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MMRC

Center Medical Mycology Research

ANNUAL REPORT OF MEDICAL MYCOLOGY RESEARCH CENTER, CHIBA UNIVERSITY 2023

千葉大学　真菌医学研究センター　報告

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Preface for the FY2023 Annual Report

In 2023, elderly individuals 65 years or older accounted for 29% of the Japanese population. This group is at increased risk of adverse health conditions such as chronic obstructive pulmonary disease, other long-term respiratory problems, malignancies, and treatment-related opportunistic fungal infections. Growing attention is being paid worldwide to the risks of imported fungal infections arising from international trade and globalization, as well as to the life-threatening risks of pulmonary aspergillosis and mucormycosis in patients with COVID-19 infection. Moreover, Candida auris is an emerging threat to the global health. In October 2022, the World Health Organization published its fungal priority pathogens list to guide research, development, and public health actions. This publication was an emergency call to action for fungal infection research. These circumstances underscore the growing role and significance of the Medical Mycology Research Center (MMRC).

In FY2021, the MMRC was granted extended certification as the National Joint Usage/Research Center for Fungal Infection by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT). To support research, education, and clinical activities in the academic, public, and private sectors, the MMRC actively promotes joint projects and the use of shared facilities. As part of the MEXT' s National Bioresource Project, the MMRC collects, stores, provides, and genetically analyzes pathogenic fungi and actinomycetes. In tandem with these activities, research groups of the MMRC pursue independent basic, applied, and clinical research projects. Furthermore, the Division of Clinical Research and the Division of Infection Control and Prevention of the MMRC have been supporting the specialized outpatient clinic for invasive fungal infections at Chiba University Hospital since 2014 and 2016, respectively. The MMRC established the first biosafety level 3 laboratory at Chiba University in 2015, and a germ-free animal facility in 2018.

To facilitate research on pathogens, infection, and immunity, the MMRC conducts joint research with other organizations at Chiba University, including the Graduate School of Medicine, University Hospital, Graduate School of Pharmaceutical Sciences, and Graduate School of Science. The results of these projects will inform clinical practice. Several MMRC faculty members serve as adjunct faculty staff of the Research Institute of Disaster Medicine, which was established in 2021. The MMRC partners with this institution to accelerate research and treatment related to emerging pandemics and post-disaster infections. The MMRC continued joint academic activities with overseas centers in FY 2023, leveraging its international cooperative network.

The MMRC pursued its FY2023 mission with emphases on the following areas of activity: (i) initiatives as a joint usage/research center and bioresource center; (ii) fundamental research on pathogens, infection, immunity, and genome-wide analysis; (iii) clinical research and development; and (iv) support for junior researchers. We thank the Scientific Council members, collaborating external researchers, and other stakeholders for their continued support.

February 2024

Chihiro Sasakawa, PhD,
Director of the MMRC

はじめに

我が国の2023年の総人口に占める65歳以上の高齢化比率は29％となり，この高齢社会においては慢性閉塞性肺疾患（COPD）等の呼吸器病や悪性腫瘍，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，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コール症，また近年ではカンディダ・アウレ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．このような背景のもとで，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し多様化しています．

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・医療活動等を積極的に推進してまいりました．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，独立研究グループリーダーによる基盤研究・開発研究・臨床研究も積極的に行われています．同時に2014年および2016年には，臨床系の２分野（臨床感染症分野，感染制御分野）により付属病院において感染症に関連する専門外来が開設され，また2015年には本学初のBSL-3施設，さらに2018年には無菌動物施設が整備されました．

本センターは，学内においても千葉大学の感染症・免疫・病原体の研究と医療活動の更なる活性化を図るため，医学研究院，付属病院，薬学研究院，理学研究院等と活発に共同研究を展開しています．また2021年に学内に設置された「災害治療学研究所」に本センターも参画し，大規模災害（地震や津波等）に随伴する呼吸器疾患と感染症の研究と治療に向けた取り組みも始めています．本センターでは，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，2023年度も海外の真菌研究拠点と国際共同研究を活発に行いました．

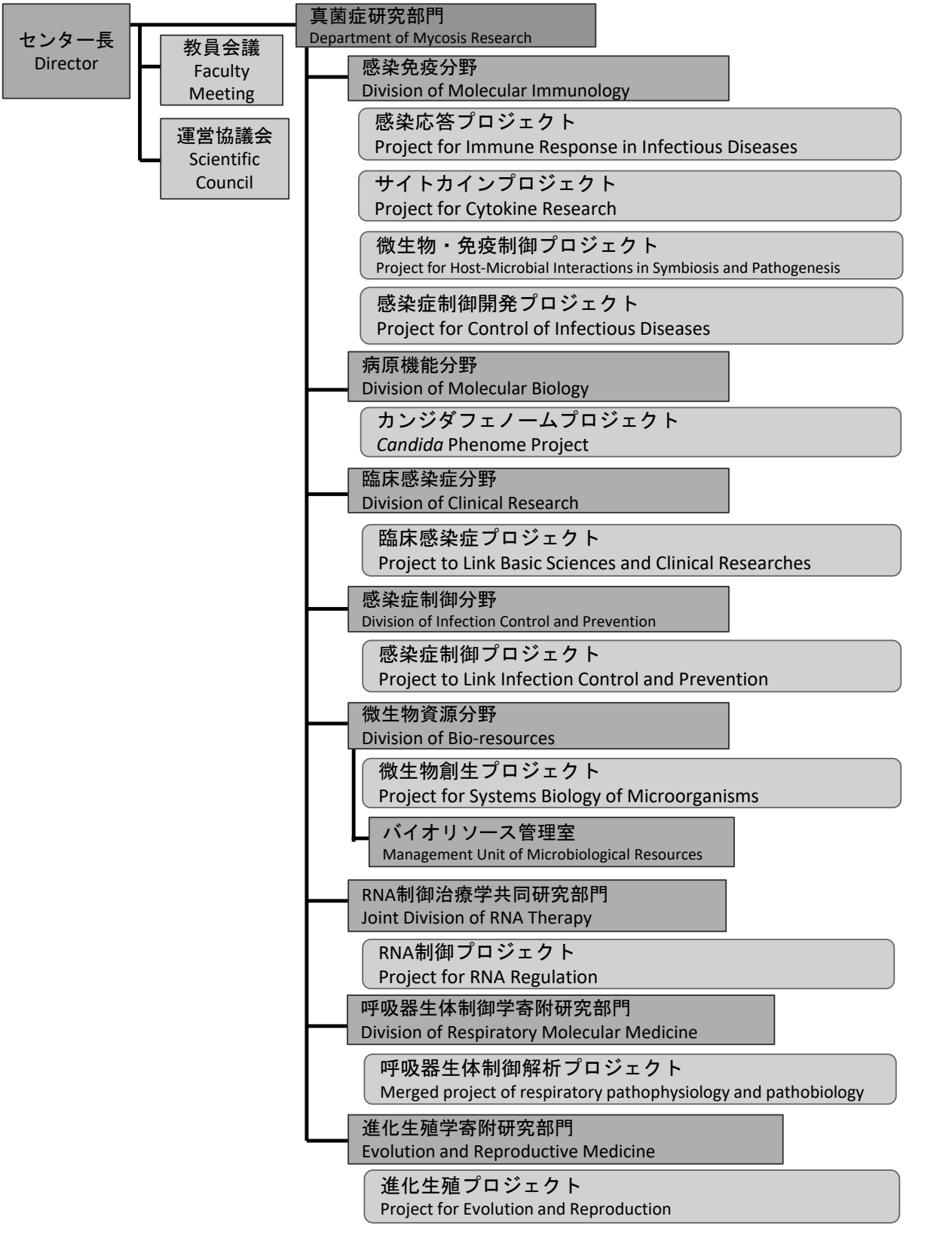
以上のように，本センターは，「共同利用・共同研究拠点及びバイオリソース中核拠点事業」，「病原体・感染・免疫・ゲノム情報研究」，「臨床・開発研究」，「若手育成」の４つを柱として2023年度も活動が行われ，ここに本センターの運営協議会の委員の方々をはじめ，多くの共同研究者の方々にも深く感謝申し上げますとともに，引き続きご協力とご指導を賜りますようお願い申し上げます．

2024年２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	Yuna Aoki（2023.3～on maternity/paternity leave）	技術職員	青木　友那
Research Technician	Kaho Kato	技術職員	加藤　香穂
Research Promotion Technician	Miyuki Takizawa（～2023.3）	技術補佐員	滝沢みゆき
Research Promotion Technician	Yukari Inada（2023.3～）	技術補佐員	稲田由圭里

1. Functional analysis of host proteins that are responsible for induction of anti-viral innate immunity.

Onomoto K, Aoki Y, Kato K, Ban M, Suzuki Y, Sakai M, Kobori T, and Yoneyama M.

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

In the previous studies, we revealed that viral infection induces RLRs to accumulate in cytoplasmic granular-like structure, antiviral stress granule（avSG）, and avSG plays a critical role as a platform for initiating RIG-I-mediated type I interferon-inducing signaling. We are now analyzing several RNA-binding proteins and avSG-localizing proteins that play a role in regulating RIG-I-mediated signal activation. In addition, we are analyzing molecular interaction between host factors and viral proteins in response to SARS-CoV-2 infection using the Bio-safety level 3（BSL3）facility of MMRC.

2. Identification of natural compounds targeting SARS-CoV-2.

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

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

More than 300 middle-molecular compounds prepared by the Faculty of Pharmaceutical Sciences, Chiba University, were screened for antiviral activity against SARS-CoV-2 by

examining the effects on virus-induced cytotoxic activity (CPE). Four middle-molecular compounds showed significant antiviral activity against SARS-CoV-2 infection. Among them, two were implicated in antiviral activity by inhibiting the activity of papain-like proteases of SARS-Cov-2. The results indicate that four plant-derived middle-molecular compounds are candidates for developing new antiviral agents to treat COVID-19.

3. Development of inhalant nucleic acid drugs for COVID-19.

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

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

In collaboration with research groups at Gifu University, the University of Tokyo, and Meijo University, we analyzed novel nucleoside analogs for developing anti-COVID-19 nucleic acid drugs. We evaluated the antiviral activity and cytotoxicity of siRNA and antisense nucleic acid (ASO) targeting SARS-CoV-2 *in vitro*. The results showed that ASOs with enhanced stability and cell permeability by introducing modified nucleic acids reduced the viral load without any cytotoxic activity.

Publications

1) Im JH, Duic I, Yoshimizu SH, Onomoto K, Yoneyama M, Kato H, Fujita T: Mechanisms of length-dependent recognition of viral double-stranded RNA by RIG-I. *Sci Rep*, 13(1): 6318, 2023

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], Wakatsuki M[1], Yoshida K[1], Yabe R[1], Torigoe S[2], Yamasaki S[1, 2, 3, 4], Barber GN[5], 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.
[4] Division of Molecular Design, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan.
[5] Department of Cell Biology, University of Miami, Miller School of Medicine, Miami, Florida, USA

Aspergillus fumigatus is a ubiquitous, yet potentially pathogenic, mold. The immune system employs innate receptors, such as dectin-1, to recognize fungal pathogens, but the immunological networks that afford protection are poorly explored. Here, we investigated the role of dectin-1 in anti-*A. fumigatus* response in an experimental model of acute invasive aspergillosis. Mice lacking dectin-1 presented enhanced signs of inflammation, with increased production of inflammatory cytokines and neutrophil infiltration, quickly succumbing to the infection. Curiously, resistance did not require T/B lymphocytes or IL-17. Instead, the main effector function of dectin-1 was the preservation of the NK cell population in the kidneys by the provision of the cytokine IL-15. While the depletion of NK cells impaired host defense in wild-type mice, IL-15 administration restored antifungal responses in dectin-1 deficient mice. Our results uncover a new effector mechanism for dectin-1 in anti-*Aspergillus* defense, adding an alternative approach to understand the pathophysiology of this infection.

Publications

1）Yoshikawa FSY, Wakatsuki M, Yoshida K, Yabe R, Torigoe S, Yamasaki S, Barber GN, Saijo S.
Dectin-1/IL-15 Pathway Affords Protection against Extrapulmonary Aspergillus fumigatus Infection by Regulating Natural Killer Cell Survival. J Innate Immun. 15(1):1-15. 2023

2）Hideki Yamamoto, Chikako Tomiyama, Sho Yamasaki, Shinobu Saijo, Yoichiro Iwakura, Kazuyoshi Kawakami.
Involvement of Dectin-2 in the Innate Inflammatory Response Triggered by Influenza Virus Hemagglutinin. Advances in Infectious Deseases. 13:478-497. 2023

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

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

Akira Haku[1], Bonita McCuaig[1], Qiongyuan Zhang[1], Yuni Sun[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.

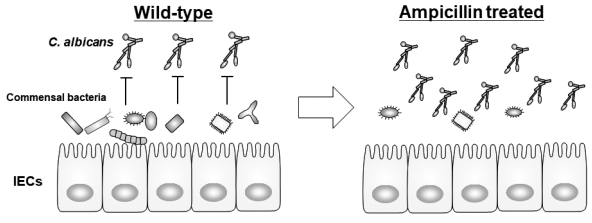


Fig 1. Commensal bacteria prevent the colonization of *C. albicans* in the gut

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.

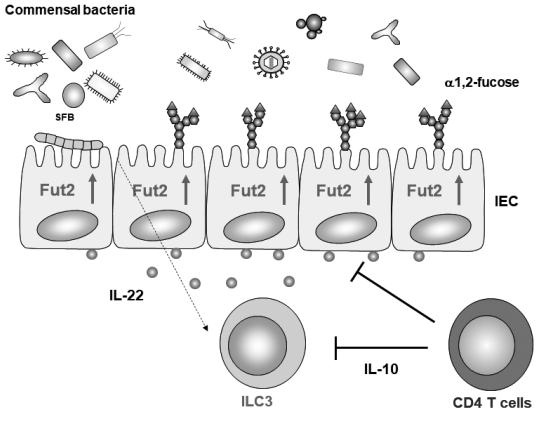


Fig 2. The inductive and regulatory mechanism of epithelial α1,2-fucose

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

1) McCuaig B and Goto Y. Immunostimulating Commensal Bacteria and Their Potential Use as Therapeutics. Int. J. Mol. Sci, 24:15644, 2023

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, we identified bacterial proteins targeted by compounds identified from our own natural product library and investigated their mechanisms of action and effects on bacterial cells.

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が標的とする細菌タンパク質を同定し，その作用機序や休眠細胞への影響について検討した．

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. Physalin H, Physalin B, and Isophysalin B suppress the quorum-sensing function of *Staphylococcus aureus* by binding to the key response regulator AgrA

Junpei Yamaguchi[1], Teruhisa Manome[2], Yasumasa Hara[2, 3], Yuriko Yamazaki[4], Yuumi Matsuoka[5], Masami Ishibashi[2, 3], Akiko Takaya[1, 3, 6]

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Staphylococcus aureus pathogenesis, including methicillin-resistant *S. aureus* (MRSA), depends on the expression of many toxins and other factors controlled by the quorum sensing system (QS), encoded on the virulence accessory gene regulator (*agr*) locus on its genome. In this study, we identified Physalin H, Physalin B, and Isophysalin B, phytochemicals belonging to the class of withanolides that can be found in plants of Solanaceae family, as novel Agr-QS modulators. The results of biological analysis and *in vitro* protein-DNA binding assays were suggested that these physalins suppress gene expression related to the Agr-QS system by inhibiting the binding of the key response regulator AgrA to the agr promoters, leading to a reduction in the function of hemolytic toxins downstream of these gene expressions in MRSA. Furthermore, although Physalin F suppressed gene expression in the Agr-QS system, its anti-

hemolytic activity was lower than the three physalins. On the other hand, five physalins isolated from the same plant as physalins with activity to Agr-QS suppression did not reduce bacterial Agr-QS activity but inhibited AgrA binding to DNA *in vitro*. As a result of the docking simulation, the direction in which physalin interacts with the DNA binding site of AgrA was classified into three docking states. The carbonyl oxygens at C-1 and C-18 of physalins, which have Agr-QS suppression activity, were directed to residues N201 and R198 of AgrA, respectively, whereas these carbonyl oxygens of physalins, without Agr-QS suppression activity, were oriented in different directions. Furthermore, 100-ns molecular dynamics simulations revealed that the hydrogen bond formed between the carbonyl oxygen at C-15 of physalins and L186 of AgrA functions as an anchor, sustaining the interaction between the carbonyl oxygen at C-1 of physalins and N201 of AgrA. Taking these findings together, it is suggested that Physalin H, Physalin B, and Isophysalin B inhibit the interaction of AgrA with the agr promoters by stable binding to the DNA binding site of AgrA, resulting in a suppression in Agr-QS function of *S. aureus*.

