している６遺伝子が見出された．これらの遺伝子はいずれも機能未知であった．本研究により，アゾール感受性に関する*A. fumigatus*の新たな因子群の同定につながった．RttAの機能や細胞内局在についての情報はまだ得られておらず，その影響を受ける６遺伝子についても全く知られておらず，*A. fumigatus*のアゾール感受性に関するさらなる基礎的研究につながると期待する．

研究課題　’23−03

Contribution of Dectin-2 and IL-17 to the host defense in experimental sporotrichosis by *Sporothrix brasiliensis*

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研究成果

Sporothrix brasiliensis was recently discovered as a new causative agent of the deep mycosis sporotrichosis but associated to more severe and lethal infections. The mechanisms behind its higher virulence, however, are still largely ignored. In this project we want to investigate how the antifungal immune response promoted by dectin-1, dectin-2 and IL-17 is shaped against *S. brasiliensis*.

We initially established in our lab two murine models of sporotrichosis, disseminated infection by intravenous inoculation, and localized infection by subcutaneous infection: the first being lethal while the latter being self-resolving.

We observed that dectin-1 and dectin-2 are essential for resistance against deep sporotrichosis (Figure 1), important for limiting fungal spread across different organs. However, their effector mechanism is not linked to IL-17 response.

IL-17, while also required for host defense, acts as a second wave of protection in the disseminated model and tend to show a more localized action, important for wound healing of superficial lesions (Figure 2).

In our next steps, we aim to elucidate the molecular mechanisms involved in each defense branch.

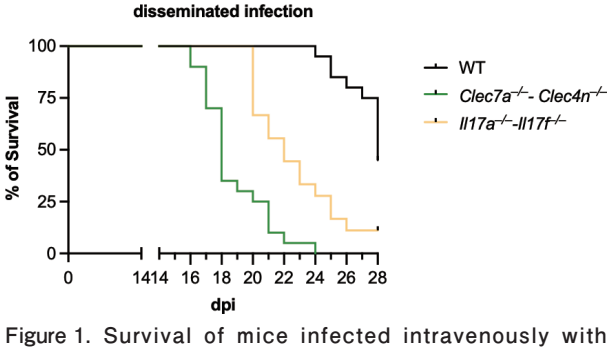

Figure 1. Survival of mice infected intravenously with *S. brasiliensis*. Lack of dectin-1/dectin-2 or IL-17A/F increase susceptibility.

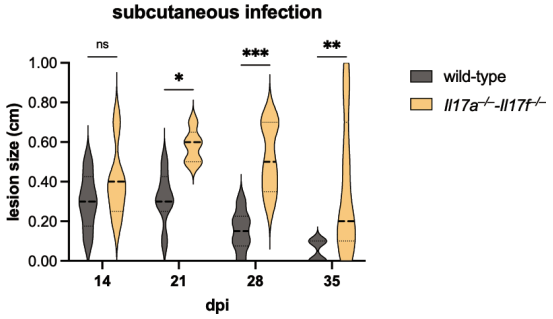

Figure 2. *S. brasiliensis*-induced lesions in mouse skin. Lack of IL-17A/F delays lesion healing

研究課題　’23－04

Proteome analysis of microRNA-mediated response induced by viral infection

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プロテオーム解析によるウイルス感染によるmicroRNAを介した複雑な応答機構の解明

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研究成果

レトロウイルスが哺乳類の細胞に感染すると，生体をウイルスから防御するために，抗ウイルス免疫応答が誘導される．この時に主に働くウイルスセンサータンパク質はRIG-I like receptors（RLRs）であり，受入教員である米山教授らが発見した分子である．発現誘導されたIFNは細胞外に分泌され，受容体を介して周辺細胞へも働いて，数百のIFN誘導遺伝子群（IFN-stimulated gene, ISG）の発現を誘導し，ウイルス複製を抑制するように働く．一方で，ウイルス感染細胞におけるアポトーシスによる細胞死は，感染細胞が自滅することで周辺の細胞を守るという意味をもつ．RLRsにはRIG-I, MDA5, LGP2といった３つの因子が存在する．いずれもISGに含まれる因子であり，RIG-IとMDA5はそれぞれ異なるウイルスRNAを認識しIFNを誘導するのに対し，LGP2はその機能が不明であった．本共同研究では，まずLGP2の機能を明らかにした．ウイルス感染早期の反応として，RNAサイレンシングの主要因子であり，二本鎖RNA結合タンパク質であるTRBPが，発現誘導されたLGP2と相互作用し，TRBPによって生合成されるはずであったmicroRNAの生合成を阻害することを明らかにした．成熟化が阻害されると，microRNAによるRNAサイレンシングは起こらなくなるが，その標的遺伝子はアポトーシス関連遺伝子群であったため，その発現が阻害されなくなることでアポトーシスが誘導されることを報告した．しかし，この反応は可逆的であり，この状態が継続すれば免疫不全を誘導する可能性も考えられる．そこで，さらに後期の反応として，アポトーシスで活性化されたカスパーゼがTRBPを切断することを発見し，それによって非可逆的な細胞死の誘導がおこることを本年度の論文として報告した．本共同研究課題では，それをさらに発展させる．すなわち，TRBP以外のmicroRNAと相互作用する二本鎖RNA結合タンパク質についても，ウイルス感染時におけるISGとの相互作用が変動するのかをマススペクトロメトリーを用いたプレテオーム解析によって明らかにし，システマティックなmicroRNAによる遺伝子制御機構について検討している．

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研究課題　'23－05

Following the genomic evolution towards drug resistance and biofilm formation in *Candida glabrata*

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研究成果

Candida species are the leading cause of disseminated fungal infections, with *Candida glabrata* ranking as the second most common cause of invasive candidiasis mostly due to its ability to quickly acquire resistance to the widely used antifungal fluconazole, as well as its capability to form stable biofilms.

