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千葉大学　真菌医学研究センター　報告

26

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Content
目　　次

Preface for FY 2022 Annual Report
はじめに

Organization
機構図

Project for Immune Response in Infections Diseases
感染免疫分野 米山教授 感染応答プロジェクト ---- 4

Project for Cytokine Research
感染免疫分野 西城准教授 サイトカインプロジェクト ---- 6

Project for Host-Microbial Interactions in Symbiosis and Pathogenesis
感染免疫分野 後藤准教授 微生物・免疫制御プロジェクト ---- 9

Project for Control of Infectious Diseases
感染免疫分野 高屋准教授 感染症制御開発プロジェクト ---- 11

Candida glabrata Phenome Project
病原機能分野 知花准教授 カンジダ・グラブラータフェノームプロジェクト ---- 13

Project of Clinical Investigation
臨床感染症分野 渡邉准教授 臨床感染症プロジェクト ---- 16

Project for Infection Control and Prevention
感染症制御分野 石和田教授 感染症制御プロジェクト ---- 21

Project for Systems Biology of Microorganisms
微生物資源分野 高橋准教授 微生物創生プロジェクト ---- 26

Management of Unit of Microbiological Resources
微生物資源分野 矢口室長 バイオリソース管理室 ---- 28

Project for RNA Regulation
RNA制御治療学共同研究部門 原口特任准教授 RNA制御プロジェクト ---- 34

Merged project of respiratory pathophysiology and pathobiology
呼吸器生体制御学研究部門 巽特任教授・呼吸器生体制御学共同研究部門
寺田特任教授 呼吸器生体制御解析プロジェクト ---- 36

Project for Evolution and Reproduction
進化生殖学寄附研究部門 生水特任教授 進化生殖プロジェクト ---- 42

Ministry of Education, Culture, Sports, Science and Technology
National BioResource Project “Pathogenic Microorganisms”
文部科学省 ナショナルバイオリソースプロジェクト「病原微生物」---- 45

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」 47

The project for prophylaxis, diagnosis, and treatment for aspergillosis and the other mycoses in aged and neonate patients
高齢者・新生児アスペルギルス症制圧へ向けた予防・診断・治療開発プロジェクト 49

AMED/JICA Science and Technology Research Partnership for Sustainable Development（SATREPS）
"The establishment of a research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones in Brazil and Japan"
AMED/JICA 地球規模課題対応国際科学技術協力プログラム（SATREPS）
「ブラジルと日本の薬剤耐性を含む真菌感染症診断に関する研究とリファレンス協力体制強化プロジェクト」 51

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」 53

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"
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） 54

Research Institute of Disaster Medicine
災害治療学研究所 55

The training course of pathogenic fungi
真菌医学研究センター病原真菌講習会 56

miRaX Therapeutics K.K.
ミラックスセラピューティクス株式会社 57

2021 Fiscal Year Cooperative Research Program Report
令和３年度 共同利用・共同研究報告 58

The 9th Global Network Forum on Infection and Immunity
感染症研究グローバルネットワークフォーラム2022 77

2022 Scientific Meetings & Seminars
2022年講演会 79

Preface for FY 2022 Annual Report

As the Japanese population rapidly ages, the risks of serious opportunistic fungal infections are increasing, particularly among patients with chronic obstructive pulmonary disease (COPD), hematopoietic malignancies, and other immunocompromising chronic conditions. Moreover, 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.

In FY 2021, the Medical Mycology Research Center (MMRC) was granted extended certification as the National Joint Usage/Research Center for Fungal Infection by the Minister 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 at the MMRC pursue independent basic, applied, and clinical research projects. The MMRC has been supporting clinical practice at two outpatient clinics of Chiba University Hospital that specialize in invasive fungal infections (one started in 2014 and the other in 2016).

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 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 2022, leveraging its international cooperative network.

The MMRC will continue to be a pioneer in antifungal medicine and infection prevention research, emphasizing the following areas of activity: (i) initiatives as a joint usage/research center and bioresource center, (ii) fundamental research on infections and immunity, (iii) clinical research on infection prevention and control, and (iv) support for junior researchers.

January, 2023

Chihiro Sasakawa, PhD,
Director of the MMRC

はじめに

我が国はすでに超高齢社会にあり，慢性閉塞性肺疾患（COPD）等の呼吸器病や造血器悪性腫瘍，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が益々大きな脅威となっています．一方で経済のグローバル化に伴う輸入真菌症に加え，コロナ禍においてはCOVID-19感染症患者に合併する肺アスペルギルス症や致死性のムコール症も国際的な新たな脅威となっています．

このような背景で，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・医療活動を積極的に推進しています．また本センターでは，文部科学省のナショナルバイオリソースプロジェクト（NBRP）として，病原真菌や放線菌の収集・保存・ゲノム情報解析・分与等の活動を行なっています．一方で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・臨床研究を推進し，さらに2014年および2016年以来，臨床系の２分野が付属病院において感染症に関連する専門外来を開設しました．また2015年には本学初のBSL-3施設を整備し，2018年には無菌動物施設も立ち上げました．一方，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．さらに2021年に設立された「災害治療学研究所」にも参画し，大規模災害やコロナパンデミックに随伴する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積極的に活用して，2022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．

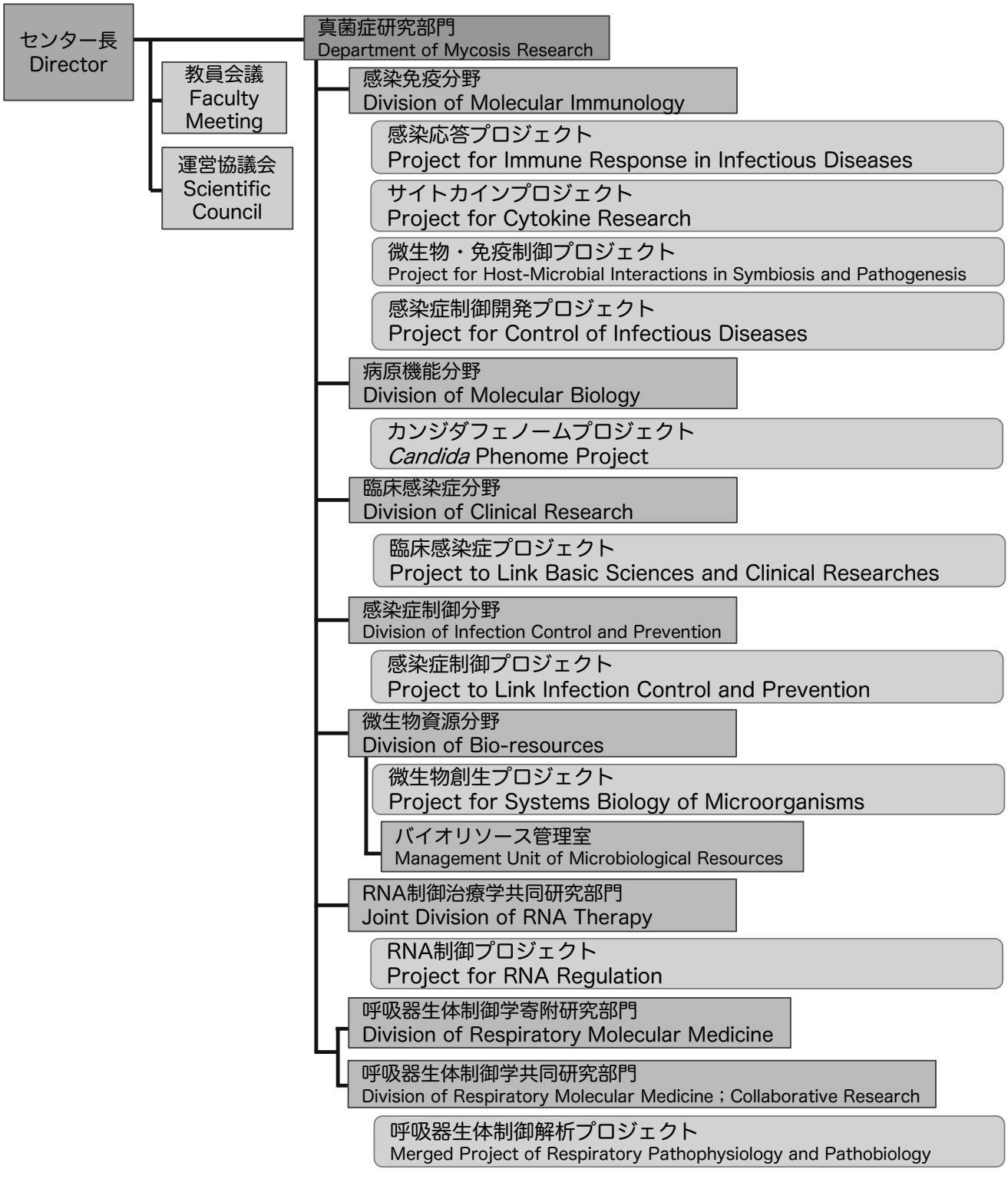
以上のように，本センターは，「共同利用・共同研究拠点及びバイオリソース中核拠点」，「感染症・免疫基盤研究」，「感染症臨床研究」，「若手育成」の４つを柱として，今後も真菌医学および感染症研究の発展に先導的な役割を果たす所存でございます．

2023年１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	技 術 職 員	青木　友那
Research Promotion Technician	Miyuki Takizawa	技 術 補 佐 員	滝沢みゆき
Visiting Researcher	Yoshihiko Muromaki（2022.10.1～）	外部機関共同研究員	室巻　良彦

1.　Inactivation Effects of Iodine on SARS-CoV-2

Onomoto K[1], Yoneyama M[1], Asakura S[2, 5], Matsumoto S[3], and Kaiho T[4, 5]

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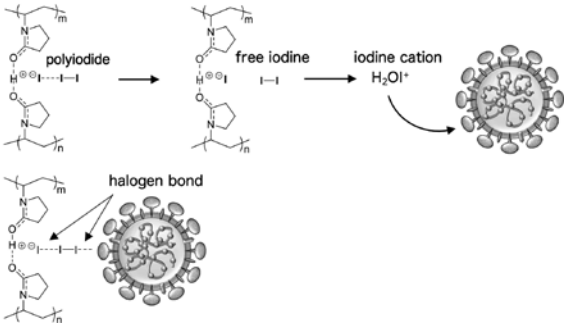
[4] Godo Shigen Co., LTD., 2-12-6, Kyobashi, Chuo-ku, Tokyo 104-0031, Japan

[5] Chiba Iodine Resource Innovation Center, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Iodine-based disinfectants have recently been reported to have an inactivating effect on severe acute respiratory syndrome coronavirus-2（SARS-CoV-2）. However, there are still many unclear points concerning their inactivation abilities since iodine exists in various forms. In this study, we examine the inactivating effect on SARS-CoV-2 of different forms of iodine, including free iodine, iodide ion, and polyiodide ion, such as triiodide ion and povidone-iodine, which are the main constituents of iodine-based disinfectants. The results indicate that although iodide ion is not involved in the inactivation of SARS-CoV-2, iodine complex and polyiodide significantly contribute to the inactivation, in addition to free iodine, which was known as the main component of disinfection activity.