2. Pharmacological effects of Koetjapic acid from *Sandricum indicum* on bacteria

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As a factor in maintaining bacterial homeostasis, the AAA$^+$ protease ClpXP is widely conserved. Under physiological conditions, ClpX, which has ATPase activity, unfolds the substrate and transfers it to ClpP, which has peptidase activity, resulting in proteolysis. In recent years, it has been reported that some compounds bind directly to ClpP and allow ClpX-independent protease activity and it has been shown that this pathway provides antibacterial activity. In this study, we screened ClpP-activating compounds using our unique natural products library and investigated their relationship with antibacterial activity. We purified *Salmonella* ClpP and constructed a compound screening system using FITC casein degradation as an indicator. As a result of investigating 1163 compounds, 4 compounds were shown to active ClpP proteolysis. Based on the screening results for the growth inhibitory activity of *Staphylococcus aureus*, it was suggested that Koetjapic acid (KA) isolated from *Sandoricum indicum* may inhibit the growth of *S. aureus* along with ClpP activity. 4 structural analogs of KA also isolated from *Sandricum indicium* did not activate ClpP, and the growth inhibition activity of Staphylococcus aureus was lower than that of KA. The re-extracted KA activated ClpP in a concentration-dependent manner, suggesting that KA has a structure that specifically binds to ClpP. Taking these findings together, it is suggested that KA is involved in antibacterial activity by binding to a bacterial target protein.

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Candida glabrata phenome project

知花ＰＩ（カンジダ・グラブラータフェノーム）プロジェクト

研究概要（Summary）

Using the systematically constructed full genome mutant library in pathogenic yeast *Candida glabrata*, we are performing development of anti-fungal drugs, gene identification and functional analyses involved in pathogenicity.

病原性酵母カンジダ・グラブラータの全遺伝子改変株を利用し，抗真菌薬の開発ならびに病原性に関する遺伝子の特定と機能解析を進めている．

Associate Professor	Hiroji Chibana	准　教　授	知花　博治
Research Technician	Azusa Takahashi	技 術 職 員	高橋　　梓
Research Fellow	Michiyo Sato	特 任 助 教	佐藤美智代
Grand Fellow	Masashi Yamaguchi	グランドフェロー	山口　正視
Research Promotion Technician	Kaname Sasamoto	技 術 補 佐 員	笹本　　要
Research Promotion Technician	Keiko Nakano	技 術 補 佐 員	中野　恵子
Research Promotion Technician	Kazue Tsuda	技 術 補 佐 員	津田　一恵

Evaluation of antifungal selective toxicity using *Candida glabrata ERG25* and human *SC4MOL* knock-in strains.

Keiko Nakano, Michiyo Okamoto, Azusa Takahashi-Nakaguchi, Kaname Sasamoto, Masashi Yamaguchi, Hiroji Chibana

With only four classes of antifungal drugs available for the treatment of invasive systemic fungal infections, the number of resistant fungi is increasing, highlighting the urgent need for novel antifungal drugs. Ergosterol, an essential component of cell membranes, and its synthetic pathway have been targeted for antifungal drug development. Sterol-C4-methyl monooxygenase (Erg25p), which is a greater essential target than that of existing drugs, represents a promising drug target. However, the development of antifungal drugs must consider potential side effects, emphasizing the importance of evaluating their selective toxicity against fungi. In this study, we knocked in *ERG25* of Candida glabrata and its human ortholog, *SC4MOL*, in *ERG25*-deleted *Saccharomyces cerevisiae*. Utilizing these strains, we evaluated 1181-0519, an Erg25p inhibitor, that exhibited selective toxicity against the *C. glabrata ERG25* knock-in strain (Fig. 1). Furthermore, 1181-0519 demonstrated broad-spectrum antifungal activity against pathogenic *Candida* species, including *Candida auris*. The approach of utilizing a gene that is functionally conserved between yeast and humans and subsequently screening for molecular target drugs enables the identification of selective inhibitors for both species.

PF1163B, a known inhibitor of *C. albicans* Erg25p, distinguishes itself from PF1163A by lacking an additional hydroxyl group on its side chain. PF1163B exhibits a broader spectrum of activity compared to PF1163A and has been reported to have slight inhibitory effects on *C. glabrata*. To confirm the usefulness of PF1163B in our study, we conducted a growth inhibition comparison involving BY4741, Sc(hERG25), and Sc(CgERG25) in the presence of PF1163B (Figure 1A). The results indicated slight inhibitory activity against all three strains, although the IC50 was not reached even at the highest concentration of 138 μM (Table 2). Another compound, 1181-0519 (N-[(2E)-2-

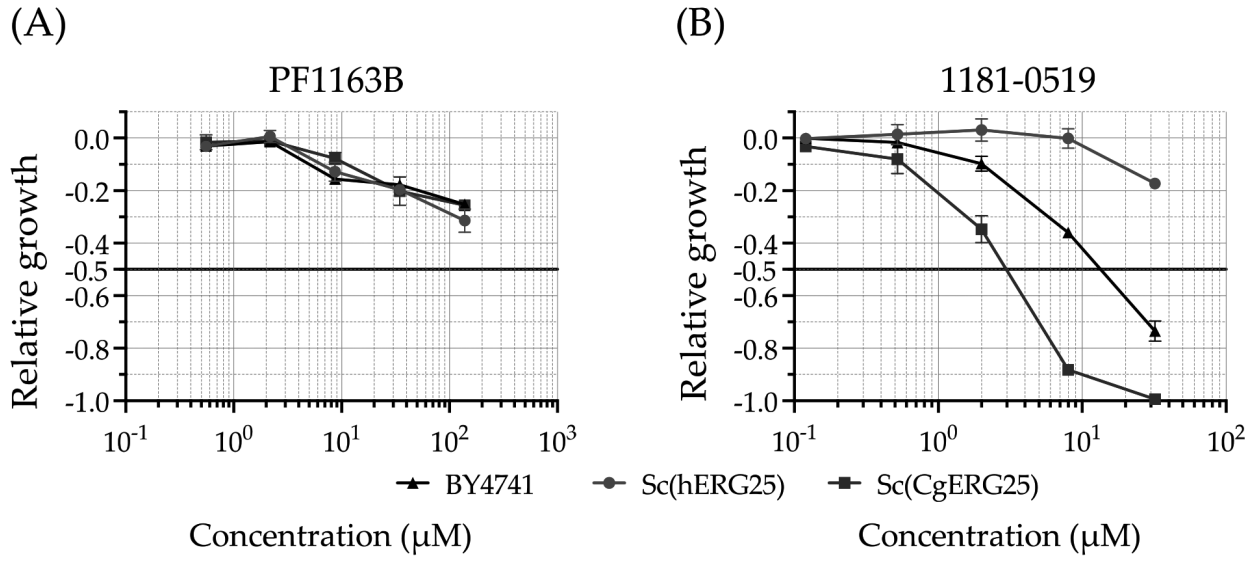

Figure 1. Evaluation of growth inhibition by compounds targeting Erg25p. Relative growth inhibition against knock-in strains (A) with PF1163B and (B) with 1181-0519. Sc(hERG25) and Sc(CgERG25) strains were grown in the two drugs. In both plots, the x-axis denotes the con-centration of the respective drugs, while the y-axis represents the "Relative growth," calculated as the area under the curve (AUC) relative to the absence of the drug. The data are based on the average of three replicates, with error bars indicating the standard deviation.

[(4-nitrophenyl) hydrazinylidene] propyl] acetamide) (Figure S6B), has been reported to possess inhibitory activity against Erg25p in *S. cerevisiae*. Thus, we compared its growth inhibitory effects on BY4741, Sc(hERG25), and Sc (CgERG25) (Figure 2B). The IC50 values for BY4741 and Sc(CgERG25) were 13 μM and 3 μM, respectively, whereas for Sc(hERG25), the IC50 was greater than 32 μM (Table 2). Consequently, we observed growth inhibition of 1181-0519 against Sc(CgERG25) but not against Sc(hERG25).

Evaluation of a novel *FKS1* R1354H mutation associated with caspofungin resistance in *Candida auris* Using the CRISPR-Cas9 System.

Maiko Kiyohara, Taiga Miyazaki, Michiyo Okamoto, Tatsuro Hirayama, Koichi Makimura, Hiroji Chibana, Nana Nakada, Yuya Ito, Makoto Sumiyoshi, Nobuyuki Ashizawa, Kazuaki Takeda, Naoki Iwanaga, Takahiro Takazono, Koichi Izumikawa, Katsunori Yanagihara, Shigeru Kohno, Hiroshi Mukae

Outbreaks of invasive infections, with high mortality rates, caused by multidrug-resistant Candida auris have been reported worldwide. Although hotspot mutations in FKS1 are an established cause of echinocandin resistance, the actual contribution of these mutations to echinocandin resistance remains unknown. Here, we sequenced the FKS1 gene of a caspofungin-resistant clinical isolate (clade I) and identified a novel resistance mutation (G4061A inducing R1354H). We applied the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system to generate a recovered strain (H1354R) in which only this single nucleotide mutation was reverted to its wild-type sequence. We also generated mutant strains with only the R1354H mutation introduced into C. auris wild-type strains (clade I and II) and analyzed their antifungal susceptibility. Compared to their parental strains, the R1354H mutants exhibited a 4- to 16-fold increase in caspofungin minimum inhibitory concentration (MIC) while the H1354R reverted strain exhibited a 4-fold decrease in caspofungin MIC. In a mouse model of disseminated candidiasis, the in vivo therapeutic effect of caspofungin was more closely related to the FKS1 R1354H mutation and the virulence of the strain than its *in vitro* MIC. The CRISPR-Cas9 system could thus aid in elucidating the mechanism underlying drug resistance in C. auris.

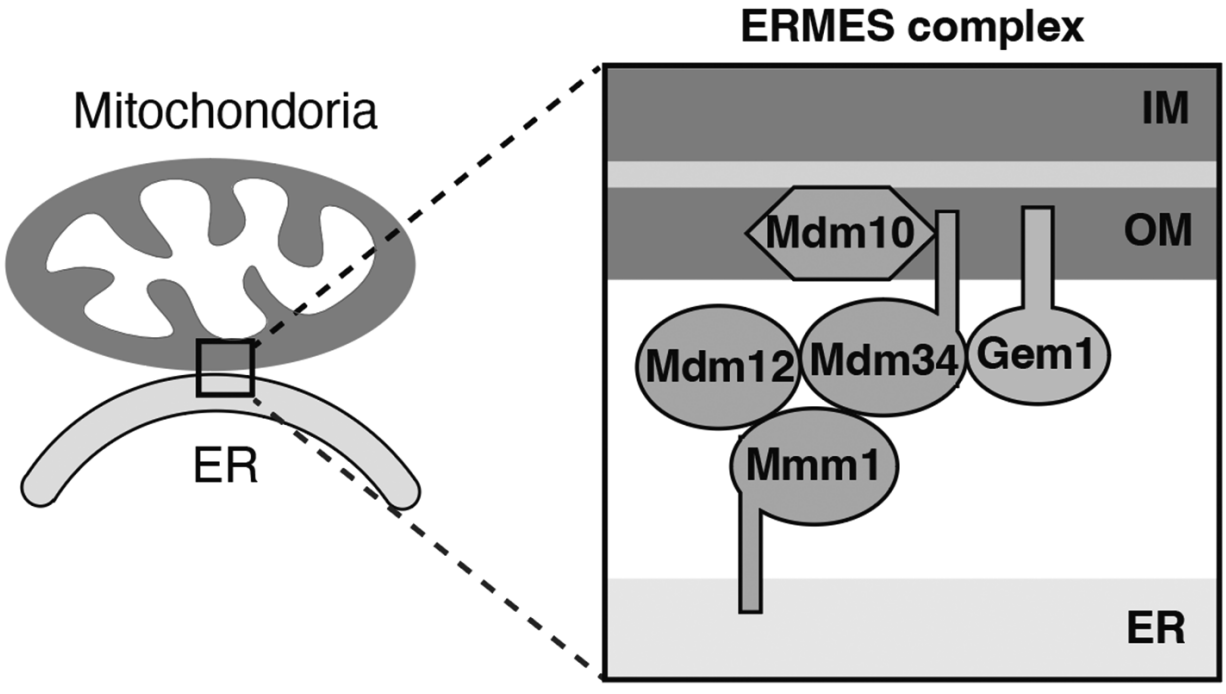

Figure 2. Schematic diagram of the ERMES com-plex. OM, outer membrane. IM, inner membrane.

In *Candida glabrata*, ERMES component *GEM1* Controls mitochondrial morphology, mtROS, and drug efflux pump expression, resulting in azole susceptibility.

Michiyo Okamoto, Keiko Nakano, Azusa Takahashi-Nakaguchi, Kaname Sasamoto, Masashi Yamaguchi, Miguel Cacho Teixeira, Hiroji Chibana

Mitochondrial dysfunction or morphological abnormalities in human pathogenic fungi are known to contribute to azole resistance; however, the underlying molecular mechanisms are unknown. In this study, we investigated the link between mitochondrial morphology and azole resistance in *Candida glabrata*, which is the second most common cause of human candidiasis worldwide. The ER-mitochondrial encounter structure (ERMES) (Fig. 2) complex is thought to play an important role in the mitochondrial dynamics necessary for mitochondria to maintain their function. Of the five components of the ERMES complex, deletion of *GEM1* increased azole resistance. Gem1 is a GTPase that regulates the ERMES complex activity. Point mutations in *GEM1* GTPase domains were sufficient to confer azole resistance. The cells lacking *GEM1* displayed abnormalities in mitochondrial morphology, increased mtROS levels, and increased expression of azole drug efflux pumps encoded by *CDR1* and *CDR2*. Interestingly, treatment with N-acetylcysteine (NAC), an antioxidant, reduced ROS production and the expression of *CDR1* in Δ*gem1* cells. Altogether, the absence of Gem1 activity caused an increase in mitochondrial ROS concentration, leading to Pdr1-dependent upregulation of the drug efflux pump Cdr1, resulting in azole resistance.

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Project of Clinical Investigation

渡邉ＰＩ（臨床感染症）プロジェクト

研究概要（Summary）

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. 350 cases in 2023）. 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 *Aspergillus* species and *Scedosporium* species, 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.

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. 2023年の全国の医療機関からの依頼件数は350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, その後も積極的に共同研究を継続している.

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Research Assistant Professor	Teppei Arai	特任助教	新居　鉄平
Research Assistant Professor	Hidetaka Majima	特任助教	馬嶋　秀考
Grand Fellow	Hideaki Taguchi	グランドフェロー	田口　英昭
Research Technician	Kyoko Yarita	技術職員	鎗田　響子
Research Promotion Technician	Yukiko Tsuchiya	技術補佐員	土屋由紀子
Research Promotion Technician	Yasuko Koga	技術補佐員	古賀　育子
Research Promotion Technician	Kyoko Inoue	技術補佐員	井上　京子

1. Validity of Platelia *Aspergillus* IgG and *Aspergillus* precipitin test to distinguish pulmonary aspergillosis from colonization

Shinfuku K, Suzuki J, Takeda K, Kawashima M, Morio Y, Sasaki Y, Nagai H, Watanabe A, Matsui H, Kamei K.