Through this joint collaboration, the role of a Mediator Complex（MC）tail subunit, CgPgd1, in the control of both azole resistance and susceptibility was scrutinized. Surprisingly, *CgPGD1* deletion leads to azole susceptibility in solid media and to azole resistance in liquid media. RNA-sequencing analysis was used to identify the CgPgd1-controled transcriptome-wide response to fluconazole in these two environmental conditions. CgPgd1 was found to regulate the expression of 454 and 658 genes when fungal cells were grown in the presence of fluconazole in solid or liquid medium, respectively. Among them, 36 candidate genes whose expression is antagonistically controlled by CgPgd1 in solid and liquid media were identified as possible causes of the dual effect of CgPgd1 depending on the environmental condition.

Also, we conducted an analysis of the phenotypic variability in biofilm formation among a collection of clinical isolates of *C. glabrata*. We then combined a comparative genomics approach with experimental microevolution of these clinical isolates toward a more robust biofilm phenotype, aiming to pinpoint key regulators and effectors of biofilm formation in *C. glabrata*. Our findings reveal that the specialization toward enhanced biofilm formation occurs rapidly, accompanied by genome alterations, adhesin modifications, and the accumulation of variations in effectors operating at various regulatory levels, spanning from epigenetic to post-translational mechanisms. We successfully identified genes with predictive roles in biofilm formation, including uncharacterized adhesins from the EPA and PWP gene families, as well as transcription factors and telomeric silencing proteins. These genes hold promise for future exploration as targets for disrupting biofilms and as markers of biofilm evolution.

Overall, this joint collaboration continues to provide new clues on new mechanisms involved in azole resistance/susceptibility and biofilm formation evolution in fungal pathogens.

発表論文

One joint paper was published in 2023, and two additional papers are being prepared for publication.

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研究課題　’23－06

Distribution and molecular phylogenetic analysis of *Macrorhabdus ornithogaster* causing Avian Gastric Yeast Disease in zoo-raised birds

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動物園飼育環境下の鳥類におけるAvian Gastric Yeast症原因真菌の分布状況および分子系統分類に関する研究

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研究成果

鳥類のAvian Gastric Yeast（AGY）症の原因真菌*Macrorhabdus ornithogaster*について，家庭飼育の愛玩鳥以外では，分布の実態は明らかになっていないことから，動物園飼育鳥類での分布実態の把握および菌種内の系統分類を行った．

協力動物病院および動物園から，症状の有無にかかわらず，計272個体からの糞試料の提供を受け，液体培養を行った．家庭飼育下罹患個体の２試料（ブンチョウおよびマメルリハ）からは，*M. ornithogaster*の大量培養および高分子DNA抽出に成功し，NGS解析によりドラフトゲノム配列を取得した．さらに，培養液抽出DNAを鋳型とし，サンガーシーケンスまたはNGSアンプリコンシーケンスを実施して，本菌のrDNA ITS1領域塩基配列を決定した．家庭飼育下罹患個体の７鳥種（マメルリハ・セキセイインコ・キエリクロボタンインコ・オカメインコ・キンカチョウ・ブンチョウ・ボタンインコ），動物園飼育下個体の４鳥種（ルリコンゴウインコ，ケープペンギン，エミュー，シロフクロウ）由来サンプルから配列の取得に成功し，GenBankデータベースからのダウンロード配列とともに系統解析を行った．その結果，今回決定した*M. ornithogaster*配列データは，サンプル採取機関および由来個体の飼育下・野生に関わらず宿主の鳥種の分類ごとに２つの単系統群「インコ目クレード」および「非インコ目クレード」を形成した．「非インコ目クレード」に属する*M. ornithogaster*は，野生または飼育下個体の，地理・年代的に離れた地点で取得された互いに独立性の高いスズメ目等複数目由来の配列が含まれたことから，本クレード内の*M. ornithogaster*は宿主特異性が比較的低く，インコ目以外の幅広い鳥の系統に感染するリスクがあることが示唆された．

研究課題　’23－07

Analysis of major drug efflux pumps in azole-resistant dermatophytes

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白癬菌においてアゾール剤排出の中核的機能を果たしているポンプの解析

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研究成果

感染症における原因微生物の薬剤耐性化がグローバルな脅威となる中，皮膚糸状菌（白癬菌）の感染によって起こる白癬においても薬剤耐性菌の蔓延が明らかとなっている．白癬の中核治療薬の１つであるアゾール系抗真菌薬（アゾール）に対する本菌の薬剤耐性化の原因の１つは，アゾールを特異的に排出する薬剤排出トランスポーター（ポンプ）の過剰発現である．本研究では，国内で発生する白癬の原因菌の大半を占める*Trichophyton rubrum*において，アゾール排出の中核的機能を果たしている薬剤排出ポンプの制御に関わる分子の同定を進めた．申請者らが分離したアゾール耐性*T. rubrum* TIMM20092では，ABC型薬剤排出ポンプMDR2やMDR3の過剰発現が確認されている．そこで，2023年度は，*Candida*属酵母や*Aspergillus*属糸状菌などで報告されているアゾール排出ポンプの制御因子（遺伝子）に関する情報を基に，*T. rubrum*のゲノムシーケンス情報を解析し，MDR2やMDR3の制御に関与している可能性のあるTERG_01042遺伝子を見出した．TERG_01042遺伝子の発現産物は*Aspergillus fumigatus*で見つかったABC型薬剤排出ポンプabcG1の発現を制御する転写因子atrRのオーソログの可能性がある．申請者らは，ゲノム編集技術を用いてTIMM20092のゲノムに存在するTERG_01042遺伝子の破壊を試み，目的とする破壊株の作出に成功した．