<Povidone iodine>



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

Onomoto K, Aoki Y, Ban M, Sakai M, Suzuki Y, Luo J, 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 biosafety level 3 (BSL3) facility of MMRC.

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

Aoki Y, 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. 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.

Publications

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2) Onomoto K, Yoneyama M, Asakura S, Matsumoto S, Kaiho T. Inactivation effects of iodine on SARS-CoV-2. J Antibacterial Antifungal Agents. 50: 97-103, 2022.

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	特　任　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. Epidermal clearance of *C. albicans* is mediated by IL-17 but independent of fungal innate immune receptors

Iwasawa MT[1], Miyachi H1, Wakabayashi S[1], Sugihira T[2], Aoyama R[2], Nakagawa S[1], Katayama Y[1], Yoneyama M[3], Hara H[4], Iwakura Y[3, 5, 6], Matsumoto M[7, 8], Inohara N[7, 8], Koguchi-Yoshioka H[2], Fujimoto M[2, 9], Gabriel Núñez G[7, 8],

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IL-17 plays important roles in host defense against *Candida albicans* at barrier surfaces and during invasive infection. However, the role of IL-17 in host defense after colonization of the epidermis, a main site of *C. albicans* infection, remains poorly understood. Using a murine model of epicutaneous candidiasis without skin abrasion, we found that skin inflammation triggered by epidermal *C. albicans* colonization was self-limiting with fungal clearance completed by day 7 after inoculation in wild-type mice or animals deficient in IL-17A or IL-17F. In contrast, marked neutrophilic inflammation in the epidermis and impaired fungal clearance were observed in mice lacking both IL-17A and IL-17F. Clearance of *C. albicans* was independent of Dectin-1, Dectin-2, CARD9 (caspase-recruitment domain family, member 9), TLR2 (Toll-like receptor 2) and MyD88 in the epidermal colonization model. We found that group 3 innate lymphoid cells (ILC3s) and γδT cells were the major IL-17 producers in the epicutaneous candidiasis model. Analyses of $Rag2^{-/-}$ mice and $Rag2^{-/-}Il2rg^{-/-}$ mice revealed that production of IL-17A and IL-17F by ILC3s was sufficient for *C. albicans* clearance. Finally, we found that depletion of neutrophils impaired *C. albicans* clearance in the epidermal colonization model. Taken together, these findings indicate a critical and redundant function of IL-17A and IL-17F produced by ILC3s in host defense against *C. albicans* in the epidermis. The results also suggest that epidermal *C. albicans* clearance is independent of innate immune receptors or that these receptors act redundantly in fungal recognition and clearance.

3. 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]

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[5] Department of Cell Biology, University of Miami, Miller School of Medicine, Miami, Florida, USA

Aspergillus fumigatus (*A. 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.

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2）Makusheva Y, Chung SH, Akitsu A, Maeda N, Maruhashi T, Ye XQ, Kaifu T, Saijo S, Sun H, Han W, Tang C, Iwakura Y. The C-type lectin receptor Clec1A plays an important role in the development of experimental autoimmune encephalomyelitis by enhancing antigen presenting ability of dendritic cells and inducing inflammatory cytokine IL-17. Exp Anim. 71(3): 288-304. 2022.

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
Graduate student	Akira Haku	大学院生	白　　旭
Graduate student	Qiongyuan Zhang	大学院生	張　瓊元
Graduate student	Yuni Sun	大学院生	孫　宇霓
Research Promotion Technician	Sawako Domae	技術補佐員	堂前　清香
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1. Commensal bacteria and host immune system regulate fungal colonization in the gut

Akira Haku, Yoshiyuki Goto

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.

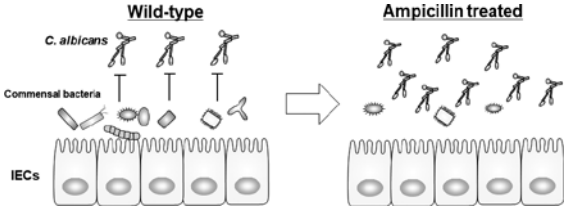

Fig 1. Commensal bacteria prevent the colonization of *C. albicans* in the gut

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

Yoshiyuki Goto

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.

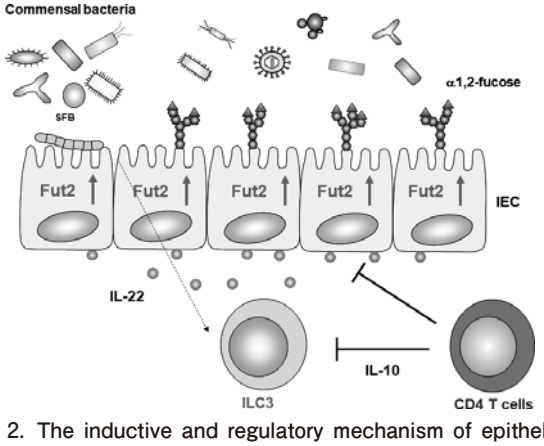

Fig 2. The inductive and regulatory mechanism of epithelial α1, 2-fucose

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2) Goto Y. Unique symbiont-derived sphingolipids: Dietary amino acids source branch formation. Cell Host Microbe. 30: 3-5, 2022

Project for Control of Infectious Diseases

高屋（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 fiscal year, we searched the natural product library for compounds that can reduce persisters and found several candidate compounds.

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．本年度は天然物ライブラリーから細菌休眠状態を制御できる化合物を探索し，複数の候補化合物を見出した．

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. Molecular mechanism of Physalin H to suppress the Agr-quorum sensing system of methicillin-resistant *Staphylococcus aureus*

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

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

An infection caused by methicillin-resistant *Staphylococcus aureus*（MRSA）is a global health problem that is difficult to treat because of antibiotic resistance. One major virulence modulator of *S. aureus* is the accessory gene regulator（Agr）-quorum sensing（QS）system that coordinates cell behavior in response to bacterial density. Inhibition of the Agr-QS system possibly reduces MRSA pathogenesis by decreasing virulence. This study aims to identify the small molecules that reduce the expression of the Agr-QS system from our natural product library. Several products were obtained as candidates from a screening of the natural product library using a strain with a reporter gene that depended on the Agr-QS system. Among them, physalin H, isolated from the *Physalis minima*（Eggplant）, suppressed the Agr-QS system in the type *agr* I strain and in the different type *agr* III strain. AgrA is a transcriptional regulator to bind the P3 promoter and has highly conserved sequences among all types of agr. The electrophoretic mobility-shift assay revealed that purified C-terminal AgrA protein could bind to the DNA fragment of the P3 promoter. In contrast, its binding was abolished when physalin H was added together. These results suggest that physalin H suppresses the Agr-QS system by preventing the

interaction of AgrA with the P3 promoter.

2. New polyoxygenated cyclohexenes isolated from *Uvaria rufa* and cinnamtannin B1 isolated from *Nephelium hypoleucum* with TRAIL-resistance-overcoming activity

Kritamorn Jitrangsri[1, 2], Yasumasa Hara[2], Akiko Takaya[2], Masami Ishibashi[2]

[1] Faculty of Pharmacy, Silpakorn University, Thailand
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Bioactivity-guided isolation of *Uvaria rufa* leaves and *Nephelium hypoleucum* bark resulted in the successful isolation of nine compounds (1-9) bearing a new polyoxygenated cyclohexene scaffold: zeylenol-6-shikimate (1), (-)-zeylenol (2), microcarpin A (3), uvarigranol F (4), quercetin (5), kaempferol (6), and p-coumaric acid (7) from *U. rufa*, and epicatechin (8) and cinnamtannin B1 (9) from *N. hypoleucum*. The structures of the isolated compounds were elucidated using various spectroscopic techniques. All compounds, except for 7, exhibited weak-to-strong TRAIL-resistance-overcoming activity and an increased gastric cancer cell line (AGS) inhibition by 17-32% as compared to the treatment with the compounds alone. Compounds 3 and 9 were studied for their ability to overcome TRAIL resistance using western blot analysis, which indicated that they sensitized AGS cells to apoptosis via both extrinsic and intrinsic pathways by increasing the expression of several proapoptotic proteins (cleaved caspase −3, −8, and −9) and by decreasing the expression of anti-apoptotic protein Bcl-2.

Publications

1) Kritamorn J, Hara Y, Takaya A, Ishibashi M. New polyoxygenated cyclohexenes isolated from *Uvaria rufa* and cinnamtannin B1 isolated from *Nephelium hypoleucum* with TRAIL-resistance-overcoming activity. *Phytochemistry Letters* 52: 7-9. 2021.
2) Hara Y, Watanabe K, Takaya A, Ebihara I, Manome T, Arai M, Yaguchi T, Ishibashi M. Two bioactive compounds, Uniformides A and B, isolated from a culture of *Nocardia uniformis* IFM0856T in the presence of animal Cells. *Organic Letters* 24: 5867-5867 2022.

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

Summary（研究概要）

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.