When *Aspergillus*, an ubiquitous, saprophytic fungus, is detected in respiratory tract specimens collected from chronic respiratory disease patients, it is important to determine whether it is a true infection or colonization. We investigated the usefulness of the Bio-Rad Platelia *Aspergillus* IgG (Platelia Aspergillus IgG) enzyme-linked immunosorbent assay (ELISA) method and the *Aspergillus* precipitin test to distinguish pulmonary aspergillosis from colonization. Between January 2017 and November 2021, 51 confirmed, untreated pulmonary aspergillosis (33 chronic pulmonary aspergillosis [CPA] and 18 allergic bronchopulmonary aspergillosis [ABPA]) and 77 colonization patients were included in this study. At first, the conventional cutoff value was utilized in assessing the validity of the two antibody tests for distinguishing pulmonary aspergillosis from colonization. The Platelia *Aspergillus* IgG cutoff value was then reevaluated to fit this situation. Finally, differences in test accuracy dependent on *Aspergillus* species were assessed for both antibody tests by comparing cases with *Aspergillus fumigatus* complex and those with non-*fumigatus Aspergillus* complex. Both antibody tests demonstrated significantly higher positive rates for pulmonary aspergillosis ($P < 0.0001$) than colonization. The cutoff value should be 15.7 arbitrary units (AU)/mL to best distinguish infection from colonization, which was higher than the conventional value of 10 AU/mL. The diagnostic sensitivity of Platelia *Aspergillus* IgG for the non-*fumigatus Aspergillus* complex was inferior to the *A. fumigatus* complex ($P = 0.019$). In conclusion, both *Aspergillus* antibody tests were valid to distinguish infection from colonization, although we should note the higher cutoff value for Platelia *Aspergillus* IgG and the lower sensitivity in cases of non-*fumigatus Aspergillus* infection.

2. In vivo efficacy of pitavastatin combined with itraconazole against *Aspergillus fumigatus* in silkworm models.

Majima H, Arai T, Kamei K, Watanabe A.

Azole resistance in *Aspergillus fumigatus* is a worldwide concern and new antifungal drugs are required to overcome this problem. Statin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, has been reported to suppress the growth of *A. fumigatus*, but little is known about its in vivo antifungal effect against *A. fumigatus*. In this study, we evaluated the in vivo efficacy of pitavastatin (PIT) combined with itraconazole (ITC) against azole-susceptible and azole-resistant strains with silkworm models. Prolongation of survival was confirmed in the combination-therapy (PIT and ITC) group compared to the no-treatment group in both azole-susceptible and azole-resistant strain models. Furthermore, when the azole-susceptible strain was used, the combination-therapy resulted in a higher survival rate than with ITC alone. Histopathological analysis of the silkworms revealed a reduction of the hyphal amount in both azole-susceptible and azole-resistant strain models. Quantitative evaluation of fungal DNA by qPCR in azole-susceptible strain models clarified the reduction of fungal burden in the combination-therapy group compared with the no-treatment group and ITC-alone group. These results indicate that the efficacy of PIT was enhanced when combined with ITC in vivo. As opposed to most statins, PIT has little drug-drug interaction with azoles in humans and can be used safely with ITC. This combination therapy may be a promising option as an effective treatment in clinical settings in the future.

3. A Novel Combination of mutations in Insig and Cyp51A confers multi-azole Resistance to *Aspergillus fumigatus*.

Arai T, Takahashi H, Majima H, Watanabe A.

Objectives: *Aspergillus fumigatus* is an opportunistic pathogenic fungus that causes aspergillosis. Azole antifungal

agents play a pivotal role in the treatment of aspergillosis. Recently, the incidence of azole-resistant *A. fumigatus* in clinical settings and the environment is rising and is becoming a serious problem worldwide. Some mutations in the fungus that cause azole resistance have been reported, and other unknown molecular mechanisms of azole resistance in *A. fumigatus* would exit. In recent years, several reports have shown that focusing on multiple gene phenotypes is essential to elucidate the whole picture of azole drug resistance mechanisms in *A. fumigatus*. In this study, we report that mutations in Insig, a regulator of lipid metabolism, contribute to azole drug resistance in concert with Cyp51A mutations.

Method: The comparative genomic analysis was performed between strains with the same cyp51A mutation (Gly448Ser) and different azole resistance patterns isolated sequentially from same patients to find novel factors associated with azole resistance. The mutant alleles were replaced with wild-type alleles in clinical isolates by the CRISPR-Cas9 system to find novel factors associated with azole resistance. Antifungal susceptibility tests were performed according to CLSI-M38.

Results: Four *A. fumigatus* strains isolated from two different patients were studied. Patient A: The MICs of the 1st isolate of itraconazole (ITCZ), voriconazole (VRCZ), posaconazole (PSCZ) and isavuconazole (ISCZ) were 2, > 8, 1 and 8, whereas the ones of 2nd isolate were 8, > 8, 1 and > 8, respectively. The numbers of short tandem repeats of these two strains were identical, and same mutation (Gly448Ser) in Cyp51A was confirmed. Patient B: The MICs of the 1st isolate of ITCZ, VRCZ, PSCZ and ISCZ were 1, 1, 0.25 and 0.5, whereas the ones of 2nd isolate were 4, > 8, 0.5 and 8, respectively. The numbers of short tandem repeats of these two strains are identical. 2nd isolate confirmed the mutation (Gly448Ser) in Cyp51A. The genome comparison analysis revealed mutations in the gene encoding insulin-inducible protein (Insig) in the two multi-azole resistant strains carrying the Gly448Ser mutation in Cyp51A. The mutations found in Insig were a nonsense mutation (Trp320*) and an unfinished mutation (cDNA502-533del). Replacing the mutated insig gene with the wild-type gene in these clinical isolates restored susceptibility to ITCZ and ISCZ. On the other hand, susceptibility to VRCZ was unchanged. Replacing the wild-type insig gene with the mutated insig gene in the experimental strain Afs35 did not change susceptibility to azoles.

Conclusions: This study identified a novel genetic alteration associated with azole resistance. The Insig mutation contributes additively to azole resistance in concert with the Cyp51A mutation, but not by itself. These results indicate that focusing on the phenotypes of multiple genes is essential to gain a complete picture of the azole resistance mechanism in *A. fumigatus*.

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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 sero-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
Adjunct Research Technician	Tomoko Ogawa	非常勤技術職員	小川　知子
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. Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2022

Ishiwada N[1, 2], Shinjoh M[1], Kusama Y[2], Arakawa H[3], Ohishi T[3], Saitoh A[2, 3], Suzuki A[3], Tsutsumi H[3], Nishi J[3], Hoshino T[3], Mitsuda T[3], Miyairi I[2, 3], Iwamoto-Kinoshita N[4], Kobayashi H[4], Satoh K[4], Shimizu A[4], Takeshita K[4], Tanaka T[4], Tamura D[4], Tokunaga O[4], Tomita K[4], Nagasawa K[4], Funaki T[4], Furuichi M[4], Miyata I[4], Yaginuma M[4], Yamaguchi Y[4], Yamamoto S[4], Uehara S[3], Kurosaki T[3], Okada K[3], Ouchi K[3]

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The members of the Japanese Society for Pediatric Infectious Diseases and the Japanese Society of Pediatric Pulmonology have developed Guidelines for the Management of Respiratory Infectious Diseases in Children with the objective of facilitating appropriate diagnosis, treatment and prevention of respiratory infections in children. The first edition was published in 2004 and the fifth edition was published in 2022. The Guideline 2022 consists of 2 parts, clinical questions and commentary, and includes general respiratory infections and specific infections in children with

underlying diseases and severe infections. This executive summary outlines the clinical questions in the Guidelines 2022, with reference to the Japanese Medical Information Distribution Service Manual. All recommendations are supported by a systematic search for relevant evidence and are followed by the strength of the recommendation and the quality of the evidence statements.

2. Importance of toxin genes and polymerase chain reaction-based open reading frame type analyses for severe *Staphylococcus aureus* infection in children

Takeuchi N[1], Ishiwada N[1]

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This study analyzed 26 *Staphylococcus aureus* strains, including 16 methicillin-resistant *S. aureus* (MRSA) and 10 methicillin-susceptible *S. aureus* (MSSA), collected from eight medical institutions in the Chiba Prefecture that requested a toxin gene analysis between 2015 and 2021. A total of 14 Panton-Valentine leukocidin (PVL) positive strains were identified, including MSSA. PVL-positive strains were classified into seven types according to polymerase chain reaction-based open reading frame typing (POT); of these types, three POT MRSA strains have not been previously reported, and one has been previously reported as PVL-negative. Some strains tested positive for both PVL and toxic shock syndrome toxin 1. One POT type was identified in both PVL-positive and PVL-negative strains. To the best of our knowledge, this is the first report on the regional spread of highly pathogenic *S. aureus* strains based on the POT method in children from multiple medical institutions. This method is useful for estimating the spread of toxin gene-carrying strains in the community owing to its association with toxin genes. As the number of PVL-positive strains in Japan increases, it is important to analyze the isolates of severe *S. aureus* infections in children by combining toxin gene analyses with the POT method.

3. Increase in prevalence of *Streptococcus pneumoniae* serogroup 24 in children upon introducing 13-valent pneumococcal conjugate vaccine in Japan

Ohkusu M[1], Takeshita K[1], Takeuchi N[1], Ishiwada N[1]

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After introducing the 13-valent pneumococcal conjugate vaccine (PCV13) for children, a change in the prevalence of different *Streptococcus pneumoniae* serotypes that cause invasive pneumococcal diseases (IPDs) has been observed. The prevalence of vaccine serotypes has decreased and that of non-vaccine serotypes has increased. Currently, serogroup 24 has become one of the major non-vaccine serotypes causing IPDs in children in Japan. The aim of this study was to characterize clinical and genomic features of *S. pneumoniae* serogroup 24 strains isolated from sterile body sites in Japanese children. Serotyping, multi-locus sequence typing and genomic analysis of capsular polysaccharides of 61 strains of serogroup 24 were performed from 2015 to 2021. Among the 61 strains, 36, 23 and two belonged to serotypes 24F, 24B and 24C, respectively. The 24F sequence type (ST) 2572 and 24B ST 2572 were the major serotypes and sequence types observed from 2015 to 2019. By contrast, 24F ST 162 and 24B ST 2754 were the two major serotypes and sequence types observed after 2020. Two strains of serotype 24C were detected for the first time in Japan. Sequence analysis of the *abpA* gene, which plays a role in the synthesis of capsular polysaccharides in *S. pneumoniae*, was performed to distinguish different strains of serogroup 24. After the introduction of PCV13 in Japan, serogroup 24 has become one of the most prevalent non-vaccine serotypes causing IPDs in children. This serogroup has not been targeted in the next-generation pneumococcal conjugate vaccines. Therefore, monitoring of *S. pneumoniae* serogroup 24 that causes IPDs in children is essential.

4. Impact of Janus kinase inhibitors on antibody response to 13-valent pneumococcal conjugate vaccine in patients with rheumatoid arthritis

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Objectives: To evaluate the antibody response to 13-valent pneumococcal conjugate vaccine (PCV13) in patients with rheumatoid arthritis receiving Janus kinase inhibitors (JAKIs).

Methods: Fifty-three patients receiving methotrexate (MTX; n = 10), JAKI (n = 20), or MTX + JAKI (n = 23) were vaccinated with PCV13. Serum concentrations of immunoglobulin G (IgG) antibodies to 13 pneumococcal serotype capsular polysaccharides were quantified before and 4-6 weeks after vaccination. Positive antibody response was defined as a 2-fold or more increase in IgG concentrations from prevaccination levels.

Results: After vaccination, IgG concentrations significantly increased in all treatment groups (P <0.001), but fold increases (postvaccination to prevaccination ratios) were different among treatment groups (9.30 for MTX, 6.36 for JAKI, and 3.46 for combination therapy). Positive antibody response rates were comparable between the MTX group (90%) and the JAKI group (95%) but lower in the MTX + JAKI group (52.2%). In a multivariable logistic regression analysis, the combination therapy was the only factor associated with a reduced antibody response to PCV13. No severe adverse events were observed in any treatment group.

Conclusion: Although JAKIs do not impair PCV13 immunogenicity in rheumatoid arthritis patients, the combination of MTX with JAKI can reduce the antibody response in this patient population.

5. Bacteriological and molecular characterization of temperature- and CO_2-dependent *Streptococcus pneumoniae* serotype 24F ST162 isolated from Japanese children

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Streptococcus pneumoniae serotype 24F is one of the most prevalent non-vaccine serotypes that causes invasive pneumococcal disease (IPD) in many countries, including Japan. This study aimed to analyze the bacteriological and molecular characteristics of serotype 24F sequence type (ST) 162, which has been increasingly isolated from pediatric patients with IPD in Japan recently. The examination of growth conditions, sequencing of genes associated with the CO_2 dependence, and antimicrobial susceptibility testing at 35℃ under 5% CO_2 were performed for 10 isolates obtained from Japanese children with IPD caused by serotype 24F ST162. All isolates failed to grow at 35℃ in ambient air; however, they showed growth at 30 ℃ in ambient air and at 35℃ under 5% CO_2. Sequencing of murF involved in cell wall synthesis indicated that all isolates had a single amino acid substitution, $MurF^{A179V}$. Additionally, all isolates were sensitive to penicillin G and resistant to trimethoprim-sulfamethoxazole. Furthermore, 25 non-capnophilic strains were obtained from all CO_2-dependent isolates, and their murF sequences were compared. Thirteen of the 25 non-capnophilic strains displayed a different amino acid substitution, $MurF^{V179A}$, whereas the other 12 presented the previously described $MurF^{A179V}$. This suggests that a proportion of the CO_2-dependent phenotype of serotype 24F ST162 may have been conferred by $MurF^{A179V}$; however, the mechanism for the CO_2 dependence of these isolates warrants

further investigation. The CO_2-dependent serotype 24F ST162 pneumococcal isolates shared common characteristics in terms of growth patterns, molecular basis, and antimicrobial susceptibility; therefore, future epidemiological trends of this clone must be closely monitored.

6. Large-scale questionnaire survey of parents and guardians on antimicrobial resistance using group health checkups for infants and toddlers in Japan

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Background: It is important to improve the knowledge of antimicrobial resistance (AMR) among parents and guardians, to promote AMR stewardship in pediatrics. However, a large-scale survey on parents' knowledge and awareness of AMR has not yet been conducted in Japan. Furthermore, the current status of knowledge and awareness is unknown. Infant and toddler health checkups are large-scale administrative activities that approximately all children and their parents undergo in Japan. Therefore, we conducted a knowledge and awareness survey using a questionnaire during the group health checkups.
Methods: All parents and guardians who participated in the group health checkups (4-month, 1.5-year, and 3-year) in Chiba City during the year were targeted. Parents' knowledge and awareness of AMR and their wishes for future information on AMR were surveyed using a one-choice questionnaire.
Results: The questionnaire collection rate was 87.5% (16,663/19,047), and the valid response rate was 77.0% (14,674/19,047). Of the parents, 37.2% answered that "antibiotics are not effective for colds." However, 58.9% answered that they "had never heard of the drug-resistant bacteria." While 8.3% of parents answered that they "sometimes want my child to be prescribed antibiotics even if the doctor deemed it unnecessary," 46.1% of parents answered that "they were unaware of whether their children were prescribed antimicrobials."
Conclusions: Knowledge and awareness of AMR among parents in Japan are inadequate, and there is room for improvement. Continuous awareness-raising activities combining multiple methods are needed in the future.

7. Novel STAT1 Variants in Japanese Patients with Isolated Mendelian Susceptibility to Mycobacterial Diseases

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Purpose: Heterozygous dominant-negative (DN) STAT1 variants are responsible for autosomal dominant (AD) Mendelian susceptibility to mycobacterial disease (MSMD). In this paper, we describe eight MSMD cases from four kindreds in Japan.

Methods: An inborn error of immunity-related gene panel sequencing was performed using genomic DNA extracted from whole blood samples. The identified variants were validated using Sanger sequencing. Functional analysis was evaluated with a luciferase reporter assay and co-transfection assay in STAT1-deficient cells.