研究課題　’23－08

Pathophysiological analysis of aspergilloma

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アスペルギローマの病態解析

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研究成果

2023年度は，アスペルギローマ周囲の泡沫化マクロファージを詳細に解析し，アスペルギローマの排除を担うマクロファージの性質の解明を試みた．留置した菌球周囲のIba1陽性マクロファージは，菌球留置後０週：0.02％，1-4週：3.5％，8-16週：7.5％と経時的に上昇していた（p = 0.0021）．３ヶ月の経過で好中球は菌球内部まで浸潤していた一方で，Iba1陽性マクロファージは菌球内部へは浸潤せず，菌球の周囲を取り囲む形で集簇していた．菌球周囲に集簇したマクロファージは３ヶ月間の慢性の経過で泡沫化しており，Modified Gomori Methenamine-Silver Nitrate染色では，泡沫細胞内にアスペルギルスの残骸と思われるデブリが観察された．さらにOil-red O染色で泡沫細胞内は赤く染色され，泡沫細胞内部は脂質で充満していることが確認された．Oil-red O染色陽性面積は，菌球留置２週間後：0.09％，14週間後0.37％と上昇しており（p = 0.029），経時的に泡沫細胞の増加を確認した．さらにマクロファージの表面マーカーを免疫染色で評価したところ，アスペルギローマ周囲の泡沫細胞はIba1とPU.1に陽性で，F4/80, CD163, CD206に陰性であった．アテローム性動脈硬化プラークを含む泡沫細胞は，一般にM1様マクロファージと考えられており，今回の所見と一致すると思われた．一方，周囲組織内の常在マクロファージはすべてのマーカーで陽性であった．これらの結果は，菌球の周囲に集まったマクロファージは組織内の常在マクロファージとは異なることを示している．

研究課題　’23－09

Evaluation of siderophore type antifungal derivative

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シデロフォア型抗真菌薬誘導体の薬効試験

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研究成果

シデロフォア型抗真菌薬誘導体を用いて, *Candida auris*を含む既存の抗真菌薬に対する耐性株６種11株のカンジダ属病原真菌を対象に詳細な活性試験を実施した結果, *C. glabrata*に対して, 既存薬と比較して約２倍の強い活性（MIC: 0.03 μg/ml）が新規誘導体で確認されている. さらに, *C. auris*に対しては, 既存薬APS2397のMICが＞50 μg/mlであるのに対し新規誘導体ではMIC: 1-4 μg/mlの新たな高い活性が示され, 既存薬では得られなかった*C. auris*に対する有望な抗真菌活性が確認されている. これらの成果を踏まえ, 令和５年度には, *in vivo*での薬効評価試験に取り組んだ. *C. glabrata*の感染後の生存率を指標とする評価系を構築するために, 適切な免疫抑制剤の投与方法について詳細な条件検討を行った. その結果, Balb/cマウスにシクロフォスファミドを事前に投与し易感染状態にすることで, *C. glabrata*を接種後約半数のマウスが死亡する条件を設定することができた. さらに, この実験系では, ミカファンギンの投薬により死亡率を０％に抑えるコントロールを設定することができた. *C. auris*を用いた*in vivo*評価系の構築を進めたが, シクロフォスファミドと新規誘導体の併用により, マウスの死亡率が上昇したため, さらなる条件検討が必要であるという重要な知見が得られた. この結果から, 今後の研究において, より安全で効果的な治療法の開発に向けた貴重な指針を得ることができた.

研究課題　’23－10

Screening of yeast protein synthesis inhibitor from Chiba University compound library

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千葉大学化合物ライブラリーを用いた酵母タンパク質合成阻害剤のスクリーニング

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研究成果

【背景・目的】千葉大学人工化合物ライブラリー由来の化合物Ｃは, ある種の病原性真菌, 及びパン酵母*Saccharomyces cerevisiae*に対して生育阻害を示す. 本研究では, 遺伝学的な実験手法が高度に駆使できる*S. cerevisiae*を用いて化合物Ｃの標的分子の同定を目指す. 始めに薬剤耐性に関わる遺伝子を12個破壊し多剤感受性となった株（12Δ株）の化合物Ｃ感受性を調べたところ, 12Δ株は野生株に比べ化合物Ｃに対し極めて高い感受性を示したことから, 本株を出発点として, ①化合物Ｃ耐性変異株の探索, 及び②マルチコピーサプレッサーの単離, という２つの手法で標的分子の同定を目指した.

【方法と結果】①化合物Ｃ耐性変異株の単離：２μg/mLの化合物Ｃ含有SD培地に12Δ株を塗布し, 生存率が30～40％となるよう紫外線照射を行い, 15株の化合物Ｃ耐性株を単離した. これらの株を元株と性別が異なる12Δ株と掛け合わせて二倍体を作製し, 一つの二倍体由来の４つの胞子を顕微鏡下で分離し, 各配偶子の表現型を調べた（四分子解析）. その結果, 得られた耐性株のうち

２株は，四分子解析で得られた４つの配偶子の化合物Ｃ耐性:感受性が２：２の分離を示し，染色体上の一箇所の変異が化合物Ｃ耐性の獲得に寄与することが示唆された．今後は化合物Ｃ耐性をもつ配偶子に対して戻し交雑を数回行った後，全ゲノムシーケンスにより化合物耐性に関わる変異の同定を目指す．

②マルチコピーサプレッサーの単離：*S. cerevisiae*の多コピーゲノムライブラリーを12Δ株に形質転換し，２μg/mL，及び４μg/mLの化合物Ｃ含有SD-leucine培地に塗布した．生育したコロニー20株から回収したプラスミドを12Δ株に再形質転換した結果，化合物Ｃへの耐性付与が確認された７個のプラスミドは，同時に化合物Ｃを含まない培地上での12Δ株の生育速度を早める効果が見いだされた．興味深いことにこれらのプラスミドの生育促進効果は12Δを宿主にした場合にのみ観察され，野生株では見られなかった．これらのプラスミドに挿入されている断片を解読したところ，全てに酵母の16本の染色体のいずれかのセントロメア配列（CEN）が含まれることが明らかとなった．ゲノムライブラリーのベースとなったYEp13ベクターは，*S. cerevisiae*が保持している２μm DNAを母体としており，セントロメアを含む染色体やプラスミドとは異なる仕組みで複製・保持される．

【考察】CENを含むYEp13プラスミドによる化合物Ｃへの耐性付与と生育促進効果の関係は現時点で不明であるものの，このCENによる株特異的な生育促進効果については現在までに報告はなく，12Δ株を用いたことで初めて見いだされた現象であり，ABCトランスポーターやその転写因子など12個の因子のいずれかの欠失により，YEp13に挿入されたCENによって補われる何らかの異常が起きていると考えられる．今後は多角的な解析により12個の遺伝子のどれが本現象に関与しているのかを明らかにしていきたい．

研究課題　’23－11

Antibacterial/antimicrobial activity analysis of newly developed macrolide antibiotics

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新規マクロライド系抗菌剤の抗真菌活性ならびに抗細菌活性研究