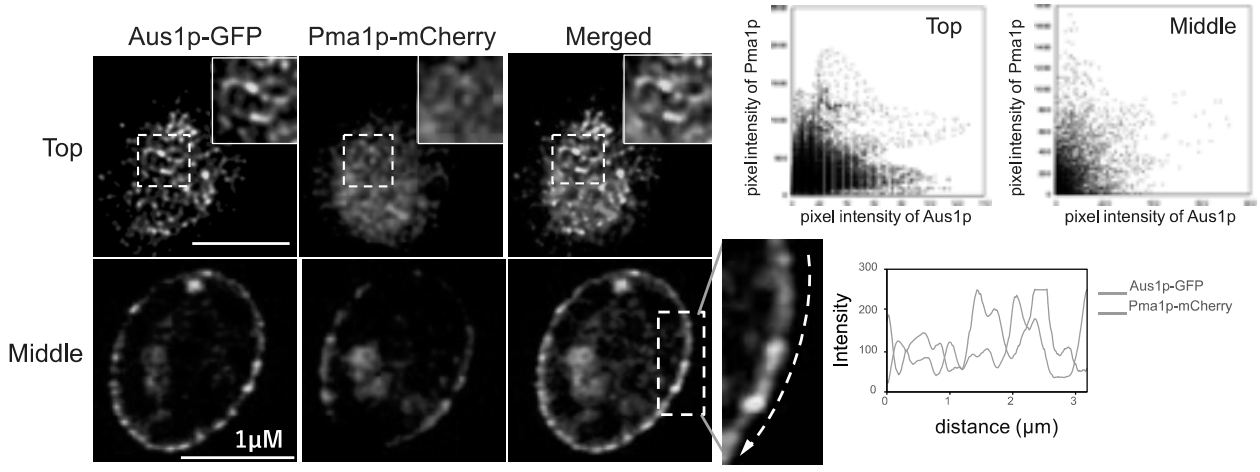
病原性酵母カンジダ・グラブラータの全遺伝子改変株を利用し，抗真菌薬の開発ならびに病原性に関する遺伝子の特定と機能解析を進めている．

Associate Professor	Hiroji Chibana	准　教　授	知花　博治
Research Technician	Azusa Takahashi	技 術 職 員	高橋　　梓
Research Assistant Professor	Michiyo Sato	特 任 助 教	佐藤（岡本）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

Erg25 Controls Host-Cholesterol Uptake Mediated by Aus1p-Associated Sterol-Rich Membrane Domains in *Candida glabrata*

Michiyo Okamoto, Azusa Takahashi-Nakaguchi, Kengo Tejima, Kaname Sasamoto, Masashi Yamaguchi, Toshihiro Aoyama, Minoru Nagi, Kohichi Tanabe, Yoshitsugu Miyazaki, Hironobu Nakayama, Chihiro Sasakawa, Susumu Kajiwara, Alistair J P Brown, Miguel C Teixeira, Hiroji Chibana

The uptake of cholesterol from the host is closely linked to the proliferation of pathogenic fungi and protozoa during infection. For some pathogenic fungi, cholesterol uptake is an important strategy for decreasing susceptibility to antifungals that inhibit ergosterol biosynthesis. In this study, we show that *Candida glabrata ERG25*, which encodes an enzyme that demethylates 4, 4-dimethylzymosterol, is required for cholesterol uptake from host serum. Based on the screening of *C. glabrata* conditional knockdown mutants for each gene involved in ergosterol biosynthesis, *ERG25* knockdown was found to decrease lethality of infected mice. ERG25 knockdown impairs the plasma membrane localization of the sterol importer Aus1p, suggesting that the accumulated 4, 4-dimethylzymosterol destabilizes the lipid domain with which Aus1p functionally associates. *ERG25* knockdown further influences the structure of the membrane compartment of Can1p (MCC)/eisosomes (ergosterol-rich lipid domains), but not the localization of the membrane proteins Pma1p and Hxt1p, which localize to sterol-poor domains. In the sterol-rich lipid domain, Aus1p-contining domain was mostly independent of MCC/eisosomes, and the nature of these domains was also different: Ausp1-contining domain was a dynamic network-like domain, whereas the MCC/eisosomes was a static dot-like domain. However, deletion of MCC/eisosomes was observed to influence the localization of Aus1p after Aus1p was transported from the endoplasmic reticulum (ER) through the Golgi apparatus to the plasma membrane. These findings suggest that *ERG25* plays a key role in stabilizing sterol-rich lipid domains, constituting a promising candidate target for antifungal therapy.



WT_Aus1G/Pma1R cells were observed at the cell surface (Top) and transverse region (Middle) using a high-resolution confocal fluorescence microscope in real time. Each area enclosed by the dashed lines also is provided as a magnified image. In the magnified image of the transverse region, intensity profiling of GFP (green) and mCherry (magenta) on the plasma membrane was carried out in the direction shown by the arrow. Scatterplots of green and magenta pixel intensities in each panel were indicated.

Ultrastructural Examination of Mouse Glomerular Capillary Loop by Sandwich Freezing and Freeze-Substitution.

Masashi Yamaguchi, Azusa Takahashi-Nakaguchi, Katsuyuki Uematsu, Hiroyuki Yamada, Michiyo Sato-Okamoto, Hiroji Chibana

Sandwich freezing is a method of rapid freezing by sandwiching specimens between two metal disks and has been used for observing exquisite the close-to-native ultrastructure of living yeast and bacteria. Recently, this method has been found to be useful for preserving cell images of glutaraldehyde-fixed animal and human tissues. In the present study, this method was applied to observe the fine structure of mouse glomerular capillary loops. Morphometry was then performed, and the results were compared with the data obtained by an in vivo cryotechnique, which may provide the closest ultrastructure to the native state of living tissue. The results show that the ultrastructure of glomerular capillary loops obtained by sandwich freezing-freeze-substitution after glutaraldehyde fixation was close to that of the ultrastructure obtained by in vivo cryotechnique not only in the quality of cell image but also in quantitative morphometry. They indicate that the ultrastructure obtained by sandwich freezing-freeze-substitution after glutaraldehyde fixation may more closely reflect the living state of cells and tissues than conventional chemical fixation and dehydration at room temperature and conventional rapid freezing-freeze-substitution of excised tissues without glutaraldehyde fixation. Sandwich freezing-freeze-substitution techniques should be used routinely as a standard method for observing the close-to-native ultrastructure of biological specimens.

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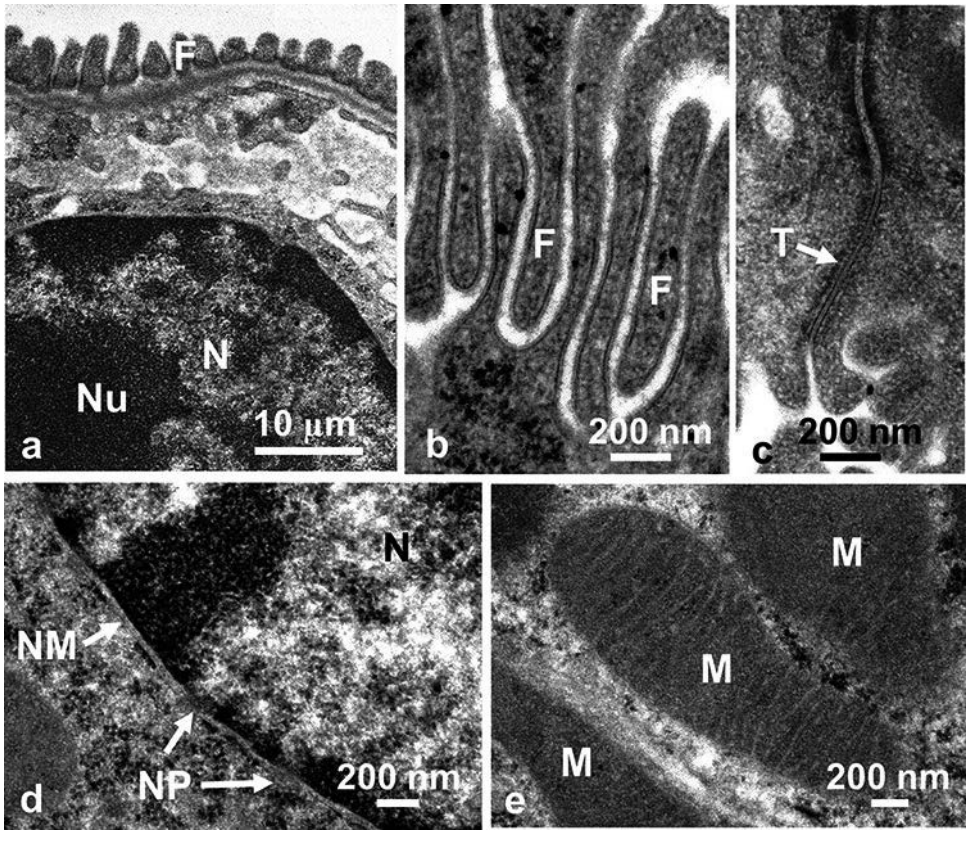
3）Okamoto M, Takahashi-Nakaguchi A, Tejima K, Sasamoto K, Yamaguchi M, Aoyama T, Nagi M, Tanabe K, Miyazaki Y, Nakayama H, Sasakawa C, Kajiwara S, Brown AJP, Teixeira MC, Chibana H:



Ultrathin sections of mouse kidney prepared by sandwich freezing and freeze-substitution after glutaraldehyde fixation. (a) Nucleus and nucleolus of endothelial cell and foot processes of glomerular capillary loop. (b) Tangential section of foot processes of glomerular capillary loop. (c) Tight junction. (d) Nuclear membrane and nuclear pores. (e) Mitochondria. F, foot process; M, mitochondria; N, nucleus; NM, nuclear membrane; NP, nuclear pore; Nu, nucleolus; T, tight junction.

Erg25 Controls Host-Cholesterol Uptake Mediated by Aus1p-Associated Sterol-Rich Membrane Domains in *Candida glabrata*. Front Cell Dev Biol, 24; 10: 820675, 2022.

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Project of Clinical Investigation

渡邉ＰＩ（臨床感染症）プロジェクト

Summary（研究概要）

We have been doing basic and clinical research primarily on fungal infections while seeing patients in the Specialty Clinic for Fungal Infections at the University Hospital. Working as the Reference Center for fungal infections, 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. 200 cases in 2022). Concerning research activities, we are investigating various aspects of systemc mycoses with many universities, hospitals, and medical institutions such as NIID. The main research topics are: the mechanisms and the epidemiology of antifungal resistance of *Candida* species and *Aspergillus* species, the development of their diagnostic methods, and establishment of a consensus on treatment outcome definitions of Chronic pulmonary aspergillosis.