Results: Patient 1.1 was a 20-month-old boy with multifocal osteomyelitis and paravertebral abscesses caused by Mycobacterium bovis bacillus Calmette-Guérin (BCG). Although the paravertebral abscess was refractory to antimycobacterial drugs, the addition of IFN-γ and drainage of the abscess were effective. Intriguingly, his mother (patient 1.2) showed an uneventful clinical course except for treatment-responsive tuberculous spondylitis during adulthood. Patient 2.1 was an 8-month-old boy with lymphadenopathy and lung nodules caused by BCG. He responded well to antimycobacterial drugs. His mother (patient 2.2) was healthy. Patient 3.1 was a 11-year-old girl with suspected skin tuberculosis. Her brother (patient 3.2) had BCG-osis, but their mother (patient 3.3) was healthy. Patient 4 was an 8-month-old girl with left axillary and supraclavicular lymphadenopathy associated with BCG vaccination. Kindreds 1, 2, and 3 were shown to have novel heterozygous variants (V642F, R588C, and R649G) in STAT1, respectively. Kindred 4 had previously reported heterozygous variants (Q463H). A luciferase reporter assay in STAT1-deficient cells followed by IFN-γ stimulation confirmed that these variants are loss-of-function. In addition, with co-transfection assay, we confirmed all of these variants had DN effect on WT STAT1.

Conclusion: Four kindred MSMD subjects with 3 novel variants and 1 known variant in STAT1 were identified in this study. AD STAT1 deficiency might be prevalent in Japanese patients with BCG-associated MSMD.

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Project for Systems Biology of Microorganisms

高橋ＰＩ（微生物創生）プロジェクト

研究概要（Summary）

Our research areas are Bioinformatics and Systems Biology. Our Bioinformatics approach aims to deeply and clearly understand massive biological experiment data, e. g., sequence data by next generation sequencers. Systems Biology aims to understand how biological systems work and help the experimental design mainly by mathematical modelling approach.

我々はコンピュータ解析によって, 次世代シーケンサーを含む様々な生物実験で得られる大量データからの新規生物学的知見の創出, 並びに, 数理モデルアプローチによる生命現象の解明に取り組んでいます. 大量データによる生命の「構成要素の理解」, 数理モデルによる「挙動の理解」という二つのコンセプトの下, 病原真菌を含む微生物を対象に細胞機能の分子レベルでの理解を目指しています.

Associate Professor	Hiroki Takahashi	准　教　授	高橋　弘喜
Research Assistant Professor	Jun-ichi Ishihara	特 任 助 教	石原　潤一
Research Assistant Professor	Saho Shibata（2023.4 ～）	特 任 助 教	柴田　紗帆
Research Promotion Technician	Machiko Zen	技術補佐員	全　真知子
Research Promotion Technician	Emi Shirai（2023.6 ～）	技術補佐員	白井　江美

1. Investigation of the relationships between heterogeneity against environmental stresses and pathogenicity in pathogenic fungi *Aspergillus fumigatus*

Saho Shibata, He Xiaohui, Yu Lu, Jun-ichi Ishihara, 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. Systems biology for understanding the stress responses in bacteria

Jun-ichi Ishihara, Hiroki Takahashi

It is conceivable that the heterogeneity could be one of the adaptation mechanisms to a diverse of environments in bacteria. We address the heterogeneity of bacteria by two approaches; one is the systems biology approach where we derive the mathematical model and conduct the simulation of transcriptional regulation in metal response, and second is the microfluidic device to directly measure the single cell behavior of bacteria. We launched the assembling of device and succeeded the microfluidic device which could be useful to detect the single cell behavior.

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

Jun-ichi Ishihara, Masaki Nagayama, 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．

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1. Polymerase chain reaction-based methods for the detection of heat-resistant ascomycetous fungi.

Yoshioka I[1, 2], Mori Y[1], Fahal AH[3], Siddig EE[3], Kaneko S[4, 5], Yaguchi T[1].

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Background

Filamentous fungi of the genus *Madurella* are the primary causative agents of mycetoma, a disease observed in tropical and subtropical regions. Since early diagnostics based on a morphological approach are difficult and have many shortcomings, a molecular diagnostic method suitable for rural settings is required. In this study, we developed the loop-mediated isothermal amplification (LAMP) method to present a foundational technique of the diagnosis of *Madurella* spp. (*M. mycetomatis*, *M. pseudomycetomatis*, *M. tropicana*, and *M. fahalii*), the common causative organisms of eumycetoma.

Principal findings

We successfully designed a primer pair targeting the rDNAs of three *Madurella* spp. excluding *M. fahalii*, and detected up to 100 fg of genomic DNA extracted from isolates of *M. mycetomatis* and 1 pg of *M. pseudomycetomatis* and *M. tropicana*, within one hour. Second, a primer pair specific to *M. mycetomatis*, the most common causative species, or *M. fahalii*, a drug-resistant species, was constructed, and the detection limit of both primer pairs was 1 pg. The designed primers accurately distinguished 16 strains of the genus *Madurella* from various fungal species known to cause mycetomas.

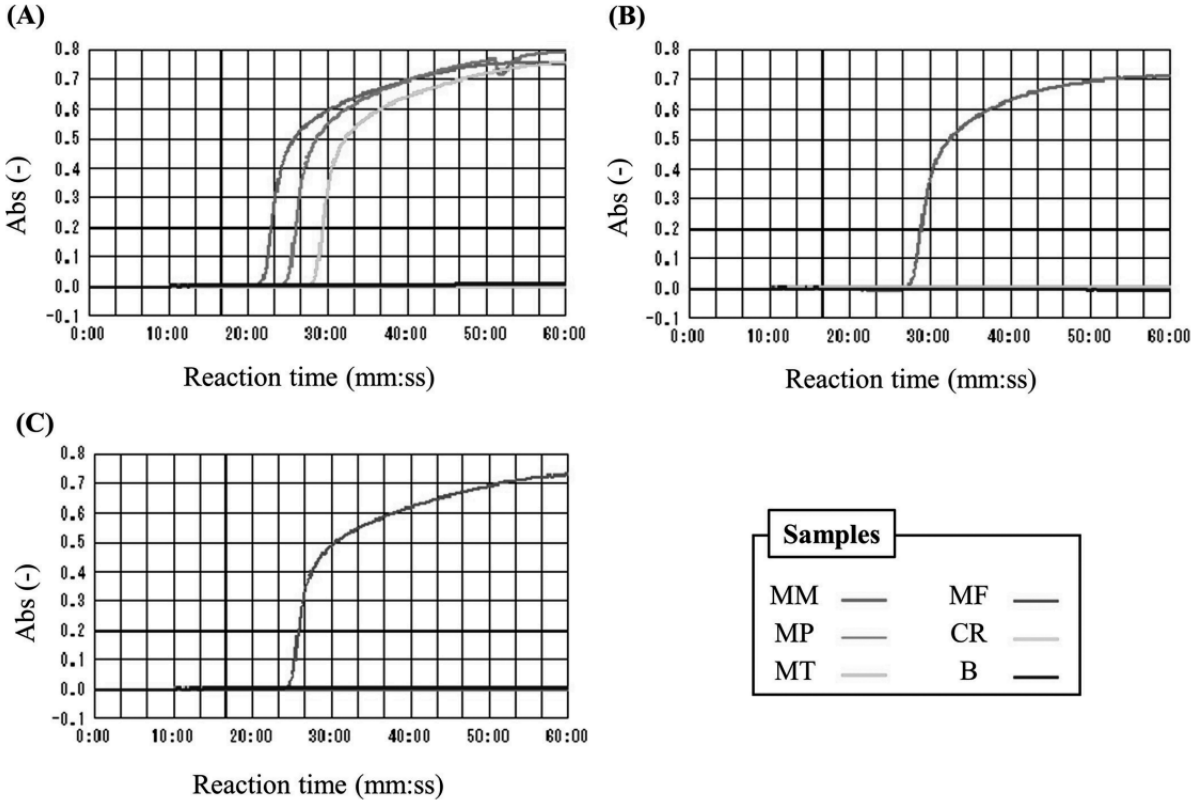

FIG. 1. The specificity of designed LAMP primers, (A) PV, (B) PM and (C) PF. 10 ng of the extracted genomic DNAs derived from *Madurella* spp. were used as templates. LAMP reaction was performed at 63℃ for 60 min, and the turbidity of the reaction solution was monitored as an indicator of amplification. As for primer PV, the genomic DNA of *Chaetomium rectangulare* was also used. The sample legends of the plots were represented on the bottom right panel. Abbreviations: MM, *M. mycetomatis* IFM 46458; MP, *M. pseudomycetomatis* IFM 46460; MT, *M. tropicana* CBS201. 38^{T}; MF, *M. fahalii* CBS129176^{T}; CR, *Chaetomium rectangluare* CBS 126778^{T}; B, Blank sample with a buffer used for DNA dilution

Conclusion

In summary, we established the first model of a LAMP detection method that rapidly and sensitively detects and identifies *Madurella* isolates for clinical diagnostics. Moreover, the combined designed primer sets could identify mycetoma-causing strains simultaneously.

2. Polymerase chain reaction-based methods for the detection of heat-resistant ascomycetous fungi.

Yaguchi T[1].

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There is increasing incidence of food spoilage and health hazards caused by heat-resistant fungi belonging to the genera *Byssochlamys*, *Thermoascus*, and *Neosartorya*, among others. Their ascospores cannot be sterilized by heating the food. The microbiological risk assessment studies of these fungi during the production of food and beverages indicated that these fungal species or genera in food are associated with different health risks. Therefore, it is necessary to distinguish *Byssochlamys*, *Thermoascus*, and *Neosartorya* from other fungi in the food industry. These genera can be identified by sequence analysis of housekeeping genes such as β-tubulin, but the process is costly and time-consuming. Therefore, rapid and simple PCR-based methods have been developed using specific primer sets for genus- or species-level identification. PCR amplification products are observed to be specific for each of these genera or species and do not cross-react with other fungi associated with food spoilage and

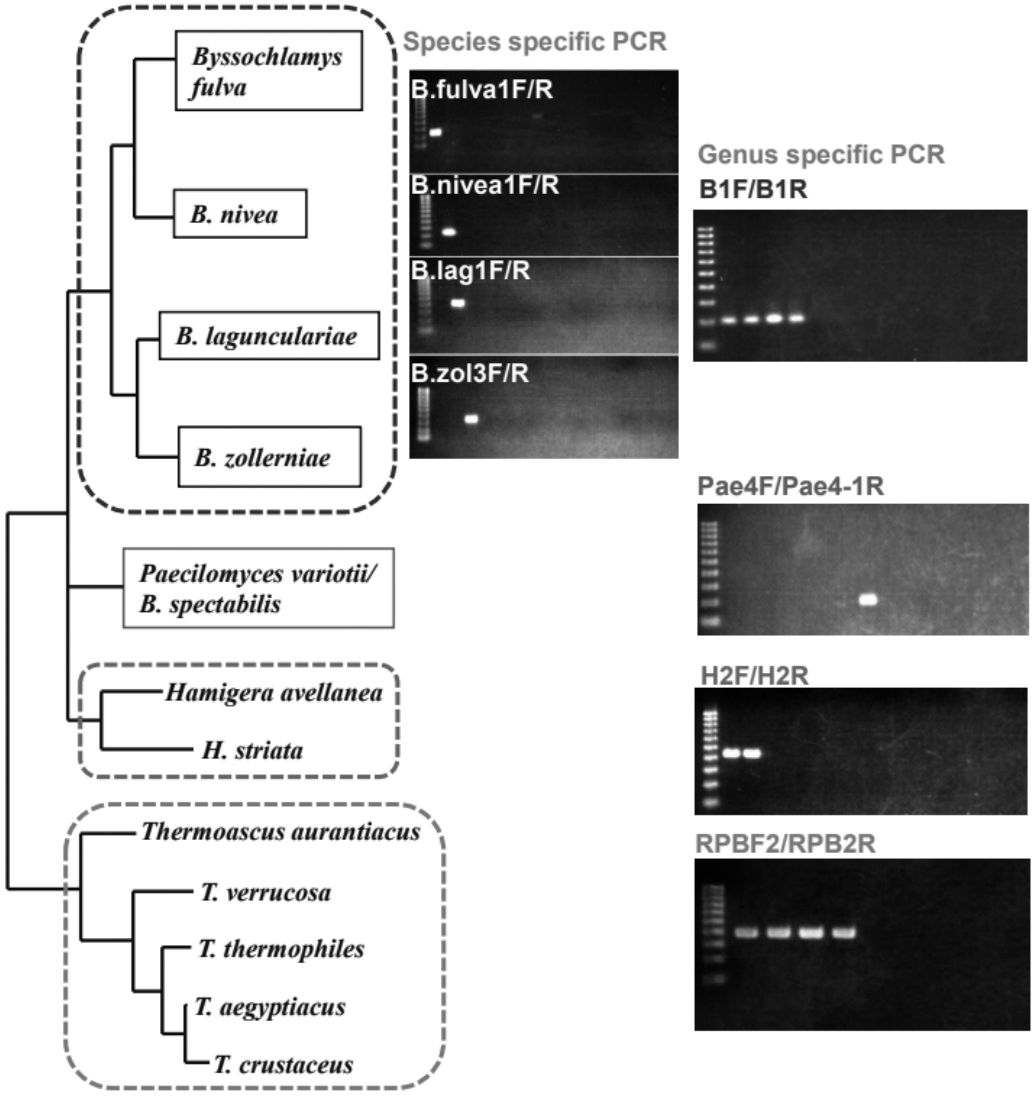

FIG. 2. Genus and species specific PCR on *Byssochlamys*, *Thermoascus* and *Hamigera*. This figure was produced on the basis of data of Nakayama et al., 2012, Hosoya et al., 2012, 2014.

environmental contamination. These identification methods are simple, rapid, and highly specific, making them feasible for use in the quality management of food production plants.

3. Bifusicoumarins A-D: Cytotoxic 3S-dihydroisocoumarins from the entomopathogenic fungus *Cordyceps bifusispora* (NBRC 108997).

Elshamy AI[1], Mohamed TA[2], Yoneyama T[3], Noji M[3], Ban S[4], Imagawa H[3], Efferth T[5], Hegazy M-EF[2, 5], Umeyama A[3].

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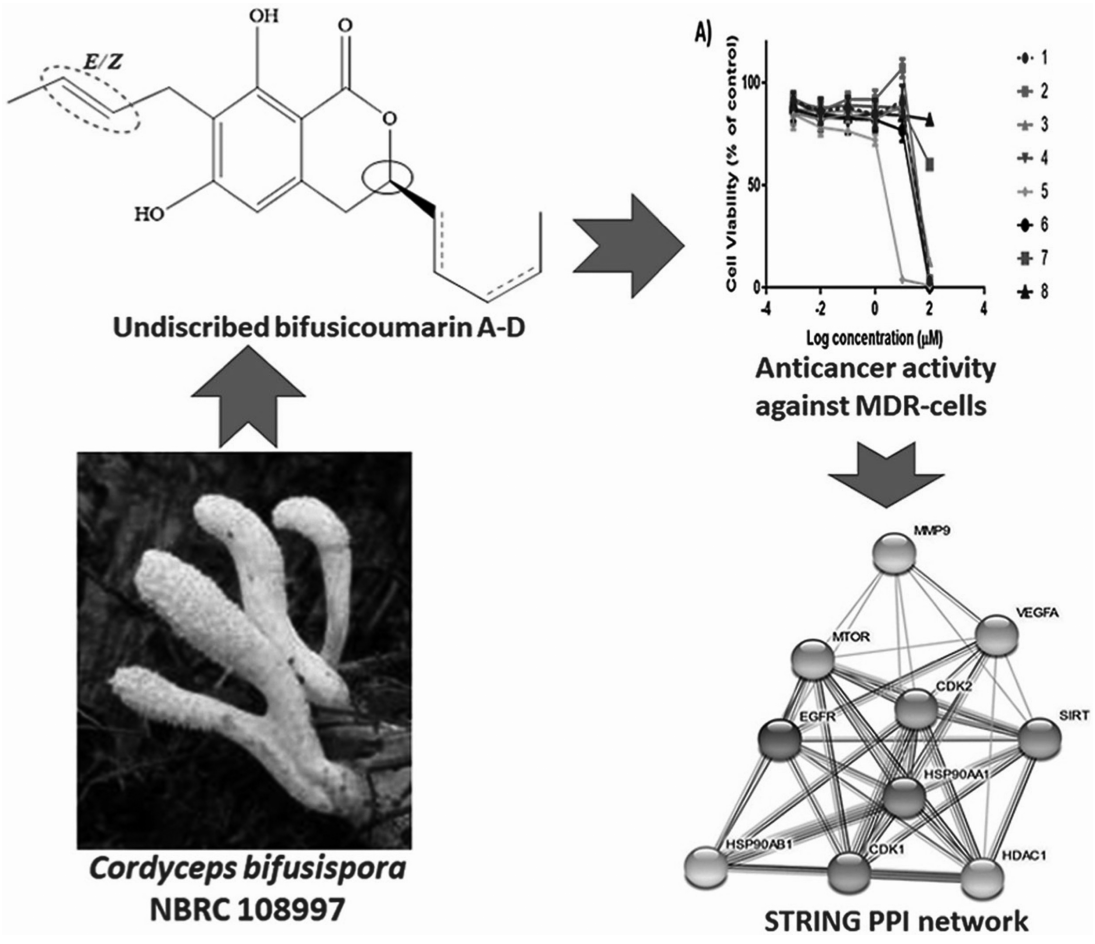

FIG. 3. Graphical abstract.