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研究成果

これまでの共同研究で，ユーシェアリライド（天然24員環マクロライド）と比較し，カンジダ，アスペルギルスなどに対する抗真菌活性およびMRSAを含むグラム陽性菌に対する抗細菌活性が，いずれも高いユーシェアリライド類縁体を見出し，その供給法を確立した．さらに構造活性相関解析から，ユーシェアリライドに含まれるホスホリルコリン基が活性の発現に必須の官能基であることを見出した．すなわち，活性発現の端緒となるユーシェアリライド分子の細胞膜表面への静電的な結合におけるコリン残基の関与が示唆された．

2023年度はユーシェアリライドのラクトン環のサイズが活性に与える影響を調査する目的で，環構造の異なる化合物の合成および真菌に対する薬理活性評価を行なった．その結果，抗菌剤メチノリドと同程度の環サイズとしたマクロライドは真菌に対して活性を示さないことが明らかになった．したがって，抗真菌活性の発現においてユーシェアリライドの環構造が必要である可能性が示唆された．

なお，本研究では，すでに新規抗真菌活性ならびに抗細菌活性物質を得ているが，特許出願前のためその合成法ならびに構造等の情報は非公開としている．

研究課題　’23－12

Development of anti-fungal agents that target ergosterol biosynthesis

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エルゴステロール合成経路を標的とする抗真菌薬の開発

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研究成果

新たな抗真菌薬の開発のため, 理化学研究所でのスクリーニングで見出した, エルゴステロール合成経路を標的とすることが予測された化合物について, 令和４年度に理化学研究所では実験室酵母の遺伝子改変株を用いた遺伝学的および生化学的解析を実施した. 令和５年度には, 当該化合物の作用機序解明を行い, 抗真菌薬としての可能性を検証した.

真菌感染症は, 世界各地で発生率が増加しているにも関わらず, 臨床で使用可能な抗真菌薬の数には限りがあり, 耐性菌の出現もさらなる脅威となっている. よって, 既存の医薬品とは作用機序の異なる, 新たな抗真菌薬の開発が喫緊の課題となっている. 代表者（理化学研究所）は実験室酵母であるサッカロミセスセレビシエに対して増殖阻害活性を示す化合物としてCP1およびCP2（以下,「CP化合物」と称する）を見出した. 代表者らが開発した, サッカロミセスセレビシエの遺伝子改変株ライブラリーを用いた「酵母ケミカルゲノミクス法」により, CP化合物はエルゴステロール合成経路を標的とすることが予測された. そこで, 千葉大学真菌医学研究センターとの共同研究により, CP化合物の作用機序解明に取り組み, 既存の抗真菌薬の標的分子とは異なる分子を標的とすることが支持された.

研究課題　’23－13

Bacterial analysis of *S. pneumoniae* isolated from pediatric invasive disease in Yogyakarta

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研究成果

The purpose of the research is to observe invasive pneumococcal disease（IPD）incidence, serotype distribution, and antibiotic susceptibility of *Streptococcus pneumoniae* isolated from pediatric IPD patients in Yogyakarta, Indonesia. Taking advantage of this joint research program, we accepted the PhD student from Indonesia to MMRC in October 2023. We conducted a molecular analysis of multidrug resistant *S. pneumoniae*（MDRSP）serotypes 19A and 19F isolated from Indonesia. Results have shown that the macrolide resistant genes were highly found in MDRSP isolates. The data of the MDRSP strains serotype 19F and 19A in this study can aid in the implementation and monitoring of pneumococcal vaccination in Indonesia.

研究課題　’23－14

Development of VRE infection control under acidic and basic environment

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酸性および塩基性環境下におけるVRE感染制御法の開発

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研究成果

昨年度までに，申請者らは代表的な多剤耐性細菌として知られるバンコマイシン耐性腸球菌（VRE）に対し，塩基性環境下においてV型ATPase阻害剤が増殖阻害効果を示すことを見出していた．本年度は，in vitroにおいて酸性環境下でVREの増殖阻害効果が報告されている化合物であるニクロサミドに着目し，マウスのVRE腸管感染モデルを用いてin vivoにおける感染阻害効果を検証した．その結果，ニクロサミド単剤投与群は非投与群と比較して，腸管におけるVRE数を有意に低下させることを見出した．さらに，V型ATPase阻害剤とニクロサミドを同時にマウスに経口投与し，VRE感染に対するこれらの化合物の併用効果を検証した．その結果，腸管におけるVRE数は，非投与群と比較して併用投与群において有意に低下し，V型ATPase阻害剤およびニクロサミドの単剤投与群と比較しても有意に低下することを見出した．以上の結果は，ニクロサミドがVREの腸管感染に対して阻害効果があることを示唆しており，V型ATPase阻害剤との併用による多剤耐性細菌に対する新たな治療戦略の開発に繋がることが期待される．

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研究課題 ’23－15

Screening of novel genes involved in biofilm formation and antifungal resistance in *Aspergillus fumigatus*

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アスペルギルスのバイオフィルム形成および抗真菌薬耐性に関連する新規遺伝子群の探索

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研究成果

深在性真菌症の中でも*Aspergillus fumigatus*を主要病原菌とするアスペルギルス症は増加傾向にあり，予後が非常に悪い．近年，アスペルギルスのバイオフィルム形成がアスペルギルス感染に関与することが示唆されている．特にアスペルギローマの菌糸塊に見られる菌糸周囲には厚い細胞外マトリクスが観察されている．このようなバイオフィルムを形成する状態では，いくつかの抗真菌薬に対する感受性が低下する現象が示され，難治性の原因の１つになっていると考えられる．しかしながら，バイオフィルム形成，および，それによる抗真菌薬耐性の詳細な分子メカニズムは不明な点が多い．本研究では，バイオフィルム形成に関わる新規遺伝子を同定し，抗真菌薬耐性との関連性を明らかにすることを目的とする．2023年度では，*A. fumigatus*の全遺伝子を対象としたCRISPR/Cas9ゲノム編集技術を用いたスクリーニング系を構築し，血清存在下で発現が変動する遺伝子の同定を試みた．