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. 2022年の全国の医療機関からの真菌症治療相談依頼件数は200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, 慶應大学病院など国内のさまざまな研究機関, 医療施設と協力して臨床・基礎研究を行っており, 難治性真菌症の感染機構や診断・治療法の開発研究を進めている. 中でもカンジダ症およびアスペルギルス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, 診断法についての研究を進め, 多くの画期的な論文を発表するなど高い成果を挙げた. また, 慢性肺アスペルギルス症の治療効果判定についての国際的コンセンサスのとりまとめをおこなった.

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.

Professor	Katsuhiko Kamei（～2022.3.31）	教　　授	亀井　克彦
Associate Professor	Akira Watanabe	准　教　授	渡辺　　哲
Research Assistant Professor	Teppei Arai	特任助教	新居　鉄平
Research Assistant Professor	Hazim O. Abdelgalil Khalifa（～2022.3.31）	特任助教	ハジム O. A カリファ
Research Assistant Professor	Hidetaka Majima（2022.4.1～）	特任助教	馬嶋　秀考
Researcher	Akio Toh-e（～2022.3.31）	研　究　員	東江　昭夫
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	Rio Seki（～2022.3.31）	技術補佐員	関　　里亜
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（～2022.3.31, 2022.10.1～）	技術補佐員	井上　京子

1. Azole and echinocandin resistance mechanisms and genotyping of *Candida tropicalis* in Japan: cross-boundary dissemination and animal-human transmission of *C. tropicalis* infection.

Khalifa HO, Watanabe A, Kamei K

Objectives: To assess the prevalence and genetic basis of antifungal resistance mechanisms as well as the genotyping of *Candida tropicalis* from clinical and non-clinical sources in Japan.
Methods: Eighty *C. tropicalis* isolates, including 32 clinical isolates recovered from 29 patients and 48 non-clinical isolates recovered from 24 different sources (animals and the environment) were evaluated. All isolates were tested phenotypically for resistance to a wide range of antifungals and genotypically for resistance mechanisms to azole and echinocandin. Furthermore, all the isolates were genotyped by multilocus sequence typing (MLST).
Results: Phenotypically, 30.2% (16/53) of the isolates were azole-resistant, with high levels of azole resistance among clinical isolates (51.7%; 15/29) and low levels (4.2%; 1/24) among non-clinical isolates. None of the isolates were reported as echinocandin resistant, with 60.4% (32/53) of the isolates intermediate to caspofungin. Azole resistance was basically attributed to high expression levels of drug efflux transporter genes (CDR2 and CDR3), transcription factors (TAC1 and UPC2) and ergosterol biosynthesis pathway HMG gene. No FKS1 hot spot 1 (HS1) or HS2 missense mutations were detected in any of the isolates. MLST analysis revealed 36 different sequence types (STs), with the first identification of 23 new STs. Phylogenetic analysis confirmed the close relationship between the clinical and non-clinical isolates, with identifications of ST232 and ST933 among patients and marine mammals.
Conclusion: Our results confirmed the emergence of azole resistance in *C. tropicalis* in Japan. Furthermore, phylogenetic analysis confirmed the transboundary dissemination and cross-transmission of *C. tropicalis* between humans and animals.

2. Prevalence of Antifungal Resistance, Genetic Basis of Acquired Azole and Echinocandin Resistance, and Genotyping of *Candid krusei* recovered from an International Collection.

Khalifa HO, Hubka V, Watanabe A, Nagi M, Miyazaki Y, Yaguchi T, Kamei K

This study was designed to evaluate the prevalence of antifungal resistance, genetic mechanisms associated with in vitro induction of azole and echinocandin resistance and genotyping of *Candida krusei*, which is intrinsically resistant to fluconazole and is recovered from clinical and nonclinical sources from different countries. Our results indicated that all the isolates were susceptible or had the wild phenotype (WT) to azoles, amphotericin B, and only 1.27% showed non-WT for flucytosine. Although 70.88% of the isolates were resistant to caspofungin, none of them were categorized as echinocandin-resistant as all were susceptible to micafungin and no FKS1 hot spot 1 (HS1) or HS2 mutations were detected. In vitro induction of azole and echinocandin resistance confirmed the rapid development of resistance at low concentrations of fluconazole (4 μg/ml), voriconazole (0.06 μg/ml), and micafungin (0.03 μg/ml), with no difference between clinical and nonclinical isolates in the resistance development. Overexpression of ABC1 gene and FKS1 HS1 mutations were the major mechanisms responsible for azole and echinocandin resistance, respectively. Genotyping of our 79 isolates coupled with 217 other isolates from different sources and geography confirmed that the

isolates belong to two main subpopulations, with isolates from human clinical material and Asia being more predominant in cluster 1, and environmental and animals isolates and those from Europe in cluster 2. Our results are of critical concern, since realizing that the *C. krusei* resistance mechanisms and their genotyping are crucial for guiding specific therapy and for exploring the potential infection source.

3. Non-*fumigatus Aspergillus* Infection Associated with a Negative *Aspergillus* Precipitin Test in Patients with Chronic Pulmonary Aspergillosis.

Takeda K, Suzuki J, Watanabe A, Narumoto O, Kawashima M, Sasaki Y, Nagai H, kamei K, Matsui H

Aspergillus antibody testing is key for the clinical diagnosis of chronic pulmonary aspergillosis (CPA) with high sensitivity. However, false-negative results in patients with CPA might be obtained, depending on the *Aspergillus* species. The aim of this study was to investigate which factors are associated with false-negative results in *Aspergillus* precipitin tests and whether the sensitivity of precipitin tests in CPA is influenced by *Aspergillus fumigatus* and non-*fumigatus Aspergillus* species. Between February 2012 and December 2020, 116 consecutive antifungal treatment-naive patients with CPA were identified and included in this retrospective chart review. *Aspergillus* species isolated from the respiratory tract of patients were identified by DNA sequencing. Characteristics of patients with positive and negative results for *Aspergillus* precipitin tests were compared. The sensitivity of the *Aspergillus* precipitin tests was compared between patients with *A. fumigatus*-associated CPA and non-*fumigatus Aspergillus*-associated CPA. A non-*fumigatus Aspergillus* species was the only factor significantly associated with negative *Aspergillus* precipitin test results in patients with CPA in the multivariate analysis (hazard ratio, 8.3; 95% confidence interval, 3.2 to 22.1; $P < 0.0001$). The positivity of the *Aspergillus* precipitin test for patients with non-*fumigatus Aspergillus*-associated CPA was lower than that for patients with *A. fumigatus*-associated CPA (84.8% versus 37.9%; $P < 0.0001$). These results revealed that the presence of non-fumigatus *Aspergillus*-associated CPA should be considered with a negative *Aspergillus* precipitin test; this finding may prevent diagnostic delay or misdiagnosis for CPA.

4. Treatment outcome definitions in chronic pulmonary aspergillosis: a CPAnet consensus statement.

Van Braeckel E, Page I, Davidsen J, Laursen C, Agarwal R, Alastruey-Izquierdo A, Barac A, Cadranel J, Chakrabarti A, Cornely O, Denning D, Flick H, Gangneux JP, Godet C, Hayashi Y, Hennequin C, Hoenigl M, Irfan M, Izumikawa K, Koh WJ, Kosmidis C, Lange C, Lamprecht B, Laurent F, Munteanu O, Oladele R, Patterson T, Watanabe A, Salzer H

Chronic pulmonary aspergillosis (CPA) is a severe fungal infection of the lung with a high morbidity and mortality. It is usually seen in immunocompetent patients with respiratory disorders. Clinical presentation is nonspecific and often overlaps with the symptoms and the radiological pattern caused by the underlying disease. Many patients with CPA respond well to antifungal therapy, but others do not. Many aspects of this neglected disease are unknown and the disease therefore requires multinational and multicenter cooperation. To meet this challenge, the Chronic Pulmonary Aspergillosis Network (CPAnet) was established in March 2017 to promote clinically oriented research. One of the key research priorities of the network was the establishment of a consensus on treatment outcome definitions. This is important, because harmonization of endpoints will enable comparison of study results leading to a better understanding of CPA treatment and management.

5. Evaluation of the Sensititre YeastOne and Etest in Comparison with CLSI M38-A2 for Antifungal Susceptibility Testing of Three Azoles, Amphotericin B, Caspofungin, and Anidulafungin, against *Aspergillus fumigatus* and Other Species, Using New Clinical Breakpoints and Epidemiological Cutoff Values.

Melhem MSC, Coelho VC, Fonseca CA, Oliveira L, Bonfietti LX, Szeszs MW, Magri MMC, Dorneles FS,

Taguchi H, Moreira DVS, Motta AL, Batista MV, Kamei K, Shikanai-Yasuda MA

Aspergillosis is an invasive fungal disease associated with high mortality. Antifungal susceptibility testing (AFST) is receiving increasing consideration for managing patients, as well as for surveilling emerging drug resistance, despite having time-consuming and technically complex reference methodologies. The Sensititre YeastOne (SYO) and Etest methods are widely utilized for yeasts but have not been extensively evaluated for Aspergillus isolates. We obtained Posaconazole (POS), Voriconazole (VCZ), Itraconazole (ITC), Amphotericin B (AMB), Caspofungin (CAS), and Anidulafungin (AND) minimum inhibitory concentrations (MICs) for both the Etest (n = 330) and SYO (n = 339) methods for 106 sequenced clinical strains. For 84 *A. fumigatus*, we analyzed the performance of both commercial methods in comparison with the CLSI-AFST, using available cutoff values. An excellent correlation could be demonstrated for Etest-AMB and Etest-VCZ (p < 0.01). SYO-MICs of AMB, VCZ, and POS resulted in excellent essential agreement (> 93%), and > 80% for AMB, VCZ, and ITC Etest-MICs. High categoric agreement was found for AMB, ITC, and CAS Etest-MICs (> 85%) and AMB SYO-MICs (> 90%). The considerable number of major/very major errors found using Etest and SYO, possibly related to the proposed cutoffs and associated with the less time-consuming processes, support the need for the improvement of commercial methods for *Aspergillus* strains.

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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.