Cordyceps is a genus of ascomycete fungi with some of them being edible and/or having a long tradition in Chinese medicine. The chemical characterization of a solvent extract of the entomopathogenic fungus *Cordyceps bifusispora* afforded four undescribed coumarins, bifusicoumarin A-D (1–4), along with previously reported metabolites (5–8). Structural elucidation was performed via NMR, UV and HRMS analyses, X-ray single crystal diffraction and experimental ECD. A high throughput resazurin reduction assay, that measures cell viability, indicated that 5 has a IC_{50} between 1 and 15 μM for several assayed tumor lines. Moreover, a protein-interaction network indicated that *C. bifusispora* is a promising source of additional antitumor metabolites based on SwissTargetPrediction software predictions.

4. Draft genome sequence of *Aspergillus lacticoffeatus* WU-2020, a citric acid producer suitable for solid culture, belonging to *Aspergillus* section *Nigri*.

Yoshioka I[1, 2], Takahashi H[2, 3, 4], Kusuya Y[2], Yaguchi T[2], Shibata A[5], Kirimura K[1, 5].

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Aspergillus lacticoffeatus WU-2020 is a citric acid hyperproducer that is suitable for solid culture. Here, we present a high-quality draft of its genome sequence (35.9 Mb), which consists of 11 scaffolds and contains 11,490 genes. We also present the mitochondrial genome, which is 31.3 kb in length.

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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に注目し，そ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．

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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 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.

To improve efficiency of LNP encapsulating Super-S-TuD, we developed a method to add “active targeting” to LNP by modifying the surface layer of LNP with ligand molecules that have the ability to bind to target cells. It is important that the ligand molecules are located at the surface layer of the LNP for that the ligand molecules efficiently bind to the target cells. Therefore, we developed the method of binding the ligand molecule to the tip of the PEG on the surface layer of LNP. We tested several types of polyarginine peptides as ligand molecule. It was found that some polyarginine peptides cause attached LNP to form aggregates. We also found that some polyarginine peptides that did not form aggregates increased the efficiency of nucleic acid introduction into cells.

Merged project of respiratory pathophysiology and pathobiology

巽浩一郎・磯野史朗・並木隆雄・入鹿山容子(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

研究スタッフ

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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

1. Functional roles of CD26/DPP4 in bleomycin-induced pulmonary fibrosis. Physiol Rep. 2023;11:e15645.

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

Among the various histopathological patterns of drug-induced interstitial lung disease (DILD), diffuse alveolar damage (DAD) is associated with poor prognosis. However, there is no reliable biomarker for its accurate diagnosis. Here, we show stratifin/14-3-3σ (SFN) as a biomarker candidate found in a proteomic analysis. The study includes two independent cohorts (including totally 26 patients with

DAD) and controls (total 432 samples). SFN is specifically elevated in DILD patients with DAD, and is superior to the known biomarkers, KL-6 and SP-D, in discrimination of DILD patients with DAD from patients with other DILD patterns or other lung diseases. SFN is also increased in serum from patients with idiopathic DAD, and in lung tissues and bronchoalveolar lavage fluid of patients with DAD. *In vitro* analysis using cultured lung epithelial cells suggests that extracellular release of SFN occurs via p53-dependent apoptosis. We conclude that serum SFN is a promising biomarker for DAD diagnosis.

2. Partially hydrolyzed guar gum suppresses the progression of pulmonary arterial hypertension in a Su5416/hypoxia rat model.

Sanada TJ, Hosomi K, Park J, Naito A, Sakao S, Tanabe N, Kunisawa J, Tatsumi K, Suzuki T.

Background: The pathogenesis of chronic thromboembolic pulmonary hypertension (CTEPH) is considered to be associated with chronic inflammation; however, the underlying mechanism remains unclear. Recently, altered gut microbiota were found in patients with pulmonary arterial hypertension (PAH) and in experimental PAH models. The aim of this study was to characterize the gut microbiota in patients with CTEPH and assess the relationship between gut dysbiosis and inflammation in CTEPH.
Methods: In this observational study, fecal samples were collected from 11 patients with CTEPH and 22 healthy participants. The abundance of gut microbiota in these fecal samples was assessed using 16S ribosomal ribonucleic acid (rRNA) gene sequencing. Inflammatory cytokine and endotoxin levels were also assessed in patients with CTEPH and control participants.
Results: The levels of serum tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, and macrophage inflammatory protein (MIP)-1α were elevated in patients with CTEPH. Plasma endotoxin levels were significantly increased in patients with CTEPH ($P < 0.001$), and were positively correlated with TNF-α, IL-6, IL-8, and MIP-1α levels. The 16S rRNA gene sequencing and the principal coordinate analysis revealed the distinction in the gut microbiota between patients with CTEPH ($P < 0.01$) and control participants as well as the decreased bacterial alpha-diversity in patients with CTEPH. A random forest analysis for predicting the distinction in gut microbiota revealed an accuracy of 80.3%.
Conclusion: The composition of the gut microbiota in patients with CTEPH was distinct from that of healthy participants, which may be associated with the elevated inflammatory cytokines and endotoxins in CTEPH.

3. N-butyldeoxynojirimycin (miglustat) ameliorates pulmonary fibrosis through inhibition of nuclear translocation of Smad2/3.

Nakamura H, Zhou Y, Sakamoto Y, Yamazaki A, Kurumiya E, Yamazaki R, Hayashi K, Kasuya Y, Watanabe K, Kasahara J, Takabatake M, Tatsumi K, Yoshino I, Honda T, Murayama T.

Background: Sarcoidosis is a granulomatous systemic disease of unknown etiology. Mononuclear cells such as macrophages or lymphocytes in lung tissue and hilar or mediastinal lymph nodes have been recognized to play an essential role in granuloma formation in pulmonary sarcoidosis. Peripheral blood mononuclear cells (PBMCs) consist of several immunocompetent cells and have been shown to play a mechanistic role in the pathogenesis of sarcoidosis. However, the genetic modifications that occur in bulk PBMCs of sarcoidosis remain to be elucidated.
Purpose: This study aimed to explore the pathobiological markers of sarcoidosis in PBMCs by comparing the transcriptional signature of PBMCs from patients with pulmonary sarcoidosis with those of healthy controls by RNA sequencing.
Methods: PBMC samples were collected from subjects with pulmonary sarcoidosis with no steroid/immunosuppressant drugs ($n = 8$) and healthy controls ($n = 11$) from August 2020 to April 2021, and RNA sequencing was performed with the PBMC samples.
Results: Principal component analysis using RNA sequencing datasets comparing pulmonary sarcoidosis with healthy

controls revealed that the two groups appeared to be differentiated, in which 270 differentially expressed genes were found in PBMCs between sarcoidosis and healthy controls. Enrichment analysis for gene ontology suggested that some biological processes related to the pathobiology of sarcoidosis, such as cellular response to interleukin (IL)-1 and IFN-γ, regulation of IL-6 production, IL-8 secretion, regulation of mononuclear cell migration, and response to lipopolysaccharide, were involved. Enrichment analysis of the KEGG pathway indicated the involvement of tumor necrosis factor (TNF), toll-like receptor signaling, IL-17 signaling pathways, phagosomes, and ribosomes. Most of the genes involved in TNF and IL-17 signaling pathways and phagosomes were upregulated, while most of the ribosome-related genes were downregulated.

Conclusion: The present study demonstrated that bulk gene expression patterns in PBMCs were different between patients with pulmonary sarcoidosis and healthy controls. The changes in the gene expression pattern of PBMCs could reflect the existence of sarcoidosis lesions and influence granuloma formation in sarcoidosis. These new findings are important to strengthen our understanding of the etiology and pathobiology of sarcoidosis and indicate a potential therapeutic target for sarcoidosis.

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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 essential to living organisms. Through evolution, organisms have changed their reproductive strategies, from bearing many offspring with a few chances of survival to leaving a few offspring and nursing them for assured survival. In mammals, the number of eggs laid at one time has been reduced from millions in fish to one in humans. Nevertheless, humans produce nearly 7 million oocytes during the fetal period and allow more than 30 follicles to grow per cycle during adulthood. This suggests that humans have evolved a specific mechanism to reduce the number of offspring, which decreases oocyte reserve with age. Overriding this mechanism may increase oocyte reserve and enhance fertility. We are exploring this mechanism in the hope that it may serve as a new strategy for treating infertility.

生殖は生物の本質に関わる機能であり，生物進化に伴なって生殖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，ヒトは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でき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 that causes itching and vaginal discharge and is often associated with vulvar skin lesions. Although no benefit has been reported for commensal candida, we are interested in the possible merit of commensal candida because the immunological benefit of candida commensalism is reported for intestines. Vaginal candida is most common in women of reproductive age, especially during pregnancy. Candida is detected in the vaginal for 10-40% or more asymptomatic pregnant women. In Japan, universal screening for vaginal vaginosis（including a test for candida）is performed during early pregnancy. We analyze the screening data to examine the incidence of asymptomatic commensal candida, its association with bacterial vaginosis, and its contribution to vaginal discharge or other symptoms or signs. We also examine the possible effects of vaginal candida on pregnancy outcomes,

2. Sex differences in the COVID-19.

Messenger RNA COVID-19 vaccines are effective in preventing severe diseases. After the implementation of the mRNA vaccines, the real-world survey revealed several adverse events that are rare but severe and unique to the mRNA vaccines: pericarditis in young males, cerebral venous sinus thrombosis, and Guillain-Barre syndrome. There are gender differences in the incidence of adverse effects, as in the incidence of COVID-19. The sex-dependent differences in the endocrine environment, such as estrogens and androgens, and immunity may be responsible for the differences in adverse events. We focus on vulvar ulcers as an unrecognized

adverse effect of COVID-19 vaccines. Based on Hill's criteria, we showed a close association between vulvar ulcer and vaccine use to identify the ulcer as an adverse effect of the vaccine. We are also analyzing public databases such as VAERS to prove that acute vulvar ulcer is a rare but unique adverse event in young women.

3. Evolution-based approach to infertility.

Autoantibodies may affect reproductive function. Some antiphospholipid antibodies are responsible for recurrent pregnancy loss. Antinuclear antibody-positive individuals may lose the number of oocytes more rapidly than usual, leading to premature ovarian insufficiency; however, the details remain to be determined. We screened infertile patients for antinuclear antibodies to explore which types of antinuclear antibody is associated with fertility outcomes. We have found the titer-dependent association of anti-centromere antibodies to IVF failure. Anti-centromere antibodies are characteristic autoantibodies in scleroderma and have been suggested to be associated with decreased fertility in scleroderma. Therefore, our findings are consistent with the previous observation that scleroderma patients often have long periods of infertility. We are currently investigating the mechanism of action of centromere antibodies using a mouse *in vitro* maturation model. Furthermore, we are developing a new therapy to target anti-centromere antibodies. This treatment improved the development of a good-quality blastocyst and the rate of live births.

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

文部科学省　ナショナルバイオリソースプロジェクト「病原真核微生物」

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	FY2019	FY2020	FY2021	FY2022	FY2023*
Collection	579	886	837	702	235
Preservation	24, 899	25, 785	26, 591	24, 689	24, 908
Provision	1, 112	222	1, 319	846	623

*: to 31th Aug. , 2023

FIG. 1. Exhibition at the 46th Annual Meeting of the Molecular Biology Society of Japan.

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菌とその情報の共有し，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．

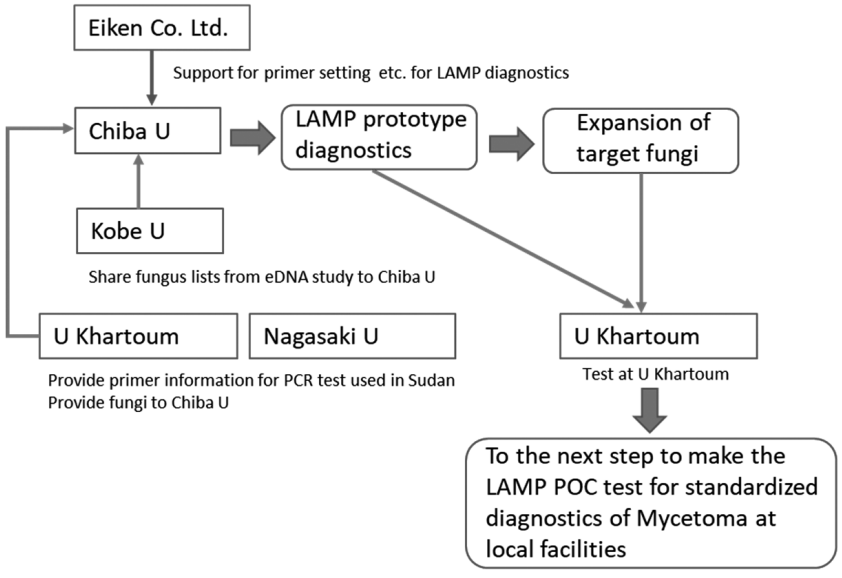

FIG. 1. Finding POC diagnosis using LAMP method of mycetoma infection.

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

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」

Infections caused by multidrug-resistant bacteria are becoming a global problem because they are difficult to control with existing antibacterial drugs. The development of new infectious disease control drugs is an urgent issue. Recent studies have revealed that the emergence of antimicrobial-tolerant cells, "persisters," which have not acquired resistance genes, contributes to the emergence of drug-resistant bacteria. Bacterial properties such as persisters are also induced by host immunity and have been suggested to be a factor in persistent and latent infections. We discovered a novel mechanism by which nosocomial multidrug-resistant *Staphylococcus aureus* alters the activity of the quorum sensing system (QS), which controls the expression of many virulence factors. Because regulation of QS contributes to long-term infection, we propose that compounds that completely inhibit QS are a target for the treatment of infectious diseases. Therefore, we identified several compounds from our natural product library that suppress the QS of *S. aureus*. For some of the compounds, we have also found target bacterial proteins. Now, we are searching for the structures of compounds that can be applied to treat infectious diseases through compound-protein docking analysis. Furthermore, compounds that increase the antimicrobial sensitivity of Gram-negative bacteria have been discovered in our natural product library, and we have been studying the compound structures and target proteins required for activity.

多剤耐性菌を原因とする感染症では，既存抗菌薬での制御が困難となることが世界的な問題であり，新たな感染症制御薬の開発が喫緊の課題となっている．薬剤耐性菌の出現には，耐性遺伝子を獲得していない抗菌薬寛容性細胞"パーシスター"の出現が寄与することが明らかとなってきた．パーシスターのような細菌性状は，宿主免疫にも誘導され，持続感染・潜伏感染の要因となることが示唆されている．我々は院内感染する多剤耐性黄色ブドウ球菌が病原因子の発現を制御するクオラムセンシングシステム（QS）の活性を変える新たな機構を見出した．QSの制御は長期感染に寄与することから，QSを完全に抑制する化合物は感染症治療の標的として重要であることを提唱している．そこで天然物ライブラリーより黄色ブドウ球菌のQSを抑制する化合物を同定した．いくつかの化合物では標的とする細菌タンパク質も見出し，化合物とタンパク質のドッキング解析などにより感染症治療に応用できるような化合物の構造を探索している．また，グラム陰性菌の抗菌薬感受性を高めるような化合物も天然物ライブラリーから見出し，活性に必要な化合物構造や標的タンパク質について新たな知見を得ている．

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 join the 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.