前年度には，全遺伝子に対するガイドRNAを設計し，これに対応するpooled oligo DNAをプラスミドベクターにクローニングすることで，変異導入用のプラスミドライブラリを作製した．このプラスミドライブラリを*A. fumigatus*の分生子に導入し，CRISPR分生子ライブラリ

を作製した．作製したCRISPR分生子ライブラリを血清存在下・非存在下で培養し，回収した菌体からDNAを抽出した．ナノポアシーケンサーMinIONを用いてガイドRNAの配列を解読し，血清存在下で有意に減少している配列を持つ遺伝子を候補遺伝子としてリスト化した．リスト化した候補遺伝子のうち，上位５種類の遺伝子について遺伝子破壊株を作製したが，血清存在下での生育に影響は見られなかった．CRISPR分生子ライブラリが全遺伝子を網羅できていないことが原因の一つと考えられたため，エレクトロポレーションの導入効率を改善し，分生子形成を経ないライブラリの作製を行うことで，CRISPRスクリーニング法の確立を目指す．これにより，血清刺激に応答するシグナル伝達機構の解明に繋げていく．

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研究課題　’23－16

Elucidating the roles of commensal-specific T cell against invading fungus

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真菌感染に対する共生細菌特異的Ｔ細胞の役割

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研究成果

本研究は，恒常的に活性化している共生細菌特異的Ｔ細胞が，体内に浸入した真菌に対する防御反応として共生細菌非特異的に機能することが可能か検討することを目的とした．

様々な哺乳類の回腸で検出されるセグメント細菌（SFB）は，高い免疫原性を有しており，SFB特異的なIL-17産生$CD4^+$Ｔ細胞（Th17細胞）を分化誘導することが知られている．イリノイ大学シカゴ校佐野研究室では，SFB特異的なTCR（TCR^{SFB}）を過剰発現するトランスジェニックマウスを獲得免疫欠損マウスRag KOマウスと交配することで，SFB特異的なTh17細胞のみを有する，SFB TCR^{Tg} Rag2 KOマウスを樹立した．我々の研究室ではSFB TCR^{Tg}として２つのTCR Tgクローン（TCR^{7B8}及びTCR^{1A2}）を有している．どちらのTCR TgもSFBに高発現しているSFB_3340 proteinに特異的であるが，そのTCRを構成するアミノ酸配列は完全に異なり，認識するEpitopeはオーバーラップするものの完全には一致しておらず，抗原特異的及び抗原非特異的なＴ細胞の応答を調べる上で優れたToolであることが期待された．令和５年度は，TCR^{7B8}及びTCR^{1A2}マウスをRag2 KO miceと交配し，TCR^{7B8} Rag2 KO mice及びTCR^{1A2} Rag2 KO miceの作製に成功した．マウス準備期間中に研究代表者のやむを得ない手術とその後の治療により，予定していた研究通りに進めることはできなかったが，今後これらのマウスを用いてＴ細胞が正常に機能しているかを検討したのち，Candida albicansを経口感染させ，経時的にSFB特異的なＴ細胞の活性化の有無をFlow cytometry analysisにより解析する予定である．

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研究課題　’23－17

Genetic analysis of SARS-CoV-2 variants and basic research for drug discovery against COVID-19

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SARS-CoV-2変異株の遺伝子解析とCOVID-19治療薬探索に向けた基礎的研究

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研究成果

新型コロナウイルスによる急性呼吸器感染症(COVID-19)は2019年末に中国武漢市付近から突然発生し，瞬く間に世界中に感染拡大した．その後，今日に至るまで変異を繰り返しながら新しい株が世界各地において波状の流行を繰り返している．これに対抗する予防策として，2021年以降には各株に対応したmRNAワクチンなど種々のワクチンが開発され，その時々において少なからぬ感染予防効果，あるいは重症化阻止を示して来たのがこの4年間であった．一方，その誘導抗体価の持続性の短さや免疫逃避する変異株への対応など多くの問題点も指摘されている．本研究は，次々と出現する変異株について流行のピーク時に逐次分離・遺伝子解析を進めながら，特にウイルス増殖を阻害できる治療薬の探索に力を注いだ．

2023年度の実際の成果としては，第8波から第9波の流行時に千葉大学附属病院に入院した患者らの鼻腔拭い液を出発材料としてVERO-E6/TMPRSS2細胞を用いてウイルス分離を行い，10株の代表的変異株（オミクロン変異株XBB. 1，GK. 1，BA. 2. 86，JN. 1株など）を得た．また臨床的に重要なものとしては，過去に臓器移植を受けたコロナ感染者から分離されたレムデシビル（RDV）耐性変異株（BA. 5系統）も含まれている．各株の帰属に関しては，従来通りSpike遺伝子領域をOneStep-RT-PCR法で増幅し遺伝子配列解析をすると共に，Illumina iSeq100を用いたNGS解析データに基づいて明らかにした．昨年度までに分離した株を加えると，合計50株程度を樹立したことになる．

治療薬探索研究としては，2023年度は治療現場への将来的実用性を念頭に，特に経口可能な薬剤に焦点を絞って調べた．その結果，ウイルスがコードする3CLプロテアーゼ阻害薬であるEnsitrelvirに加えて，静注薬であるために服薬管理が要求されるRDVに代わって，より投薬管理しやすい経口薬のRNAポリメラーゼ阻害薬VV116（国内では未承認）の抗ウイルス活性が非常に高いことを実験室レベルで確認した．またこれらの薬剤を組み合わせると，抗ウイルス効果を一段と増強できることが明らかとなり，複数の抗ウイルス剤を組み合わせる新しい併用療法の可能性が示されたものと考えている．

研究課題　’23－18

Pathological analysis of invasive infectious disease due to nontypeable *Haemophilus influenzae*

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無莢模型インフルエンザ菌による侵襲性感染症の病態解析

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研究成果

無莢膜型インフルエンザ菌（NTHi）が侵襲性感染症

を引き起こす病態の解析としてバイオフィルムに注目して研究を進めている．令和５年度はAuto-inducerとしてトリプトファン合成機構に着目し，*trpA, B, C, D, E*の欠損株を作成し，バイオフィルムの形態解析を行った．*trpA*, *trpB*欠損株ではbroth cultureでは菌は発育するが，バイオフィルムの産生をほとんど認めず，*trpC, D, E*欠損株ではバイオフィルムはしっかり形成するが，内部の細菌は死菌が増加していた．上記のように，本研究では，NTHi産生バイオフィルムが侵襲性感染症に至るメカニズムに関して新たな知見を得ている．

今後，バイオフィルム形成の過程での発現遺伝子の解析を行うため12時間後，24時間後，48時間後，72時間後でバイオフィルム内部の細菌からmRNAを回収しquorum sensing機構の解明につなげたい．

研究課題　’23－19

Identification of the transcriptional regulatory mechanism of *CgATG32*.