インフルエンザ菌の病原性解析ならびにインフルエンザ菌感染症，肺炎球菌感染症，B群溶血性レンサ球菌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
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1. Epidemiology and bacterial characteristics of invasive group B streptococcus disease: a population-based study in Japan in 2010-2020

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This is the first report on a population-based prospective study of invasive group B streptococcus (GBS) disease among children aged <15 years conducted over a period of 11 years in Japan. This study investigated the incidence and clinical manifestations of invasive GBS disease in children in Chiba Prefecture, Japan, and analysed the serotypes and drug susceptibility of GBS strains isolated during the study period. Overall, 127 episodes of invasive GBS disease were reported in 123 patients. Of these, 124 were observed in 120 patients aged <1 year, and the remaining three episodes were reported in a 9-year-old child and two 14-year-old children with underlying disease. For patients aged <1 year, the incidence rate per 1000 live births was 0.24 (0.15-0.36). The incidences of early-onset disease and late-onset disease were 0.04 (0.0-0.09) and 0.17 (0.08-0.25), respectively. The rate of meningitis was 45.2%, and the incidence of GBS meningitis

was higher than that of other invasive diseases among children in Japan. Of the 109 patients for whom prognosis was available, 7 (6.4%) died and 21 (19.3%) had sequelae. In total, 68 strains were analysed. The most common were serotype III strains (n = 42, 61.8%), especially serotype III/ST17 strains (n = 22, 32.4%). This study showed that the incidence of invasive GBS disease among Japanese children was constant during the study period. Because of the high incidence of meningitis and disease burden, new preventive strategies, such as GBS vaccine, are essential.

2. Clinical and Bacteriological Analysis of Pediatric Pneumococcal Meningitis after 13-Valent Pneumococcal Conjugate Vaccine Introduction in Japan

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Streptococcus pneumoniae is one of the leading causes of meningitis in children. In Japan, since the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13), the number of pneumococcal meningitis due to non-PCV13 serotypes in children has increased. To clarify the clinical outcomes, serotype distributions, and antimicrobial susceptibility of isolated *S. pneumoniae* strains from pediatric pneumococcal meningitis, we clinically and bacteriologically analyzed 34 cases of pediatric pneumococcal meningitis that were reported after the PCV13 introduction era in Japan. The median age at diagnosis was 1 year (range: 3 months-13 years). Ten (29.4%) patients had underlying diseases. Twenty-nine (85.3%) patients had received at least one dose of any pneumococcal vaccine. Of the 34 patients with pneumococcal meningitis, 6 had sequelae, and 4 died. Nine (26.5%) strains were resistant to penicillin; five (15%) strains to meropenem, with an MIC of 0.5 μg/mL. All strains were susceptible to vancomycin and linezolid. Daptomycin's MIC50 was 0.064 μg/mL and MIC90 was 0.094 μg/mL. Among the tested strains, only four were PCV13 serotypes. Penicillin-resistant *S. pneumoniae* was isolated from 30.0% of the patients with sequelae and death. Particularly, the proportion of serotype 10A in the sequelae and deceased cases was significantly higher than that in the complete recovery cases. We should carefully monitor the serotype and drug susceptibility of *S. pneumoniae* strains isolated from patients with meningitis after the PCV13 era and reconsider the treatment strategy to prepare against further drug-resistant pneumococcal strains.

3. Epidemiological characteristics in serotype 24 paediatric invasive pneumococcal disease according to an 11-year population-based study in Japan

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After the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13), serotype replacement has occurred in Japan, and serotype 24 has become the most common serotype in paediatric invasive pneumococcal disease

(IPD). To understand the characteristics of serotype 24-IPD in Japanese children in the post-PCV13 era, we conducted a retrospective study in children aged ≤15 years from 2010 to 2020 using a database of paediatric IPD surveillance in Chiba prefecture, Japan. We identified a total of 357 IPD cases and collected clinical information on 225 cases (24: 32 cases, non-24: 193 cases). Compared with the non-serotype 24-IPD, serotype 24-IPD was independently related to be <2 years of age [odds ratio (OR) 3.91, 95% confidence interval (CI) 1.47-10.44; P = 0.0064] and bacteremia (OR 2.28, 95% CI 1.01-5.13; P = 0.0475), as a result of the multivariate regression analysis. We also conducted a bacterial analysis, and the isolates of serotype 24-IPD had tendencies of PCG-susceptible (24: 100.0%, non-24: 61.3%; P < 0.0001) and macrolide-resistance (24: 100.0%, non-24: 87.3%; P = 0.0490). Their multilocus sequence typing was mostly ST2572 and the variants, which were unique to Japan. This tendency might have been a result of the progress made in the Japanese PCV13 immunisation programme.

4. Immunogenicity and safety of routine 13-valent pneumococcal conjugate vaccination outside recommended age range in patients with hematological malignancies and solid tumors

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Hematological malignancy and solid tumor are major risks for invasive pneumococcal disease. Thirteen-valent pneumococcal conjugate vaccine (PCV13) is recommended for immunocompromised patients aged 6 years and older and adults who had not received the vaccine previously. However, vaccination for these individuals is not publicly subsidized in Japan. We measured pneumococcal serotype-specific IgGs (Pn-IgGs) and opsonophagocytic activities (Pn-OPAs) against PCV13 serotypes (1, 3, 5, 6A, 7F, and 19A) in patients with hematological malignancies and solid tumors who were outside the recommended age range for routine vaccination at baseline and at 1 and 6 months after the first dose of PCV13. Pneumococcal serotype-specific memory B cells (Pn-MBCs) against serotype 3 were measured from a portion of the study samples. Thirty-seven patients (30 in the young patient group and 7 in the adult patient group) completed the study. Pn-IgGs were significantly elevated at 1 month post-vaccination and persisted in protection level for 6 months after the first vaccination against all six serotypes measured except serotype 3. Pn-OPAs were significantly elevated and persisted as well against all six serotypes. Pn-MBCs were measured in 10 patients, and 90% of them had at least one detectable Pn-MBC, and 70% of them showed an increased frequency of Pn-MBCs against serotype 3. No serious adverse events were observed up to 1 month after vaccination. PCV13 is thus safe and immunogenic, including against serotype 3, in patients with hematological malignancies and solid tumors outside the recommended age range for routine vaccination.

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Project for Systems Biology of Microorganisms

高橋ＰＩ（微生物創生）プロジェクト

Summary（研究概要）

Our research areas are Bioinformatics and 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	Yoko Kusuya（～2022.8）	特 任 助 教	楠屋　陽子
Research Assistant Professor	Jun-ichi Ishihara	特 任 助 教	石原　潤一
Research Promotion Technician	Machiko Zen	技 術 補 佐 員	全　真知子
Research Promotion Technician	Emi Shirai（2022.11～）	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*

Yoko Kusuya, Cai Bian, 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 and statistical analysis.

Publications

1）Bian C, Kusuya Y, Sklenář F, D'hooge E, Yaguchi T, Ban S, Visagie CM, Houbraken J, Takahashi H, Hubka V. Reducing the number of accepted species in *Aspergillus* series *Nigri*. Studies in Mycology. 102: 95-132. 2022.

2）Tsuji M, Ishihara JI, Toyoda A, Takahashi H, Kudoh S. Genome Sequence of Basidiomycetous Yeast *Mrakia gelida* MGH-2, Isolated from Skarvsnes Ice-Free Area, East Antarctica. Microbiol Resour Announc. e0106422. 2022.

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9）Alimu Y, Kusuya Y, Yamamoto T, Arita K, Shigemune N, Takahashi H, Yaguchi T. Mechanism of Polyhexamethylene Biguanide Resistance in *Purpureocillium lilacinum* strains. Biocontrol Sci. 27(3): 117-130. 2022.

Management of Unit of Microbiological Resources

バイオリソース管理室

Summary（研究概要）

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

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

Associate Professor	Takashi Yaguchi	准教授	矢口　貴志
Assistant Professor	Sayaka Ban	助教	伴　さやか
Research Technician	Junko Ito	技術職員	伊藤　純子
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Research Promotion Technician	Yu Uehara	技術補佐員	上原　ゆう

1. Mechanism of Polyhexamethylene Biguanide Resistance in *Purpureocillium lilacinum* strains.

Alimu Y[1], Kusuya Y[1], Yamamoto T[2], Arita K[2], Shigemune N[2], Takahashi H[1, 3, 4], Yaguchi T[1]

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Purpureocillium lilacinum has been recently found to contaminate a 20%（200, 000 μg/mL）aqueous solution of polyhexamethylene biguanide hydrochloride（PHMB）. We aimed to elucidate the mechanism underlying the resistance of *P. lilacinum* to PHMB. First, we induced the PHMB-resistant（IR）strains IFM 67050（IR）and IFM 65838（IR）from the type strain *P. lilacinum* CBS 284.36^T via cultivation in a medium containing high concentrations of PHMB. We then analyzed the DNA sequences via Illumina sequencing to evaluate the presence of genetic mutations in IFM 65838（IR）. Further, we established an IFM 65838（IR）uridine/uracil auxotrophic strain, and using the orotidine-5'-decarboxylase gene（*pyrG*）as a selection marker, we tried to knockout a mutant gene in IFM 65838（IR）using the CRISPR-Cas9 genome-editing technique. The growth rates of IFM 67050（IR）and IFM 65838（IR）in medium containing PHMB increased, and the minimum inhibitory concentrations（MICs）against PHMB also increased（Fig. 1）. Based on the DNA sequence analysis, we found a nonsynonymous point mutation in the gene PLI-008146（G779A）in IFM 67050（IR）and IFM 65838（IR）. This point mutation leads to site combinations of splicing changes that cause partial sequences deletion（p. Y251_G281del）in the ΔPLI-008146 locus of IFM 65838（IR）, and deletion sequences include partial adenosine/AMP deaminase motif

(PF00962) orthologous to adenosine deaminase (ADA) (GeneBank: OAQ82383.1) (Fig. 2). Furthermore, the mutant gene ΔPLI-008146 was successfully knocked out from the resistance-induced strain using a novel CRISPR-Cas9 gene transformation method. A considerable reduction in growth rate and MIC against PHMB was observed in the absence of the mutant gene. Therefore, ADA may represent an important resistance factor in PHMB-resistant *P. lilacinum*.