In 2023, a new research building of the institute was opened, and Prof. Goto's lab in MMRC moved to the building to begin research activities. The division also manages the biosafety level 3 (BSL3) facilities and conducts advanced basic research and human resource development leading to the diagnosis, prevention, and treatment of infectious diseases.

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

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年には新研究棟が完成し，感染免疫分野の後藤研究室が同研究棟へ移動し，新BSL3施設を管理しながら，研究活動を開始しています．

URL: https://www. ridm. chiba-u. jp/

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 hold 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. In 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.

In addition, this year, in November and December, we jointly held "Technology training session on rapid identification of filamentous fungi using MALDI-TOF MS" with RIKEN JCM.

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して実施し，年１回定員10名で開催している．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年度は，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，外部講師を招聘したり講習内容を見直して実施した．

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，病原原虫，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．

また，本年度は新たに11月および12月に「MALDI-TOF MSを用いた糸状菌の迅速同定の技術研修会」を理化学研究所と共同で開催した．

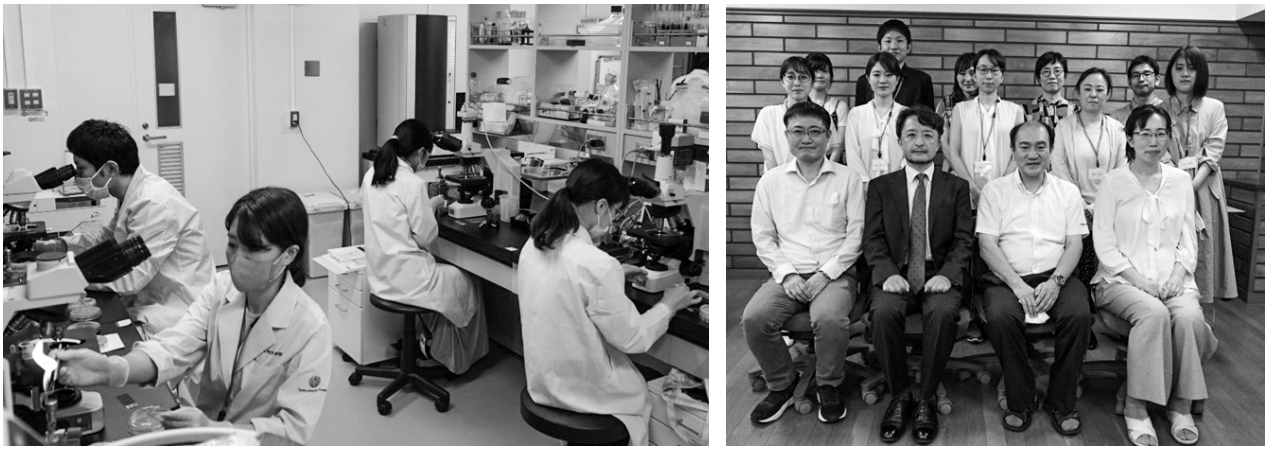

FIG 1. Scenes from the training course of pathogenic fungi.

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, 肝線維症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. j

2022 Fiscal Year Cooperative Research Program Report

令和４年度 共同利用・共同研究報告

研究課題　’22－１

Analysis of gene expression of Thl 7 cells induced by gut microorganisms

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腸内微生物によって誘導されるTh17細胞の遺伝子発現解析

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研究成果

本研究では，腸内における常在真菌の一種であるCandidaalbicansによって誘導されるTヘルパー17細胞（Th17細胞）について，遺伝的な特徴を明らかにすることを目的とし，本年度はTh17細胞の誘導機構ならびにシングルセル解析に向けた実験系の確立を進めた．申請者らは，アンピシリン処理マウスにC. albicansを経口投与することで，腸管にC. albicansが定着したマウスモデルを採用し，C. albicansが腸管粘膜固有層のTh17細胞を誘導することを見出していた．一方，この実験系ではセグメント細菌によって誘導されるTh17細胞とC. albicansによって誘導されるTh17細胞の区別が不明瞭であるため，２つの方法でC. albicans誘導性のTh17細胞を分離することを試みた．まず，C. albicansが定着し，抗生物質で腸内細菌を除去したRag2欠損マウスを準備し，さらにIL-17GFPノックインマウスの脾臓から採取したナイーブCD4T細胞を移入する ことで，粘膜固有層にTh17細胞が誘導されることを確認した．さらに，離乳前のIL-17GFPノックインマウスからアンピシリン処理を行うことで，Th17細胞誘導性のセグメント細菌の定着を防ぎ，C. albicansを定着させることで粘膜固有層にTh17細胞が誘導されることを確認した．さらに，これらの方法を用いて，腸管粘膜固有層由来のTh17細胞をセルソーターで分離する方法を確立した．以上の研究成果から，C. albicans によって誘導される腸管Th17細胞を分離する実験系を確立した．

研究課題　’22－２

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

近年，病原細菌のみならず，病原真菌における薬剤耐性化が問題となってきている．特に*Aspergillus fumigatus*のアゾール系抗真菌薬耐性化は大きな懸念を持たれている．アゾール系抗真菌薬耐性化は標的酵素Cyp51Aやそのプロモーター領域の変化によるものが知られているが，その他にも様々な要因が存在することが明らかとなってきた．しかし，Cyp51A以外の要因については十

分に明らかとなっていない．研究代表者のこれまで研究で機能未知であった*A. fumigatus*のRttAタンパク質の点変異により，アゾール系抗真菌薬への感受性が低下する現象を見出し，RttAがアゾール系抗真菌薬感受性に関連することを報告してきた．本年度の共同研究では*rttA*破壊株を取得することに成功し，この*rttA*破壊株の抗真菌薬感受性について検討した結果，アゾール系抗真菌薬への薬剤感受性が高まるとの結果が得られた．本結果は，過去の結果とも矛盾せず，RttAのアゾール感受性への寄与が確認された．*Aspergillus*属において，RttAホモログは広く見つかっているものの，ホモログには存在しているC6型転写因子の領域がRttAでは欠けている．今後，RttAの*A. fumigatus*における役割を明らかにし，どのようにしてアゾール抗真菌薬感受性に関連しているかを明らかとする予定である．

研究課題　’22－３

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

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Mitsutoshi Yoneyama
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Koji Onomoto
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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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（千葉大学医学部附属病院・感染制御部）
米山光俊
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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，急速に感染拡大した後，今日に至るまで変異を繰り返しながら世界中で猛威を奮っている．これに対抗する予防策として，mRNAワクチンなど種々のワクチンが開発され，既に大半の国民がワクチン接種を受けている．それぞれ劇的な感染予防効果を示しつつも，一方，その誘導抗体価の持続性が低いことや様々な変異株に対して効力が低下する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を逐次分離・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．

実際の成果としては，先ず2022年度中に県内で流行したピーク時を中心に千葉大学附属病院に入院され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，約20株の変異株（オミクロン変異株BA. 1, BA. 2, BA. 5, BXX株など）を得た．各株の帰属に関しては，特にSpike遺伝子領域をOneStep-RT-PCR法で増幅し，遺伝子解析データに基づいた系統樹作成によって明らかにした．昨年度に分離した株を加えると，これまでに約40株樹立したことになる．

治療薬探索研究としては，2022年度は治療の実用性を念頭に，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やPF-07321332の抗ウイルス活性が非常に高いことが分かった．この薬剤に加え，他の作用機構が異なる薬剤，例えばレムデシビルやモルヌピラビル等ウイルスRNAポリメラーゼ阻害薬との組み合わせにより，更に抗ウイルス効果を増強できることが明らかとなった．複数の抗ウイルス剤を併用する新しい治療法の可能性が示されたものと考えている．

研究課題　’22－４

Mechanism of microRNA-mediated human defense system induced by retroviral infection

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Koji Onomoto
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（Graduate School of Science and Engineering, Saitama University）
Yoshimasa Asano
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レトロウイルスによるmicroRNAを介したヒトの生体防御の分子機構解明

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米山光俊
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尾野本浩司
（千葉大学真菌医学研究センター）
高橋朋子
（埼玉大学大学院理工学研究科）
浅野吉政
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研究成果

レトロウイルスはヒトをはじめとする哺乳類の細胞へ感染すると，ウイルスセンサータンパク質を介してI型インターフェロン（IFN）を誘導して細胞を防御する．この主なウイルスセンサータンパク質はRIG-I like receptors（RLRs）であり，受入教員の米山教授らが発見した分子であり，米山教授らによって，RLRsがウイルス性RNAを認識しIFNを誘導して生体を守る機構についての研究が進められている．一方で，ウイルス感染細胞はアポトーシスによって自滅することで周辺の細胞を守る機構があることも知られている．両者はともにウイルス感染から自身を守る，哺乳類のみに保存された高次生体防御機構であるが，アポトーシスが起こるメカニズムは，これまで不明であった．RLRsにはRIG-I, MDA5, LGP2といった３つの因子が存在し，いずれもウイルス感染により発現誘導されるが，RIG-IとMDA5はそれぞれ異なる特徴をもつウイルス性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，ウイルス感染による早期の反応として，発現誘導されたLGP2がRNAサイレンシングの主要因子であるTRBP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，さらに，生合成が阻害されたmicroRNAによるRNAサイレンシングが起こらなくなるため，その標的遺伝子であるアポトーシス関連遺伝子群の発現が誘導されることを報告してきた．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の状態を誘導する可能性も考えられる．そこで，今回はさらに後期の反応を検討した．その結果，早期の反応によって誘導されたアポトーシスで活性化されたカスパーゼによってTRBPが切断され，TRBPは完全にRNAサイレンシング活性を消失し，ER ストレスを誘発することで，非可逆的な細胞死を誘導すると考えられる結果が得られた．本研究の成果については論文執筆中である．

研究課題　’22－５

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．2022年度では，A. fumigatusの全遺伝子から設計したガイドRNAに対するpooled oligo DNAをCRISPR/Cas9ゲノム編集技術による変異導入のためのプラスミドベクターにクローニングし作製したプラスミドライブラリを保有するA. fumigatusの分生子ライブラリを作製した．

大腸菌で構築したCRISPRライブラリには10371種類の異なるガイドRNA配列が含まれていることが確認できている．

大腸菌でこのCRISPRプラスミドライブラリを増幅後，エレクトロポレーション法を用いてA. fumigatusに導入した．

その後，ハイグロマイシンを含む寒天培地上で形質転換したA. fumigatusを選択し，形質転換株を選択し，分生子を回収した．回収した分生子からDNAを抽出し，ナノポアシーケンサーMinIONを使用してガイドRNAの配列を解読した．結果として，3292種類の配列が含まれていることが確認された．これは予想の約32％で，エレクトロポレーション法による導入効率が低さ，そして分生子形成に必須の遺伝子が回収できないことがその原因であると考えられる．今後は，A. fumigatusへの形質転換効率を改善し，分生子形成を経ずにライブラリを作製することを目指す．これによりCRISPRスクリーニング法を確立し，血清刺激に応答するシグナル伝達機構の解明につなげる．

研究課題　’22－６

Characterizing new effectors underlying *Candida glabrata* evolution towards drug resistance: aiming improved diagnosis and therapeutics

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研究成果

This report aims to investigate the frequency of polymorphisms and genome rearrangements as potential genetic factors contributing to *Candida glabrata* drug resistance.

Methods: To accomplish this, we analyzed genomic variation across 94 geographically diverse isolates with distinct resistance phenotypes, whose sequences are deposited in GenBank. Additionally, we sequenced the genomes of three clinical isolates, including two azole-resistant strains that did not exhibit Gain-Of-Function（GOF）mutations in the *PDR1* transcription factor gene. By comparing the genomic variations in susceptible and resistant isolates, we aimed to identify variants exclusive to resistant strains while accounting for variants arising from genome diversity.

Results: Our analysis revealed that more than half of the azole resistant isolates did not possess exclusive polymorphisms in

PDR1, suggesting the existence of alternative genetic mechanisms underlying antifungal resistance. Furthermore, we identified consistent copy number variations affecting a subset of chromosomes.

Conclusion: Through our comprehensive analysis of genomic and phenotypic variations across isolates, we successfully identified genetic changes that were specifically enriched in antifungal resistant strains on a genome-wide scale. These findings represent a crucial initial step in uncovering additional determinants of antifungal resistance. Specifically, our investigation of the newly sequenced strains suggests the involvement of a set of mutations/genes in the unconventional azole resistance phenotype.

We have been published two papers related to the evolution of drug resistance in *Candida glabrata*.

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研究課題　'22－7

Roles of Clec1a in the development of inflammatory diseases

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活性型C型レクチンファミリー分子「Clec1a」の機能解析

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研究成果

Clec1Aは，C型レクチン受容体ファミリーの一員で，細胞外領域に糖鎖認識ドメインを持つが，細胞質ドメインには既知のシグナル伝達モチーフを持たない．この分子は内皮細胞で高発現し，樹状細胞で弱く発現している．リガンドとして，Aspergillus fumigatusの菌体表面の1, 8-dihydroxynaphthalene-melaninを認識し，宿主防御に重要な役割を果たすことが報告されているが，生理的条件下におけるこの分子の役割については，未だ解明されていない．そこで，本研究ではClec1aの自己免疫疾患の発症における役割を検討する目的で，多発性硬化症の動物モデルである実験的自己免疫性脳脊髄炎（EAE）の誘導実験を行った．

その結果，Clec1a-/-マウスでは，野生型マウスと比較して，最大疾患スコアが有意に低く，病理学的にも脊髄の脱髄や炎症が軽度であることがわかった．その機構を解析する目的で免疫後のマウスのリンパ球を採取し，EAE誘導に使用した抗原で，*in vitro*リコール実験を行った．再刺激後のメモリーT細胞増殖はClec1a-/-マウスで有意に減少していたことから，Clec1a-/-マウスの樹状細胞の抗原提示能が低下していることが示唆された．また，RNA-SeqおよびRT-qPCR解析により，EAE誘導後のClec1a-/-マウスにおいて，Il17a, Il6, Il1bなどの炎症性サイトカインの発現が顕著に減少していることが明らかになり，このサイトカイン産生の減少がClec1a-/-マウスの症状改善に関与していると考えられた．以上の結果から，Clec1Aは抗原提示細胞の機能を正常に保つことで生体恒常性維持に関与している可能性が示された．

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研究課題　’22－８

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

令和３年度，共同研究初年度において，新規ASP2397誘導体を用いて，既存の抗真菌薬に対する耐性株を含む６種11株のカンジダ属病原真菌を用いて詳細な活性試験を行った．令和４年度は，*in vitro* における薬効試験を進めた．Candida glabrataに対する *in vitro* における抗真菌活性の強化が確認された．さらに天然化合物では示されなかったC. auris に対する抗真菌活性が誘導体において示された．前年度から継続しているin vivoでの薬効評価試験において，C. glabrata の感染後の生存率を指標とする評価系構築のために，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マウスに対してシクロフォスファミドを事前に投与し易感染状態にした上でC. glabrata を接種し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．尚，同実験系ではミカファンギンの投薬により死亡率 0 %とするコントロールを設定することができた．次にC. auris を用いたin vivo 評価系の構築を進めた結果，C. auris 感染下において，C. glabrata を用いた場合よりもシクロフォスファミドの投与を減量した方法によってより再現性の高い抗真菌薬の評価が可能であることがわかった．以上のように，C. glabrata とC. auris による感染モデルを使用したin vivo薬効評価試験において，免疫抑制剤のそれぞれ異なる投与方法を設定した．

研究課題　’22－９

Serotonin-producing mast cells suppress excessive ILC2 activation in fungus-associated airway inflammation

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真菌誘導性喘息モデルにおけるセロトニンによる２型自然リンパ球（ILC2）の抑制機構の解明

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研究成果

２型自然リンパ球（ILC2s）の制御にはサイトカインや神経伝達物質が関与しており，重症喘息患者ではセロトニン（5-hydroxytryptamine, 5-HT）産生が増加することが知られている．しかし，5-HTが喘息時の炎症を悪化させるのか抑制するのかという点に関しては相反する報告があり，不明な点が多く残されている．また近年，T細胞による抗原依存性喘息とILC2による抗原非依存性喘息が認識されているが，それらに対するセロトニンの影響については不明である．そこで，本研究は，ILC2による喘息における5-HTの役割の解明と，5-HT産生細胞の同定を目的とした．