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*Candida glabrata*におけるマイトファジー関連遺伝子*ATG32*の転写調節機構の解明

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研究成果

病原真菌*Candida glabrata*は鉄欠乏下でミトコンドリア選択的オートファジー（マイトファジー）を活性化させるが，その活性調節機構は不明である．鉄欠乏下で発現量が増加し，マイトファジーに必須である*ATG32*に着目し，*ATG32*の発現調節機構を解明することを本研究の目的とした．2022年度までに，エキソリボヌクレアーゼ*XRN1*が鉄依存的に*ATG32*プロモータ領域に結合し，発現調節に関与することを見出した．また，*XRN1*の遺伝子破壊株と野生株についてRNA-seqによる網羅的遺伝子発現解析およびミトコンドリア顕微鏡観察，ウェスタンブロット解析の結果から，*XRN1*遺伝子破壊株ではミトコンドリア関連遺伝子，ミトコンドリア局在タンパク質，ミトコンドリア量が全て増加していることが明らかとなった．*XRN1*のRNase活性喪失型変異タンパク発現株を作製し，*ATG32*発現解析を行った結果，変異株では*ATG32*の発現量が顕著に増加し，マイトファジー活性の亢進も認められことから，*XRN1*のRNase活性がマイトファジー活性調節に必要であることが予想された．

2023年度は，*XRN1*が関与するマイトファジーのミトコンドリア機能調節における役割を明らかにするために，*XRN1*遺伝子破壊株およびRNase活性喪失型変異タンパク発現株を用いてミトコンドリア機能指標の解析を実施した．*XRN1*破壊株およびRNase活性喪失型変異タンパク発現株では，ミトコンドリアの機能指標であるミトコンドリア膜電位が顕著に低下し，ミトコンドリア由来活性酸素種産生量の増加，ミトコンドリア量の増加が認められた．また*XRN1*破壊株およびRNase活性喪失型変異タンパク発現株から単離したミトコンドリアの解析を実施した結果，両変異株の酸素消費速度は野生株と比較して顕著に増加し，反対にATP合成活性は顕著に低下することが明らかになった．

研究課題　’23－20

Development of novel therapeutic approach for systemic persister infections

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パーシスター全身感染症克服法の開発

松岡悠美
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高橋弘喜
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研究成果

本研究チームは，2021年度AMED-CREST「感染症創薬に向けた研究基盤の構築と新規モダリティ等の技術基盤の創出」（代表：高屋明子，2021-2026）に採択されており，皮膚，および全身*Staphylococcus aureus*の感染モデルを本共同研究支援により確立することにより，パーシスターをターゲットとした候補薬剤の生体内での，評価が可能とした．また，これまでに，NICUなどで得られた院内黄色ブドウ球菌感染症と共通する菌の進化形態が，Agrクオラムセンシングの可逆性のサイレンシングによるものであることを発見した．この形質が，院内感染症のパーシスター発生や，抗生剤耐性獲得に関わっていることを見出した．一方，ヒト皮膚生着株においてはこのような機構を介した進化形態は見られなかった．本年度は，これらの院内定着に必要な形質が，*S. aureus*ゲノムメチル化によるものであることを見出し，このメチル化を制御するであろう候補遺伝子（本報告書では以下“メチル化酵素X”とする）の欠損株を作成し，形質を確認したところ，メチル化酵素Xのゲノム上欠損株はAgrクオラムセンシングの可逆性のサイレンシングの形質を示し，さらにプラスミドを用いてメチル化酵素Xを補完した株を作成すると，Agrの発現はもとの野生型の形質に復帰することが明らかとなった．メチル化酵素XはrRNAのメチル化も起こすため，この影響を除外するためAgrクオラムセンシングの可逆性のサイレンシング株のrRNAのメチル化についても解析し，rRNAのメチル化はAgrクオラムセンシングの可逆性のサイレンシングに影響していないことを確認した．本年度は本申請課題とは異なるが，これまで３名の教員が共同研究にて進めてきた皮膚の細菌叢とアレルギー発症に関する成果などを報告した．

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研究課題　’23－21

Joint Research for Fight against Rubella in Chiba City by University, Health Center and Medical Association

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千葉市における大学・行政・医師会が連携した風疹対策共同研究

玉井和人
（千葉市医師会感染症対策委員会）
落合弘章
（千葉市保健福祉局課長）

石和田稔彦
（千葉大学真菌医学研究センター）

研究成果

我が国の風疹追加的対策であるMRワクチン５期定期接種事業は，実施期間が延長したにも関わらず抗体検査・ワクチン接種実施率が未だ全国的に伸び悩んでいる．千葉市では，国事業に加え市独自事業として，妊娠希望女性や風疹抗体価の低い妊婦，これらの配偶者・家族に対する抗体検査助成，風疹抗体価が低い全ての人を対象としたMRワクチン接種助成を行っている．本年度も千葉市在住の対象者に対して行われた国事業および千葉市事業の風疹抗体検査申込書，MRワクチン接種予診票を，個人情報を削除した後に全例回収し，千葉大学にて集計・傾向を分析した．千葉市保健所，千葉市医師会と共同で問題点と改善策を検討し，事業促進に関する活動を行った．

2023年12月までに，抗体検査は58, 233件（国事業：45, 949件，千葉市事業：12, 284件），MRワクチン接種は19, 262件（国事業：9, 498件，千葉市事業：9, 764件）実施された．