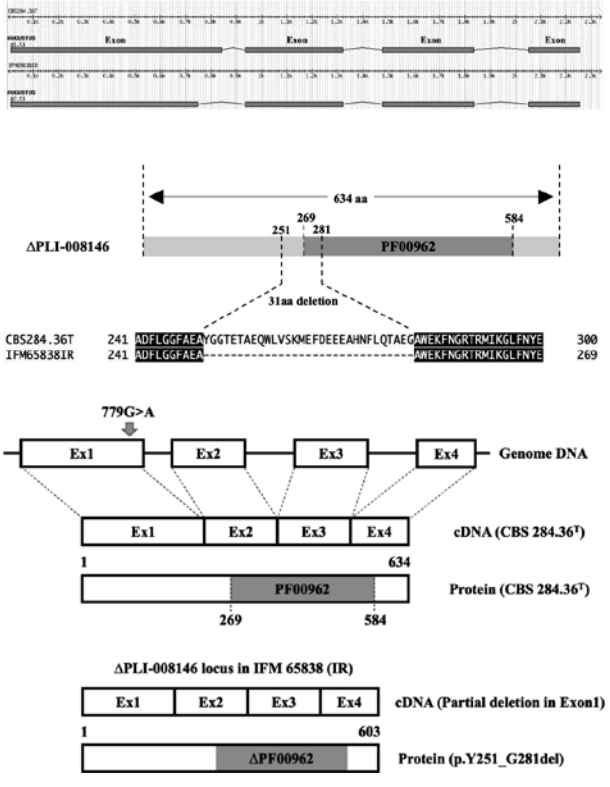

FIG. 1. The predicted protein domain structure on ΔPLI-008146 locus. The figure indicates partial deletion (p. Y251_G281del) of the adenosine/AMP deaminase motif (PF00962) in the ΔPLI-008146 locus of IFM 65838 (IR).

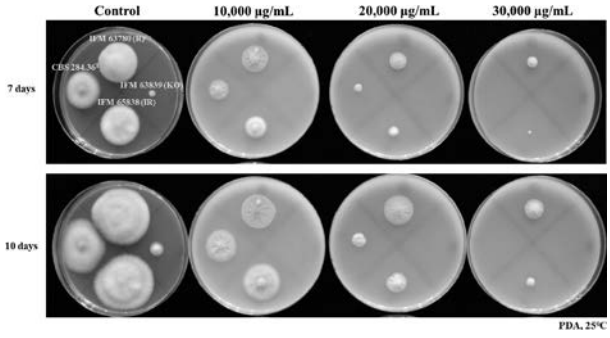

FIG. 2. Growth rate of *P. lilacinum* in different concentration of PHMB. PDA contained PHMB concentration: 0 μg/mL, 10, 000 μg/mL, 20, 000 μg/mL and 30, 000 μg/mL. Concentration of conidia suspension 106 conidia/mL. Incubated in 25℃ for 7 and 10 days. (R): PHMB-resistant strain, (IR): Induced PHMB-resistant strain, (KO): The mutant gene ΔPLI-008146 knockout strain.

2. Molecular phylogenetic study of strains morphologically identified as *Exophiala dermatitidis* from clinical and environmental specimens in Japan.

Alimu Y[1], Ban S[1], Yaguchi T[1]

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In this study, we aimed to clarify the phylogenetic distribution of *Exophiala dermatitidis* in Japan and describe the characteristics of each genotype. We examined 67 clinical and environmental isolates that were morphologically identified and preserved as *E. dermatitidis*, and confirmed the identification based on the ribosomal DNA internal transcribed spacer (ITS) region. Genotype analyses were aligned and compared using maximum likelihood phylogenetic tree analyses of the ITS1 region. Additionally, the strains of each gene type were tested for mycological characteristics, such as growth temperature, growth rate, and drug sensitivity. The 67 strains examined were isolated from Japan, the United States, Brazil, Venezuela, and China. According to the establishment of a phylogenetic tree for the ITS1 region, 45 of the 49 Japanese strains were classified as genotype A, two as genotype B, and two as genotype D (A2 according to the method of Matos et al. (2003)). Chinese strains were divided into genotypes A and D (A2), and

South American strains were classified as genotype A, B, B2, and C2, while all strains from the USA belonged to genotype A (Fig. 1). Namely, New genotype groups B2 and C2 were identified among some Japanese and Venezuelan strains. There were no specific differences among the genotypes or isolated regions in the antifungal susceptibility for all *E. dermatitidis* isolates. However, genotypes B2 and D (A2) exhibited growth at higher temperatures than the other genotypes (Fig. 2).

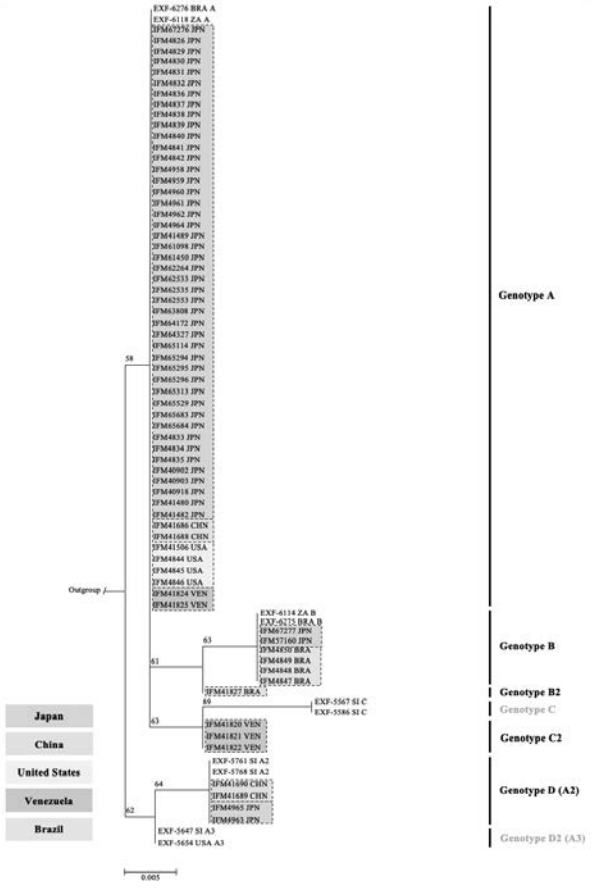

FIG. 1. The phylogenetic tree calculated by maximum likelihood method based on the sequences of the ITS1 region from the 78 members of *Exophiala dermatitidis* and relatives. *Exophiala heteromorpha* CBS 232. 33 was taken as an outgroup. The bootstrap values shown over than 50%. There were a total of 176 positions in the final dataset. BRA: Brazil, ZA: South Africa, JPN: Japan, CHN: China, USA: United States, VEN: Venezuela, SI: Slovenia.

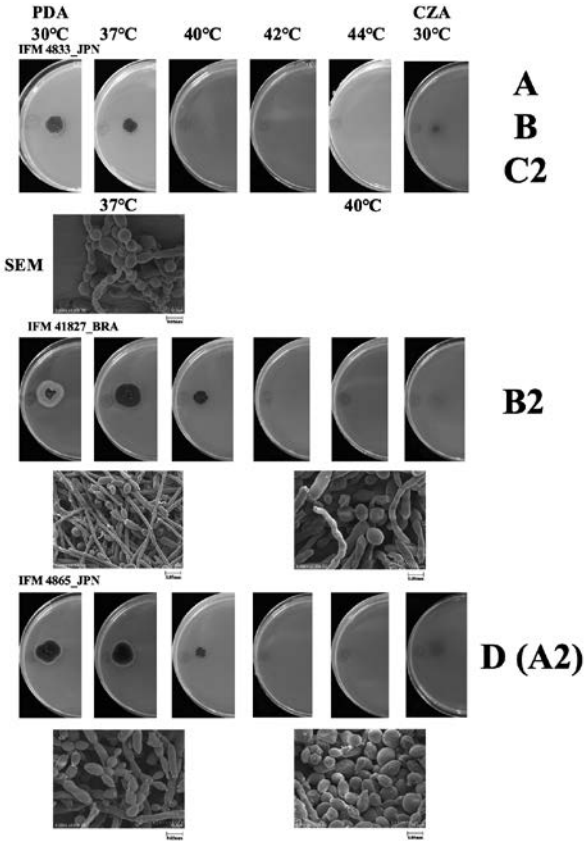

FIG. 2. Growth characterization on PDA medium. 104 cells/ml (10 µl), incubated in 30, 37, 40, 42 and 44℃ on PDA, 14 days. The hyphal and the conidia were observed by the SEM.

3. Gene amplification of *CYP51B*: a new mechanism of resistance to azole compounds in *Trichophyton indotineae*.

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Trichophyton indotineae causes dermatophytosis that is resistant to terbinafine and azole compounds. The aim of this study was to determine the mechanisms of resistance to itraconazole (ITC) and voriconazole (VRC) in strains of *T. indotineae*. Two azole-sensitive strains (ITC MIC < 0.125 mg/mL; VRC MIC < 0.06 mg/mL) and four azole-resistant strains (ITC MIC > 0.5 mg/mL; VRC MIC > 0.5 mg/mL) were used for the investigation. The expression of MDR genes encoding multidrug transporters of the ABC family for which orthologs have been identified in *Trichophyton rubrum* and those of *CYP51A* and *CYP51B* encoding the targets of azole antifungal compounds were compared between susceptible and resistant strains. *TinMDR3* and *TinCYP51B* were overexpressed in *T. indotineae* resistant strains. However, only small differences in susceptibility were observed between *TinMDR3* disruptants and parental strains overexpressing *TinMDR3*. In contrast, whole-genome sequencing revealed the creation of a variable number of *TinCYP51B* tandem repeats at the specific position of their genomes in three resistant strains. Downregulation of *TinCYP51B* by RNA interference (RNAi) restored the susceptibility of azole-resistant strains, while overexpression of *TinCYP51B* conferred resistance to a susceptible strain of *T. indotineae*. In conclusion, the reduced sensitivity of *T. indotineae* strains to azoles is mainly due to the overexpression of *TinCYP51B* resulting from additional copies of this gene.

Publications

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Project for RNA Regulation

原口（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. This project, which has started in July 2020, 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．令和２年７月に開始された本プロジェクトでは，このような遺伝子制御ネットワークの乱れの原因となる因子として，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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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

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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 investigated the method of binding the ligand molecule to the tip of the PEG on the surface layer of LNP using R8 peptide as the ligand molecule and achieved a remarkable increase in the efficiency of nucleic acid delivery. Furthermore, this LNP modified with R8 peptide showed high blood retention and accumulation in tumor tissue in xenograft mouse.