まず，真菌の一種である*Alternaria*で誘発する喘息（*Alternaria*誘発喘息）マウスモデルを用いて，5-HTの効果を検討した．また，セロトニン産生細胞を同定するために，マスト細胞欠損マウスを用いた．その結果，１）*Alternaria*誘発喘息マウスモデルで発症する炎症はILC2依存性依存性であり，その炎症は5-HT投与により停止する，２）5-HTによる炎症抑制効果は，5-HTがILC2を直接阻害することにより発揮されること，３）マスト細胞欠損マウスではILC2依存性の炎症が増悪することから，5-HTを産生する細胞はマスト細胞であること，の３点が明らかとなった．また，マスト細胞由来の5-HTによるILC2抑制機構は真菌以外の喘息誘発物質では起こらず，真菌成分依存的な反応であることもわかっており，炎症初期には5-HT陽性マスト細胞が観察されなかったことから，一連の炎症反応の終盤に5-HTがILC2の活性化を抑制している可能性が示唆された．

研究課題　’22－10

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

これまでの研究で，我々の有するアスペルギローママウスモデルと，千葉大学真菌医学研究センター臨床感染症分野の有する*gliA*（グリオトキシン産生クラスター遺伝子）欠損*Aspergillus fumigatus*と，*laeA*（二次代謝産物産生調節因子）欠損*A. fumigatus*を用いて，アスペルギルス二次代謝産物がアスペルギローマの組織侵襲に与える影響の解析を実施し，二次代謝産物が組織侵襲の重要な因子であることを見出した．

さらに，アスペルギローマのトランスクリプトーム解析およびメタボローム解析により，アスペルギローマ内でのグリオトキシン産生の証明に成功した．加えて同マウスモデルを用いて*A. fumigatus*の二次代謝産物が表現型に及ぼす影響を宿主反応の側面から解析した．*laeA*欠損株でアスペルギローママウスモデルを作成し，留置14～90日後にアスペルギローマの肉眼，病理所見と菌量を評価した．比較対象として*laeA*欠損株の親株であるAf293株も同項目を評価した．*laeA*欠損株はAf293株より分生子形成や組織侵襲が乏しく，留置14日後の平均菌量もAf293より*laeA*欠損株が低い傾向を認めた．留置14日後および30日後の病理では，Af293株では好中球浸潤が目立つ一方，*laeA*遺伝子欠損株では好中球が少なくマクロファージ浸潤が目立った．これらの結果から，アスペルギローマにおける*A. fumigatus*の二次代謝産物産生は宿主の炎症細胞浸潤に影響する可能性が示唆された．今後はサンプル数を増やし，サイトカイン解析や臨床スコアリングなど多面的に*laeA*遺伝子欠損株の病原性を評価し，またグリオトキシン非産生株を用いてグリオトキシンが病原性に与える影響を評価予定である．

研究課題　’22－11

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

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Aspergillus 呼吸器検体臨床分離株の菌種同定・薬剤感受性の検討

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研究成果

2013年の研究開始から2022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．22年度は44株のAspergillusと担子菌を検出し検討中である．

当院で治療導入前の51例の培養陽性肺アスペルギルス症（慢性肺アスペルギルス症33例，アレルギー性気管支肺アスペルギルス症18例）と呼吸器検体からAspergillus陽性となるも該当する病変がなくcolonizationと考えられた77例について，沈降抗体とBio-Rad Platelia Aspergillus IgGの２方法でアスペルギルス抗体を測定し，各抗体検査の有用性を比較検討した．またColonizationをcontrol群とした際のPlatelia Aspergillus IgGの最適なcut off値と菌種による検査精度の相違を一部の検体は本研究の遺伝子による菌種同定の結果も用いて検討した．Platelia Aspergillus IgGは陽性尤度比 7.35，陰性尤度比 0.26で沈降抗体法よりも精度は高かった．呼吸器基礎疾患がありcolonization群をcontrolとした場合のcut off値は15.7 AU/mLと高値となった．Platelia Aspergillus IgGでは肺アスペルギルス症診断の感度は，A. fumigatusで83.3%に対して，non-fumigatus Aspergillusでは45.5%でnon-fumigatus Aspergillusで有意に低い結果であった（p=0.019）．

発表論文

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研究課題　’22－12

Elucidation of antifungal resistance mechanisms in *Candida auris*

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Candida auris の抗真菌薬耐性機序の解明

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研究成果

共同利用研究の成果として発表した論文のアブストラクトを以下に示します．

多剤耐性Candida auris によって引き起こされる，高い死亡率を伴う侵襲性感染症の発生が世界中で報告されています．FKS1 のホットスポット変異はエキノカンジン耐性の原因として確立されていますが，エキノカンジン耐性に対するこれらの変異の影響は不明のままです．今回我々は，カスポファンギン耐性臨床分離株（クレードⅠ）のFKS1 遺伝子を配列決定し，新規耐性変異（R1354Hを誘導するG4061A）を同定した．我々は，クラスター化規則的に間隔をあけた短いパリンドロームリピートCRISPR-Cas9システムを利用して，この単一ヌクレオチド変異のみが野生型配列に戻った回復株（H1354R）を作製しました．また，C. auris 野生型株（クレードⅠおよびII）に R1354H 変異のみを導入した変異株を作製し，その抗真菌感受性を解析しました．親株と比較して，R1354H 変異株はカスポファンギン最小発育阻止濃度（MIC）の 4 ～ 16 倍の増加を示しましたが，H1354R 復帰株はカスポファンギン MIC の 4 倍の減少でした．播種性カンジダ症のマウスモデルでは，カスポファンギンの in vivo 治療効果は，*in vitro* MIC よりも FKS1 R1354H 変異および菌株の毒性とより密接に関連していました．したがって，CRISPR-Cas9 システムは，C. auris の薬剤耐性の根底にあるメカニズムの解明に有用であることが示されました．

研究課題　’22－13

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

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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，昨年度，院内感染症から単離された菌株の全ゲノム・全メチル化解析を行うことにより，これまで，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見出した．この形質が，院内感染症のパーシスター発生や，抗

生剤体制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た．そこで，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．

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研究課題　’22－14

Identification of 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．2021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．2022年度は，XRN1のRNase活性喪失型変異タンパク発現株と，XRN1と協調して働くRNA分解関連因子の破壊株を作製し，ATG32発現解析を行った．両変異株共にATG32の発現量が顕著に増加することを見出し，XRN1のRNase活性がATG32発現抑制に関与していることが示唆された．XRN1のRNase活性喪失型変異タンパク発現株においては，ミトコンドリア分解活性も増加していることが確認され，マイトファジー活性化にもRNase活性が関与していることが予想された．

研究課題　’22－15

Elucidation of the mechanisms of azole resistance in dermatophytes

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白癬菌に拡がるアゾール系抗真菌薬耐性化の分子メカニズムの解析

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研究成果

*Trichophyton indotineae*は，数年前に新種提案された高病原性白癬菌である．申請者らは，日本国外で分離された多数の*T. indotineae*を対象に，アゾール系抗真菌薬イトラコナゾール（ITC）およびボリコナゾール（VRC）に対する感受性を調査し，薬剤低感受性を示す複数の株を見出した．これらの株に共通する薬剤低感受性化の要因の解析を進めたところ，アゾールの作用標的の１つであるCYP51B（lanosterol 14α-demethylase）をコードする*CYP51B*遺伝子が同遺伝子座上で繰り返し重複変異を生じ（タンデムリピート形成），CYP51Bが過剰発現したことが主な原因であると結論づけた（2021年度成果）．この結論に辿り着くために実施した遺伝学的解析の中で，RNA干渉技術（RNAi）を用いてアゾール低感受性株における*CYP51B*遺伝子の発現量の低下させる試みを行った．その結果，明確な発現量の低下を示す遺伝子操作株は得られたものの，顕著な発現量の低下を起こした株の作出には至らず，遺伝子重複と遺伝子産物の過剰発現の直接的な関係性の証明には至らなかった．そこで本研究では，他の病原真菌で普及しつつあるCRISPR/Cas9システムによるゲノム編集技術を応用して，アゾール低感受性株TIMM20118のゲノムにある*CYP51B*のタンデムリピートをシングルコピーに改変した遺伝子操作株の作出を試みた．その結果，*T. indotineae*の他，*T. rubrum*でも機能するCRISPR/Cas9ゲノム編集システムの構築に成功し，TIMM20118からシングルコピーの*CYP51B*遺伝子をもつ遺伝子操作株を作出することができた．そして，作出した遺伝子操作株のITCおよびVRC感受性を解析したところ，これらのアゾールに対する感受性が顕著に改善することが明らかとなり，遺伝子重複と遺伝子産物の過剰発現の直接的な関係性が証明された．本解析結果を含む，*T. inndotineae*のアゾール低感受性化の仕組みに関する解析結果をまとめた原著論文を2023年７月に学術誌”Antimicrobial Agents and Chemotherapy”にサブミットした（現在，査読中）．

研究課題　’22－16

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

深在性真菌感染症の治療薬の選択肢は僅かに４系統しかないにも関わらず耐性株が増加しており，新規作用機序を持つ抗真菌薬の開発が求められている．エルゴステロール

の生合成経路および最終産物であるエルゴステロールはこれまでに多くの既存抗真菌薬の標的となった実績があり，さらに有用な標的分子が残されていると考えられている．理化学研究所では，実験室酵母（*Saccharomyces cerevisiae*）の遺伝子改変株を用いたスクリーニングによってエルゴステロール生合成経路に含まれるErg25（Methylsterol monooxygenase）を標的とすることが予測された化合物を見出すことができた．Erg25は，アゾールの標的分子であるErg11（Lanosterol 14-αdemethylase）よりも優れた標的分子であることが報告されている（Okamoto et al.，2022）．そこで，千葉大学において*Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. auris, C. krusei* を用いた感受性評価を実施した結果，*Candida glabrata*に対してのみ生育阻害活性を示した．次に当該化合物の作用機序解明のために，知花准教授が保有する*C. glabrataERG25* ノックダウン株を用いて当該化合物の感受性試験を行い，当該化合物に対する高感受性化が確認された．この結果によりErg25を標的分子とする上記の予測を支持することができた．今後，標的分子の同定や細胞毒性の評価，さらに類縁体の探索や合成により広域性の強化を目指したい．

研究課題　’22－17

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. We reported 3 cases of IPD in children who were admitted to Dr. Sardjito General Hospital, Yogyakarta, Indonesia between 2016 and 2019. While our first 2 patients had milder course of disease, our third patient who presented with meningoencephalitis had poor outcome. Risk factors shown in our cases were young age and malignancy history. Multiple antibiotic resistance was observed in our isolates. The fact that none of our patients have received pneumococcal vaccination marks the necessity of this vaccine especially for at-risk children.

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研究課題　’22－18

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

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研究成果

国内での風疹流行に対して2019年４月より麻しん・風しん（MR）ワクチン５期定期接種が国事業として開始

された.

千葉市では以前より市独自事業として, 妊娠希望の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きている. 私たちは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, 千葉大学真菌医学研究センターにて集計・傾向を分析した（当センター倫理審査委員会承認番号No. 18）.

2018年10月から（国事業は2019年４月から）現時点で集計を終えている2023年１月までの期間において, 抗体検査は54,691件（国事業：43,835件, 千葉市事業：10,856件）, MRワクチン接種は17,314件（国事業：9,056件, 千葉市事業：8,258件）実施された. 国事業における抗体検査の進捗率は33%と十分とは言えず, 月別実施件数の推移は伸び悩んでいたのに対して, 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, 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認められた. 抗体検査結果から得られる性別・年代別抗体陰性率（HI法で16倍以下の割合）は, 20歳代男性で48%, 女性で44%であり, 他世代に比べて高かった.

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26回日本ワクチン学会学術集会において発表した.

また, 解析結果を踏まえて, 子育て世代に対する千葉市事業促進のために, 市民および医師会に対して啓発活動を行った. 市民に対しては, テレビやラジオ等の公共放送で研究結果を紹介し, SNSを用いて制度の啓発活動を行った. 母子健康手帳交付時や乳幼児健診時, イベント時においてパンフレットを配布した（添付資料参照）. 医師会に対しては, 会員向けの勉強会を開催した. 千葉市事業を利用して産婦人科でMRワクチン接種を行う女性が多い傾向を踏まえて, 市内の産婦人科クリニックに対して追加で事業の案内を行った.

研究課題　’22－19

Analysis of the protective effects ofV-ATPase inhibitor against infection by VRE

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V-ATPase阻害剤を用いたVRE感染阻害機構の解明

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研究成果

本年度は, 生体内においてバンコマイシン耐性腸球菌（vancomycin-resistant enterococci : VRE）に対し, 申請者らが新たに見出したV-ATPase阻害剤の効果を検証した. 実験方法としては, セフォペラゾンを１週間前処理した野生型マウスに, VREを経口投与し, さらにV0ATPase阻害剤を１日２ 回, ３日間経口投与した. VRE投与３日後にマウスを解剖し, 小腸, 大腸内容物および紫便を希釈してVRE選択培地に播種し, 形成されるコロニー数を計測した. その結果, V-ATPase阻害剤非投与群と比較して, V-ATPase阻害剤投与群において, 小腸内容物中において検出されるVRE数が大きく減少することを見出した. 一方で, 大腸や糞便中に存在するVRE数はV-ATPase阻害剤投与, 非投与群で有意な差が見られなかった. V-ATPase阻害剤のVRE増殖阻害効果はpH依存性があり, 特に腸管内容物が塩基性を示す小腸において, VRE阻害効果を発揮したと考えられた.

本研究成果により, 新たに見出したV-ATPase阻害剤が, 生体内においてVREに対する増殖阻害効果を示すことが明らかとなった.

研究課題　’22－20

Evaluation of Preventable Measures Against Invasive Pneumococcal Disease in Children with Underlying Disease

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基礎疾患のある小児患者における侵襲性肺炎球菌感染症予防法の評価

大宜見　力
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研究成果

13価肺炎球菌結合型ワクチン（PCV13）の定期接種化以降, PCV13非含有血清型の肺炎球菌による感染症の増加が問題となっている. 本共同研究では, 侵襲性肺炎球菌感染症を発症した小児から分離された肺炎球菌株と罹患時の患者の肺炎球菌に対する抗体保有状況を解析し, 評価することを目的として実施している.

2022年度は, 侵襲性肺炎球菌感染症由来株４株の血清型解析を行い, 血清型は23B, 15C（２株）, 24Fでいずれもも PCV13非含有血清型であった. また, 2021年度の本共同研究で実施した胆道閉鎖症に対する肝移植後の小児のムコイド型肺炎球菌（血清型３）症例について, 血清型３に対する抗体測定とオプソニン活性測定結果を含め, 2022年度, 日本小児科学会雑誌に公表した.