国事業における抗体検査の進捗率は34％であり，前年度（33％）と比較しても実施件数は伸び悩んでいた．一方，千葉市事業の実施件数は毎月一定数の利用が継続されており，増加傾向を認め，20～30歳代男女の子育て世代が内科や産婦人科にて事業を利用している傾向が継続して認められていた．これらの結果は毎月１回ニュースレターとして千葉市および千葉市医師会にフィードバックしている．また，第27回日本ワクチン学会学術集会において発表した．

研究課題　’23－22

Analysis of toxin-producing and disinfection-resistance genes of methicillin resistant Staphylococcus aureus isolated in neonatal intensive care unit in a pediatric facility

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NICUで分離されたMRSAの消毒薬耐性能，毒素産生能の解析

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研究成果

千葉県こども病院のNICU, GCUで，2017年11月～2022年３月に臨床検体より分離されたMRSA44株（院内感染株28株，持ち込み株16株）を対象に，院内感染とPOT型，毒素産生遺伝子（*lukS-LukF*, *tst*, *eta*, *etb*），消毒薬耐性遺伝子（*qacA/B*, *smr*）との関連性について解析を進めている．毒素・消毒薬耐性遺伝子検査は2022年度に終了しており，2023年度は結果の解析と，一部菌株に対し新たに全ゲノム解析を行った．

対象となったMRSAのPOT解析では，３種類のPOT型（106-183-32; ９株，106-247-33; ６株，106-145-71; ４株）が院内感染株の68％を占めていた．毒素産生遺伝子は11株で陽性となり，９株が*tst*陽性，２株が*pvl*陽性であった（重複なし）．消毒薬耐性遺伝子は６株で陽性となり，いずれも*qacA/B*陽性であった．POT型と毒素産生遺伝子，消毒薬耐性遺伝子との関連を見ると，106-9-80の２株，106-221-120の４株，計６株で*tst*, *qacA/B*が陽性，105-145-71, 106-77-113, 110-68-33各１株，計３株で*tst*のみが陽性，106-68-33, 106-77-113の各１株，計２株で*pvl*のみが陽性であった．院内感染を生じやすい３種類のPOT型のMRSAでは，いずれの陽性株もなく，毒素産生遺伝子，消毒薬耐性遺伝子と院内感染との関連性は明らかとはならなかった．

なお，*tst*, *qacA/B*が共に陽性となった６株は，院内感染株，持ち込み株がそれぞれ３株ずつを占めた．2023年度は，この６株を対象に全ゲノム解析を実施した．今年度は結果の解析を進める予定である．

第11回感染症研究グローバルネットワークフォーラム

11th Global Network Forum on Infection and Immunity

共催：千葉大学真菌医学研究センターと千葉大学未来粘膜ワクチン研究開発シナジー拠点（cSIMVa），千葉大学地域中核・特色ある研究大学強化促進事業（J-PEAKS）

【Flash Talk & Poster Session】
日時：令和７年２月６日（木）13時00分～17時00分
場所：千葉大学医学系総合研究棟　３階　アクティブラーニングスペース＆第１講義室

【Oral Presentation】
日時：令和７年２月７日（金）９時30分～17時00分
場所：千葉大学医学系総合研究棟　４階　会議室１

組織委員長
後藤義幸　　（千葉大学真菌医学研究センター）

組織委員
Hein Min Tun（香港中文大学医学部）
米山光俊　　（千葉大学真菌医学研究センター）
石和田稔彦　（千葉大学真菌医学研究センター）
渡邉　哲　　（千葉大学真菌医学研究センター）
西城　忍　　（千葉大学真菌医学研究センター）
高屋明子　　（千葉大学薬学研究院）

研究成果

「感染症研究グローバルネットワークフォーラム」は感染症研究のネットワーク構築を目指し，当センターが中心となって平成24年度から開始され，2024年度で第11回目を迎えることとなった．本年度の国際フォーラムは，千葉大学の後藤義幸博士が組織委員長となり，「感染症とともに生きる～微生物学・粘膜免疫学・ワクチン学が共創する未来～, Synergistic Innovation of Microbiology, Mucosal Immunology and Vaccinology」をテーマとし，現在世界的に注目されている感染症，ワクチン，免疫，データサイエンス，腸内微生物研究を牽引する国内外の著名な研究者を招聘した．COVID-19やインフルエンザ等のウイルス，細菌，真菌などの微生物について，幅広い分野で世界最先端の研究成果について，ご講演いただいた．さらに，これら微生物感染時の宿主免疫応答のメカニズムについてもご紹介いただき，大変内容の濃いフォーラムとなった．

初日は45人の若手研究者によるフラッシュトークとポスター発表があり，二日目はアメリカ，香港および国内から８人の著名な研究者を招待し最先端の研究について講演を行っていただいた．二日間で延べ255人の参加があり，活発な議論を通じて新しい国際ネットワーク形成を目指した有意義な意見交換が行われた．

【開会の挨拶】
中島裕史（千葉大学）

【午前の講演】
午前の講演：Morning Session

座長：清野　宏（千葉大学未来粘膜ワクチン研究開発シナジー拠点）
１．**Rob Knight（UC San Diego（UCSD），USA）**
“Scaling microbiome studies to address global problems”

座長：米山光俊（千葉大学真菌医学研究センター）
２．**Satoshi Uematsu（Osaka Metropolitan University）**
“An enterococcal phage-derived enzyme suppresses graft-versus-host disease”
３．**Eiryo Kawakami（RIKEN）**
“Utilization of AI and data science in vaccine research”

【午後の講演】
午後の講演：Afternoon Session

座長：後藤義幸（千葉大学真菌医学研究センター）
１．**Hiutung Chu（UC San Diego（UCSD），USA）**
“Host glycans shape early-life gut colonization and immune modulation”

2．**Iliyan Iliev（Weill Cornell Medicine, USA）**
“The Evolution of Fungal Symbiosis Within the Host: An Immunological Perspective”

3．**Kenya Honda（Keio University, RIKEN）**
“Mining the gut microbiota to develop rationally designed microbial therapeutics”

座長：石和田稔彦（千葉大学真菌医学研究センター）

4．**Yoshimasa Takahashi（National Institute of Infectious Diseases）**
“Controlling antibody breadth to mutating viruses”

5．**Hein Min Tun（The Chinese University of Hong Kong, China）**
“Role of early-life gut microbiome in orchestrating immune system”