Merged project of respiratory pathophysiology and pathobiology

巽・田邉・寺田（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

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1. **Stratifin as a novel diagnostic biomarker in serum for diffuse alveolar damage.** ***Nat Commun*** **2022 Oct 4; 13 (1): 5854.**

Arakawa N, Ushiki A, Abe M, Matsuyama S, Saito Y, Kashiwada T, Horimasu Y, Gemma A, Tatsumi K, Hattori N, Tsushima K, Miyashita K, Saito K, Nakamura R, Toyoda T, Ogawa K, Sato M, Takamatsu K, Mori K, Nishiya T, Izumi T, Ohno Y, Saito Y, Hanaoka M

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. **Altered gut microbiota and its association with inflammation in patients with chronic thromboembolic pulmonary hypertension: A single-center observational study in Japan.** ***BMC Pulm Med*** **2022; 22: 138. doi:10.1186/s12890-022-01932-0.**

Ikubo Y, Sanada TJ, Hosomi K, Park J, Naito A, Shoji H, Suda R, Sekine A, SugiuraT, Shigeta A, Sakao S, Tanabe N, Mizuguchi K, Kunisawa J, Suzuki T, Tatsumi K

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. **Effects of the combination of atomoxetine and oxybutynin in Japanese patients with obstructive sleep apnea: A randomized controlled crossover trial.** ***Respirology*** **2022 Oct 2. doi:10.1111/resp.14383.**

Kinouchi T, Terada J, Sakao S, Koshikawa K, Sasaki T, Sugiyama A, Sato S, Sakuma N, Abe M, Shikano K, Hayama N, Siko Y, Ozawa Y, Ikeda S, Suzuki T, Tatsumi K

Background and objective: The possibility of combination therapy with atomoxetine (ATO) and oxybutynin (OXY)

has been suggested for obstructive sleep apnoea (OSA). However, the effectiveness of this treatment remains uninvestigated in Japanese OSA patients. Therefore, we performed a randomized, crossover, phase II, single-centre prospective trial to examine the effects of ATO-OXY therapy in Japanese OSA patients.

Methods: In total, 17 OSA patients participated in this study. The effects of one night of 80-mg ATO plus 5-mg OXY administration were compared with those of no medication administered before sleep. The primary and secondary outcomes comprised the apnoea-hypopnoea index (AHI) and nadir SpO_2, SpO_2 drop time and sleep architecture, respectively. The safety endpoints included drug side effects and adverse events.

Results: The values of AHI, nadir SpO_2, 3% oxygen desaturation index (ODI), 4% ODI, and SpO_2 drop time of <90% did not significantly differ between patients receiving ATO-OXY administration and no medication. Sleep architecture exhibited a significant change: ATO-OXY increased sleep stage N1 ($p < 0.0001$) and decreased stage N2 ($p = 0.03$), rapid eye movement ($p < 0.0001$) and sleep efficiency ($p = 0.02$). However, the subanalysis demonstrated an obvious decrease in AHI in five responder patients. Total sleep time and basal sleep efficiency tended to be lower in the responders compared with nonresponders ($p = 0.065$). No patients experienced severe adverse events or side effects.

Conclusion: Overall, ATO-OXY therapy does not reduce AHI in Japanese OSA patients, although AHI was decreased in a proportion of patients. Future studies for identifying treatment response group characteristics are warranted.

4. Transcriptome analysis of peripheral blood mononuclear cells in pulmonary sarcoidosis.

Front Med (Lausanne) 2022; 9: 822094. doi:10.3389/fmed.2022.822094.

Yoshioka K, Sato H, Kawasaki T, Ishii D, Imamoto T, Abe M, Hasegawa Y, Ohara O, Tatsumi K, Suzuki 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・長田（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 leaving many offspring for a chance of survival to leaving a few offspring and nursing them for assured survival. In vertebrates, 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 per cycle to grow during adulthood, suggesting that humans have acquired special mechanism to reduce the number of offspring during evolution. 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，コストをかけて少ない子孫を育てる方向への変化である．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機序を獲得してきたと考えられる．われわれは，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．

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1. 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 and 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.

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 difference in the endocrine environment, such as estrogens and androgens, and immunity may be responsible for the differences in adverse events. We are focusing on vulvar ulcers as an unrecognized

adverse effect of COVID-19 vaccines. We are analyzing public databases such as VAERS to prove that acute vulvar ulcer is a rare but unique adverse event in young women.

3. 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. In the present study, 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.

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

文部科学省　ナショナルバイオリソースプロジェクト「病原微生物」

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

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

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

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

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

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

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

Number of strains	FY2018	FY2019	FY2020	FY2021	FY2022*
Collection	563	579	886	837	455
Preservation	24, 459	24, 899	25, 785	26, 591	27, 036
Provision	1, 152	1, 112	222	1, 319	580

*: to 30th Dec., 2022

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を共有し，LAMP法プライマーの設計と栄研化学（株）の支援によるLAMP診断キットのプロトタイプを作成する．さらに，設備の整わない地域の医療機関での実施に向けたガイドラインを作成する．

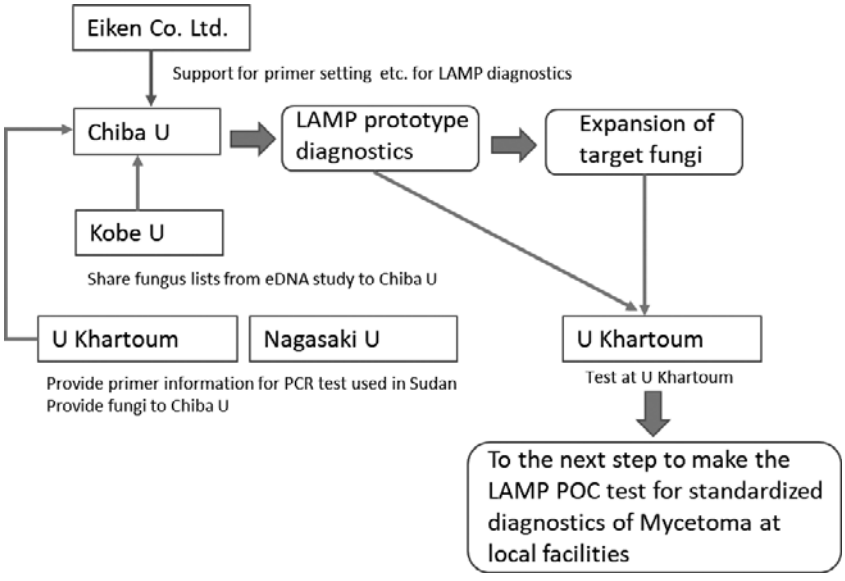

FIG.1. Finding POC diagnosis using LAMP method of mycetoma infection.

FIG.2. The Mycetoma Research Center, University of Khartoum and cooperative institutions. Chiba University is referred as a collaborator of Mycetoma Research Center (right panel; indicated by arrow)

The project for prophylaxis, diagnosis, and treatment for aspergillosis and the other mycoses in aged and neonate patients

高齢者・新生児アスペルギルス症制圧へ向けた予防・診断・治療開発プロジェクト

Aspergillosis is a disease in which *Aspergillus* spp., such as *Aspergillus fumigatus*, mainly infect the lung and often lead to death. Aspergillosis is the most common cause of death in many countries, and in Japan chronic pulmonary aspergillosis is particularly serious due to the growing number of patients particularly among COPD and the aged. In the pandemic of COVID-19, many cases of CAPA (COVID-Associated Pulmonary Aspergillosis) with high mortality were reported worldwide, and the importance of aspergillosis was reaffirmed. Our project focuses on the epidemiology of these diseases, exacerbating factors, and drug resistance (especially to azoles, the first-line drug), which is an important refractory factor in chronic infection. Another focus of our projectcs mycosis in neonates, which has tended to be overlooked until now. We aim is to find a way to control this disease by analyzing fungal infections in neonates, especially premature low-birth-weight infants, and to develope new diagnostic, therapeutic, and prophylactic methods.

The emerging azole-resistance among *A. fumigatus* has become a serious threat to our society. Mutation in Cyp51A is known to be one of the critical mechanisms of the resistance in *A. fumigatus*. We had already reported a novel mechanism of the resistance (Hmg1 mutation) (2018, 2021) along with the investigation of cyp51A gene polymorphisms to find out the difference between Japan and the other countries (2021).

This fiscal year we found that mutations in proteins involved in lipid metabolism, which have not received attention until now, are also involved in azole resistance, and presented as a new mechanism of resistance at the International Society for Medical Mycology (ISHAM2022, New Delhi, India). Furthermore, we made a comparison of assay methods of antibody titer determination, which is an important diagnostic method for aspergillosis, between the conventional preticipating method and a newly developed IgG antibody method. The result was published in Mocrobiology Spectrum (10. 1128/spectrum. 03435-22). In addition, in collaboration with a European research group, we made recommendations for determining treatment efficacy in chronic pulmonary aspergillosis and published them in a paper in European Respiratory Jounrnal (doi:10.1183/13993003.02950-2021). In terms of the basic research on pathogenic factors, we believe *Aspergillus* cell wall proteins can be new candidates for pathogenic factors, and have been making their analysis. In the current fiscal year, we obtained many groundbreaking findings to show the mechanism by which innate immunity of the host is induced by the chitin-related protein in the cell wall. These results are deeply related to the mechanism of *Aspergillus* infection and are currently submitted for publication.

For the study of deep-seated mycosis among neonates, we conducted a nationwide retrospective survey in order to determine numbers of invasive fungal infections (IFI) in Japan. Based on this background, we reported the utility of gastric aspirate fungal culture for the diagnosis of infantile fungal pneumonia caused by *Rasamsonia piperina*. We also reported neonatal meningitis and ventriculitis caused by *Aspergillus fumigatus*. The continuous monitoring of serum and cerebral fluid voriconazole concentration was proved useful for the appropriate treatment of this severe case. In this year, we analyzed two *Aspergillus fumigatus* strains isolated from neonatal invasive deep-seated mycosis. We compared above two strains using STRs analysis and found two strains were of different background strain. Environmental fungal survey in neonatal intensive care unit is needed for preventing deep-seated mycosis in extremely premature and low birth weight infant. We conducted environmental fungal survey in several NICU in Chiba prefecture and pathogenic fungi were detected. Above study results gave us the important information for the prevention, establishment of diagnosis, and treatment for aspergillosis and the other mycoses in neonate patients.