研究課題　’22－21

Pathological analysis of invasive infectious disease due to non-typeable *Haemophilus influenzae*

Kenji Gotoh
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Naruhiko Ishiwada
（Medical Mycology Research Center, Chiba University）

無莢膜型インフルエンザ菌による侵襲性感染症の病態解析

後藤憲志
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研究成果

インフルエンザ菌莢膜ｂ型ワクチン普及後に, 顕在化してきている無莢膜型インフルエンザ菌（non-typeable *Haemophilus influenzae*：NTHi）による侵襲性感染症の病態解明のため, 侵襲性感染症由来のNTHi株を用いた病原因子の解析を共同で行っている. 2021年度は, 新生児の無菌部位から分離されたNTHiのバイオフィルム産生能をplate assay法を用いて解析した. 新生児由来株は, 小児由来侵襲性感染症由来のNTHiのbiofilm産生能と比較し, 著しくバイオフィルム産生能が低かった. この結果をふまえ, 2022年度は, 新生児侵襲性感染症由来NTHi １株と新生児以外の侵襲性感染症由来NTHi １株を用いて, Drip Flow assay法での形態の評価を行った. 菌株形態の評価方法としてcoverslip上に形成されたバイオフィルムを, Live/Dead染色後およびDRAQ5染色後にconfocal microscopy用いて, 形態観測を行った. 新生児侵襲性感染症由来NTHi産生バイオフィルムは, 一部剥離を認めbiomassも少なかった. 新生児以外の侵襲性感染症由来NTHi産生バイオフィルムは, 培地の循環での影響をほとんど受けておらず, 均一なバイオフィルムが作成され, 表面にextracellular DNAの皮膜を形成しているのが確認できた. 新生児由来株は, バイオフィルム産生

とは異なるメカニズムで侵襲性感染症をきたしていると考えられた．また，これらの結果から，バイオフィルム制御システムとして分泌型ヌクレアーゼが重要である可能性が高く，*HI1296*遺伝子の発現解析を開始している．新生児以外の侵襲性感染症由来株においては，planktonic phaseとバイオフィルム内の*HI1296*遺伝子の発現の比較においては$\log_{10}$2.0以上の差を認めており，バイオフィルム内の環境下で菌が有意に発現している遺伝子であることが確認できた．一方，新生児由来株においてはplanktonic phaseとbiofilm内において*HI1296*の発現に関して，バイオフィルム内で発現を認めていたが，侵襲性感染症由来株ほどの差は認めなかった．

通常quorum-sensing機構は複数のauto-inducerで管理，維持されていることが多い．*HI1296*以外にもauto-inducerの候補を見つけるために，バイオフィルム内とplanktonic phaseにおけるRNA-Seqをさらに継続して行なった．*HI1296*以外にトリプトファン，インドール産生経路の遺伝子群が，バイオフィルム内で高頻度に発現していることを確認したが，なぜトリプトファン，インドール産生経路がバイオフィルム産生能に影響しているのかは不明である．そのためリプトファン，インドール産生経路の遺伝子群である*trpA-E*の遺伝子欠損株をそれぞれ作成し，バイオフィルムの表現系にどのように影響するのか現在検証を行っている．

研究課題　’22－22

Function and secretory mechanism of cyclic peptides produced by wide variety of fungi

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Daisuke Hagiwara
（Faculty of Life and Environmental Sciences, University of Tsukuba）
Takahito Toyotome
（Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine）
Takashi Yaguchi
（Medical Mycology Research Center, Chiba University）
Akira Watanabe
（Medical Mycology Research Center, Chiba University）
Katsuhiko Kamei
（Medical Mycology Research Center, Chiba University）

真菌類が広く多様に産生する生理活性ペプチド群の機能に応じた発現・分泌機構解明

梅村舞子
（産業技術総合研究所生物プロセス研究部門）
萩原大祐
（筑波大学生命環境系）
豊留孝仁
（帯広畜産大学獣医学研究部門）
矢口貴志
（千葉大学真菌医学研究センター）
渡辺　哲
（千葉大学真菌医学研究センター）
亀井克彦
（千葉大学真菌医学研究センター）

研究成果

Kex2-processed repeat proteins（KEP）と呼ばれるタンパク質は，ほぼ全てのカビ・キノコに広く多様に保存されており，接合因子や環状ペプチドの前駆体となる．本KEP因子の糸状菌子嚢果形成への影響を解析するため，千葉大学真菌医学研究センターが保有する糸状菌*Aspergillus nidulans*および*Neosartorya fischeri*それぞれ4株に対してゲノム解析と遺伝子発現解析を行い，*A. nidulans* A4株が有するKEP-n1因子が子嚢果形成に関与する可能性を昨年度までに見出した．本年度，液体培養・固体培養条件下での全遺伝子発現解析を追加して行ったところ，すべての条件・株において*kep-n1*遺伝子の子嚢果形成と相関した発現が観察された．また本因子破壊株では子嚢果が形成されず，マーカー復帰株では形成が確認されたため，本因子の子嚢果形成への関与が明らかになった．KEP-n1因子に由来するペプチド化合物を見出すため，子嚢果形成度の高いA4株の固体培養抽出液を加えて同因子破壊株の培養を行ったが，子嚢果形成の復帰は観察されなかった．ただ，本抽出液では子嚢果破砕を行っていないため，標的ペプチド化合物が子嚢果内に留まり作用した可能性がある．

発表論文

1）Maiko Umemura, Koichi Tamano, “How to improve the production of peptidyl compounds in filamentous fungi”, Frontiers in Fungal Biology, 3:1085624（2022）. 10. 3389/ffunb. 2022. 1085624.

研究課題　’22－23

Construction of a library of eukaryotic parasites aimed at microbial drug discovery

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Takashi Yaguchi
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微生物創薬の効率化を目指した真核生物寄生菌類ライブラリーの構築

野中健一
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矢口貴志
（千葉大学真菌医学研究センター）

研究成果

真核生物に寄生する菌類は，宿主生物に感染する過程で直接宿主に作用する低分子化合物を産生する．そのため，感染症病原体に近縁な宿主に寄生する菌類からは効率的に病原体に作用する化合物の発見が期待される．本申請では，微生物創薬の効率化を目指した真核生物寄生菌類ライブラリーの構築を行うことを目的とした．

伊豆諸島八丈島および東京都内（八王子市など），神奈川県内（横浜市など）で４種の昆虫（冬虫夏草類２種を含む），10株の担子菌類および12種の変形菌類を採集し，これらを菌類の探索源とした．希釈平板法，直接分離法などでこれらから菌類の分離を行なった結果，４種の昆虫から22株，10株の担子菌類から96株，12種の変形菌類から87株の計205株の菌類を分離した．全ての分離株について形態的特徴およびITS配列に基づき同定を行なったところ，昆虫由来株22株は９属14種，担子菌類由来株96株は28属48種，変形菌類由来株87株は36属75種に同定された．これら分離株を４種の化合物生産用培地で培養液サンプルを調製し，抗細菌・抗真菌（ヒト病原菌・植物病原菌）・殺虫などの生物活性評価を行なった．特に知見の少ない変形菌類由来株は植物病原菌に対して抗菌活性を示す割合が高い傾向にあることが明らかとなった．構築した菌類ライブラリーに分類情報，生物活性情報などを付与することで，今後の微生物創薬の効率化が図れることが期待される．

研究課題　’22－24

Characterization and ecological survey of phomopsin-producing fungi

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Sayaka Ban
（Medical Mycology Research Center, Chiba University）
Maiko Watanabe
（National Institute of Health Sciences）
Haruo Takahashi
（National Institute of Health Sciences）
Kazuhiro Hashimoto
（National Hospital Organization Sagamihara National Hospital）
Hiroyuki Nakagawa
（National Agriculture and Food Research Organization）
Takahito Toyotome
（Obihiro University）

カビ毒ホモプシン産生菌の機能解析および生態学的研究

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（東京理科大学理工学部）
矢口貴志
（千葉大学真菌医学研究センター）
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髙橋治男
（国立医薬品食品衛生研究所）
橋本一浩
（国立病院機構相模原病院）
中川博之
（農業・食品産業技術総合研究機構）
豊留孝仁
（帯広畜産大学）

研究成果

ホモプシン類は，真菌Diaporthe toxicaの代謝産物として単離・構造決定されたカビ毒で，産生菌の着生したマメ科植物を家畜が摂取すると肝障害を引き起こすことが知られている．欧州食品安全機関（EFSA）の報告によると，「ヒト及び家畜のホモプシン類への暴露量を可能な限り低く抑えることが望ましい」とされており，近年その安全性が注目されている．しかしながら，ホモプシン類産生菌の国内における分布はほとんど調査されていない．そこで本研究では，国内のマメ科植物を中心にホモプシン類産生菌の存在を調査することを目的としている．昨年度までの研究において，微生物保存機関に登録されている真菌についてホモプシン類産生能を調査したところ，Beauveria bassianaがホモプシン類合成遺伝子を保持していること，およびホモプシン類を産生することが示唆された．本年度は，B. bassianaの培養液中の代謝産物成分を固相抽出カラムにより濃縮し，高分解能質量分析装置を利用して当該化合物の構造決定を試みた．その結果，負イオン検出条件下において検出されたイオン質量に基づく推定組成式より，新規ホモプシン類を産生している可能性が考えられた．しかし，濃縮サンプルに含まれる夾雑物などの影響からフラグメントイオンの特定や構成アミノ酸に繋がる情報は得られなかったため，より詳細な検討が必要である．

感染症グローバルネットワークフォーラム2023

10th Global Network Forum on Infection and Immunity

共催：千葉大学真菌医学研究センター共同利用・共同研究拠点「真菌感染症研究拠点」

【Poster Session】
日時：令和５年２月２日（金）13時30分～15時50分
場所：千葉大学医学系総合研究棟　３階　第２講義室

【Oral Presentation】
日時：令和５年２月３日（土）　９時20分～16時10分
場所：千葉大学医薬系総合研究棟Ⅱ 地下１階 大会議室

組織委員長
澁谷和俊（東邦大学　医学部）

組織委員
宮﨑義継　（国立感染症研究所）
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）
知花博治　（千葉大学真菌医学研究センター）
矢口貴志　（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）
後藤義幸　（千葉大学真菌医学研究センター）
高橋弘喜　（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）

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，2023年度で第10回目を迎えることとなった．本年度の国際フォーラムは，東邦大学の澁谷和俊博士が組織委員長となり，「病原体と宿主との相互作用」をテーマとし，現在世界的に注目されている感染症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から細菌，抗酸菌，真菌と，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．

初日はポスター〇〇題の発表が，二日目は招待講演９題（日本・ドイツ・ブラジル・アメリカ・香港・韓国）の発表が行われた．二日間で延べｘｘｘ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】
澁谷和俊（東邦大学　医学部）

【午前の講演】
午前の講演：Morning Session

座長：高橋弘喜（千葉大学真菌医学研究センター）

1．**Joshua J. Obar（Geisei School of Medicine, USA）**
“Mda5/MAVS signaling going non-viral or not?”

2．**Gustavo H. Goldman（University of Sao Paulo, Brazil）**
“How *Aspergillus fumigatus* protects itself when producing gliotoxin”

3．**Hiroki Takahashi（MMRC, Chiba University, Japan）**
“Genomic diversity of pathogenic fungus Aspergillus fumigatus in Japan reveals the complex genomic basis of azole resistance”

【午後の講演】
午後の講演：Afternoon Session

座長：渡邉　哲（千葉大学真菌医学研究センター）

4．**Shigo Takatsuka（NIID, Japan）**
“A study of host response in post-influenza *Aspergillus* superinfection”

5．**Yasunori Miyazaki（Tokyo Medical and Dental University）**
“Allergic pneumonia due to bacteria and fungi – hypersensitivity pneumonitis -”

6．**Kiyoshi Hirahara（Chiba University）**

“Pathological tissue inflammatory memories shape the intractable pathology of chronic lung infllamation”

7．**Hein M Tun（The Chinese University of Hong Kong）**
“Gut microbiome – keystone modifier of infectious immunity and opportunities for therapy”

座長：米山光俊（千葉大学真菌医学研究センター）

8．**Eun-Kyeong Jo（Chungnam National University, Korea）**
“Autophagy and host defense against mycobacgteria”

9．**Hiroki Kato（Univeristy of Bonn, Germany）**
“MTr1 inhibition as a novel approach to tackle influenza virus”

【閉会の挨拶】
宮﨑義継（国立感染症研究所）

2023 Scientific Meetings & Seminars

2023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５年４月19日（火）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：真菌医学研究センター　臨床感染症分野
Alexandra Elizabeth Moskaluk
「Molecular characterization of feline-associated dermatophytosis」

【第２回】
※イスラエル国情不安定により，開催中止

【第３回】
日時：令和６年３月５日（火）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：真菌医学研究センター　臨床感染症分野
馬嶋秀考　特任助教
「脂質異常症治療薬スタチンが有する抗真菌作用の探求－カイコ感染モデルを用いた検証－」

「2023千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５年６月20日（火）
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）

【第２回】
日時：令和５年12月８日（金）
場所：山崎製パン企業年金基金会館（千葉県市川市市川1-3-14）
（講演１）
矢口貴志（千葉大学真菌医学研究センター准教授）
「生活環境中のカビとその対策」
（講演２）
渡邉　哲（千葉大学真菌医学研究センター准教授）
「カビがひきおこすさまざまな病気」
（講演３）
後藤義幸（千葉大学真菌医学研究センター准教授）
「腸内細菌のお話」

【第３回】
日時：令和６年３月１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）
米山光俊（真菌医学研究センター 教授）
「ウイルスと免疫」
（講演２）
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」
（講演３）
渡邉　哲（真菌医学研究センター 准教授）
「高齢者が気をつけるべき肺の病気と感染症」

「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　2023年度成果報告会」
日時：令和６年３月26日（火）～３月28日（木）
場所：オンライン開催

令和６年３月27日（水）
【特別講演】
澤　洋文（北海道大学創成研究機構ワクチン研究開発拠点拠点長）

【合同成果報告会（千葉大学真菌医学研究センター）】
鈴木純子（国立病院機構東京病院）
「Aspergillus呼吸器検体臨床分離株の菌種同定・薬剤感受性の検討」

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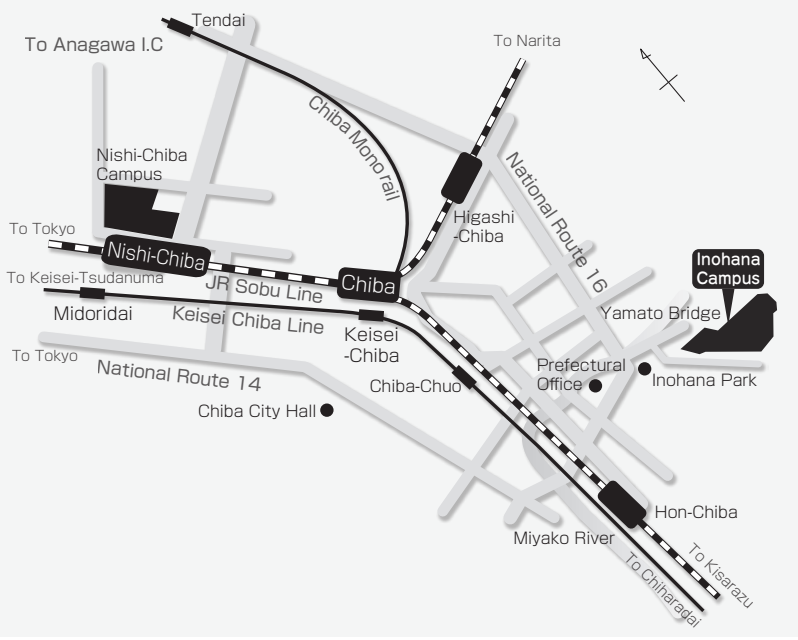

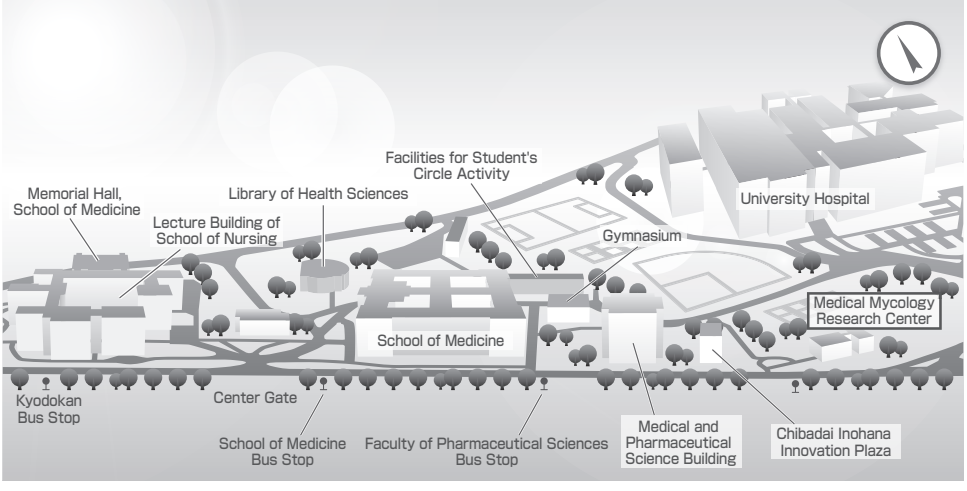

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