【閉会の挨拶】
後藤義幸（千葉大学真菌医学研究センター）

2024 Scientific Meetings & Seminars

2024年講演会

「真菌医学研究センター セミナー」

【第１回】
日時：令和６年９月27日（金）16時～17時
場所：真菌医学研究センター　大会議室，オンライン（teams）ハイブリッド開催
講師：千葉大学災害治療学研究所　感染症ワクチン開発研究部門
藤橋浩太郎　特任教授
「Controlling of Infectious Diseases by Mucosal Vaccines」

【第２回】
※開催中止

【第３回】
日時：令和６年10月25日（金）16時～17時
場所：真菌医学研究センター　大会議室，オンライン（teams）ハイブリッド開催
講師：東京大学医科学研究所　感染遺伝学分野
三宅健介　教授
「TLR responses to lysosomal nucleic acid stress」

「2024千葉大学真菌医学研究センター 市民向け公開セミナー」

【第１回】
日時：令和６年５月10日（金）
場所：ペリエホール　RoomB（千葉市中央区新千葉1-1-1 ペリエ７階）
（講演１）
石和田稔彦（真菌医学研究センター教授）
「肺炎予防のための新しいワクチン　溶連菌感染症の話題も含めて」
（講演２）
矢口貴志（真菌医学研究センター准教授）
「生活環境のカビと食品のカビ毒について」
（講演３）
渡邉　哲（真菌医学研究センター准教授）
「カビ感染症のリスクとなる呼吸器疾患」

【第２回】
日時：令和６年８月23日（金）
場所：ペリエホール　RoomB（千葉市中央区新千葉1-1-1 ペリエ７階）
（講演１）
磯野史朗（千葉大学真菌医学研究センター特任教授）
「大きないびき：病気？肥満や歯並びと関係？」
（講演２）
生水真紀夫（千葉大学真菌医学研究センター特任教授）
「不妊症の課題と最新治療」
（講演３）
巽浩一郎（千葉大学真菌医学研究センター特任教授）
「現代における漢方とは」

「東京大学医科学研究所―千葉大学真菌医学研究センター　国際共同利用・共同研究拠点事業　2024年度成果報告会」

日時：令和７年２月12日（水）～２月14日（金）
場所：オンライン開催

令和７年２月13日（木）
【特別講演】
石野智子（東京科学大学教授）

【合同成果報告会（千葉大学真菌医学研究センター）】
星野　直（千葉県こども病院）
「小児侵襲性大腸菌感染症に関する臨床的及び細菌学的検討」

小林直樹（麻布大学）
「動物園飼育環境下の鳥類におけるAvian Gastric Yeast症原因真菌の分布状況および分子系統分類に関する研究」

梅山　隆（国立感染症研究所）
「アスペルギルスのバイオフィルム形成および抗真菌薬耐性に関連する新規遺伝子群の探索」

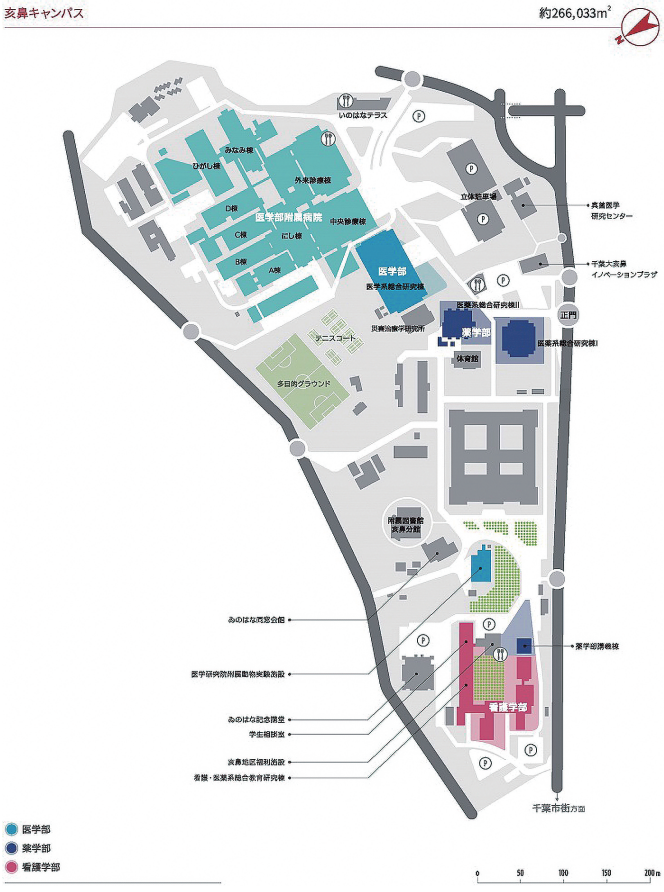
高橋朋子（埼玉大学）
「microRNA制御因子による抗ウイルス免疫応答の制御メカニズムの解明」

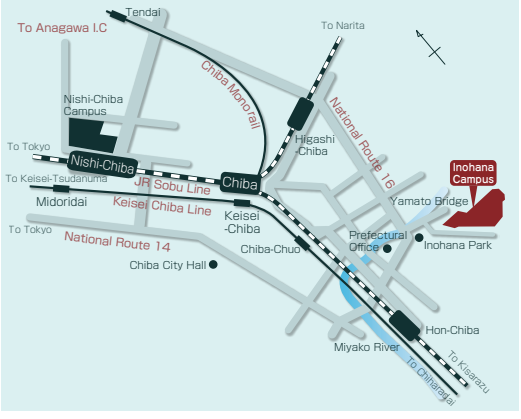
【領域３：感染症・免疫共同研究領域】
中川一路（京都大学大学院医学研究科）
「核酸に対する細胞表面受容体の解析と炎症性疾患に対する新規治療戦略の構築」

熊谷雄太郎（産業技術総合研究所）
「免疫細胞ダイレクトリプログラミング法の系統的開発と応用」

新江　賢（杏林大学）
「ダニアレルギー性気道炎症に関わる新規自然リンパ球サブセットの探索」

髙村祥子（愛知医科大学）
「疾患発症におけるTLRを介した免疫応答の関与の解明」





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