アスペルギルス症は主に*Aspergillus fumigatus*が肺を中心とした内臓に感染してしばしば死に至る疾患であり，我が国においても欧米諸国と同じく最も死亡者が多い深在性真菌症（内臓真菌症）となっている．特に我が国では高齢化が進んでいる事や慢性閉塞性肺疾患（COPD）等の慢性肺疾患が多い事から，これらに好発する慢性肺アスペルギルス症の症例が多く，本疾患の研究は社会的に特に重要である．加えて，世界中にpandemicを引き起こしたCOVID-19感染症では，致死率の高いアスペルギルス症の合併（CAPA: COVID-Associated Pulmonary Aspergillosis）が頻発し，アスペルギルス症の重要性は世界的に再認識させることとなった．本プロジェクトはこれらの疾患の疫学，増悪因子，慢性感染で重要な難治化因子となる薬剤耐性（特に主力薬剤であるアゾール薬に対する耐性）に関する研究，さらにこれまでともすれば見逃されがちであった新生児における真菌症に注目し，早産低出生体重児を中心とした新生児等の真菌症の解析等を行い，新規診断法，治療法，予防法の開発へと導く事により本疾患の制圧を目指すものである．

耐性菌はアスペルギルスにおいてもきわめて深刻な問題となりつつあるが、耐性菌研究には、数多くのさまざまな症例からの最新の検体と臨床情報の収集が不可欠である．本センターは日本感染症学会及び日本臨床微生物学会により「先進的感染症検査施設」に認定され，真菌症リファレンスセンターとして全国の医療施設から種々の検査依頼、診療サポートを引き受けていることから，多くの検体を集めることができている．令和３年度は夏から冬にかけて強烈なCOVID-19の第５波，第６波に襲われたにもかかわらず，300件を超える検査依頼があった．これらに加えて，慶應大学呼吸器内科/感染制御部・感染制御センター及びNHO東京病院も加えたより緻密な共同研究ネットワークを構築し，アスペルギルス症難治化の一因である*Aspergillus fumigatus*耐性化の研究を続けることができた．これらの活動により，アスペルギルス症の重要な診断法である抗体検査法について，従来の沈降抗体法と新規に開発されたIgG抗体測定法の比較検討を行い論文として報告した（Mocrobiol Spectr誌にて発表）．またこれまで注目されてこなかった脂質代謝に関与するタンパクの変異がアゾール薬耐性化に関与する事を明らかにし，新たな耐性機序として，その成果を国際医真菌学会（ISHAM2022, インドニューデリー）において発表した．また欧州研究グループとの共同で慢性肺アスペルギルス症の治療効果判断についての提言をまとめ，論文として発表した（Eur Respir J誌にて発表）．さらに，予防，治療や難治化克服のためには菌の病原因子に関する本質的な基礎研究が不可欠であるため，病原因子の新たな候補であるアスペルギルス細胞壁タンパク質の研究を進めてきた．今年度は更に研究を進めて宿主の自然免疫がアスペルギルス細胞壁のchitin関連タンパク質によって誘導される機序をin vitroおよびin vivoの実験によって証明するなど，多くの画期的な知見を得た．これらの成果はアスペルギルスの感染機序に深く関与するものであり，現在論文投稿中である．

新生児領域における研究では，これまでに新生児深在性真菌感染症発症状況の全国調査を実施し，初めて国内の実態を明らかにした．加えて，これまで非常に困難とされてきた乳児の糸状菌感染症の診断法に関して，胃液を使用する真菌培養での診断に成功し論文として公表した．さらに，治療が難しいとされる新生児の*A. fumigatus*による髄膜炎・脳室炎症例に対して，血中と髄液中のボリコナゾール濃度を経時的に測定することで，適切な治療を行うことができる可能性を明らかにし．論文にて公表した．また，皮膚病変を初発症状としていた超早産・超低出生体重児の髄膜炎・脳室炎２例において，分離された*A. fumigatus*のSTRs（microsatellite）解析を実施し，２症例は異なった株による感染であることを明らかにした．さらに，このような新生児における真菌症の感染源を明らかにするため，千葉県内の新生児集中治療室（NICU）の管理方法，環境中の真菌に関する調査を行い，環境中から，病原性のある真菌を検出した．これらの検討結果は，今後の新生児アスペルギルス症の予防，診断・治療法策定において極めて重要な情報を提供するものである．

AMED/JICA Science and Technology Research Partnership for Sustainable Development（SATREPS）

"The establishment of a research and reference collaborative system for the diagnoses of fungal infections including drug-resistant ones in Brazil and Japan"

AMED/JICA地球規模課題対応国際科学技術協力プログラム（SATREPS）

「ブラジルと日本の薬剤耐性を含む真菌感染症診断に関する研究と リファレンス協力体制強化プロジェクト」

The number of fungal infections has been increasing in recent years because of clinical practice advances such as hematopoietic stem cell transplantation and solid organ transplantation. Also, patients with chronic lung disease (pulmonary tuberculosis, COPD, and others) are generally susceptible to pulmonary fungal infection. Recently, many cases of invasive fungal infection in COVID-19 patients have been reported, and researchers put considerable emphasis on fungal infections. In general, fungal infections are refractory diseases, and their mortality is high. In these aspects, the impact of fungal infections is too high, not only in the medical field but also in society.

Recently, various fungi possessing resistance to antifungals have become a severe problem. In 2019, CDC in the US had listed drug-resistant fungi as one of the five "urgent threats." The emergence and increase of drug-resistant fungi are expected to lead to refractory disease and increased mortality directly. It has been reported that infections caused by drug-resistant fungi have a higher mortality rate than the ones caused by drug-sensitive fungi. However, South America, particularly in Brazil, has been little investigated and remains unclear. Given these situations, this project was started between the Medical Mycology Research Center, Chiba University, and the University of Campinas.

This project was finished in 2022. We found that mutation patterns of drug target genes in Japan are significantly different from those in Europe and Brazil. In other words, it was suggested that the mechanism of antifungal drug resistance might differ depending on the region/country, and it was confirmed that each situation should be considered when developing a method for detecting a resistance gene.

We developed resistance gene detection methods based on LAMP (Loop-Mediated Isothermal Amplification).

As a research network tool in Brazil, REDCap®, a system for data collection and management developed by Vanderbilt University, was introduced to the University of Campinas. So far, more than 300 cases of invasive mycoses of several medical institutions in Brazil have been enrolled and the registration of clinical cases are still continued.

Besides, using this consortium, a bio-resource bank for fungal strain has been established, and we continue the fungal preservation. The preserved strains are clinical fungal isolates from several hospitals and environmental isolates (from the soil, air, plants, or natural water).

真菌感染症患者数は近年増加の一途をたどっている．その背景として，免疫抑制薬の投与，造血幹細胞移植や固形臓器移植を受けている等による全身的免疫低下患者，また慢性肺疾患（肺結核症や肺気腫など）を基礎疾患に有する患者等の局所的免疫低下患者などが増えており，そのような患者の発症頻度が高いことが挙げられる．さらに新型コロナウイルス感染症（COVID-19）患者に合併した深在性真菌症症例が多数報告され，ますます重要視されている．一般に深在性真菌症は難治で致死率が高いことが知られており，その意味で，真菌感染症のインパクトは医療分野のみならず社会的にも極めて高いと言える．

さらに加えて近年，ヨーロッパ諸国を皮切りに抗真菌薬に対する耐性を有した多種多様な真菌が問題となりつつある．2019年，米国CDCは，最も差し迫った脅威とな

る５種の微生物のうちのひとつに薬剤耐性真菌をリストアップした．薬剤耐性真菌の出現，増加は疾病の難治化，致死率の上昇に直結することが予想される．実際，薬剤感性真菌による感染症よりも薬剤耐性真菌による感染症の方が致死率が高いとの報告もある．一方で，ブラジルを含めた南米での状況はほとんど調査されておらず，不明のままであったため，その早急な実態解明はまさに社会的要請である．

本プロジェクトは2022年に事業終了した．本プロジェクトではブラジルのサンパウロ州立カンピーナス大学と連携し，カンピーナス首都圏における耐性真菌による感染症の実態を明らかにし，耐性真菌の検出法を開発することを通じ，ブラジルにおける難治性真菌感染症の治療戦略を構築するとともにブラジルにおけるカンピーナス大学を中心とした耐性真菌感染症研究拠点研究ネットワークの構築を目指した．

我が国における薬剤の標的遺伝子の変異パターンは欧州及びブラジルとは大きく異なることを見出した．

また耐性遺伝子検出法としてブラジルで検出された遺伝子変異タイプの耐性株を用い，LAMP（Loop-Mediated Isothermal Amplification）法による検出法を確立した．

ブラジル国内の研究ネットワークのツールとして導入した，REDCap®（米国Vanderbilt大学が開発したデータ集積管理システム）を基盤とした真菌症の症例データベースは，多施設共同で300症例を超える真菌症の症例が集積されている．

また，このコンソーシアムを利用し，研究機関も含めた真菌株保存バンクを設立し，実際に複数の医療機関からの臨床分離株に加え，環境（土壌，空気，植物，水など）からの分離真菌株の保存が続けられている．

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. In this project, we aim to identify target factors through research on persister molecular mechanisms and to obtain compound candidates that can regulate the activity of factors through compound screening. Using a natural product library of about 1200 species, we screened compounds that inhibit the activity of bacterial factors associated with the persisters of *Staphylococcus aureus*. As a result of screening, inhibitory activity was found in several natural product compounds. To investigate the activity of compounds other than the screening strains, we used clinical isolates related to nosocomial outbreaks. We found that the compounds inhibited the activity like the screening strains. In addition, we are conducting an analysis of antibacterial drug administration in a mouse model infected with bacteria to verify the *in vivo* effects of the obtained new natural product candidate compounds.

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．本研究では，パーシスター分子機構研究を通して標的となる因子を同定し，化合物スクリーニングから因子の活性を制御できる化合物候補を得ることを目的としている．これまでに約1200種から構成される天然物ライブラリーを用いて黄色ブドウ球菌のパーシスターに関連する細菌因子の活性を阻害する化合物スクリーニングを実施した．スクリーニングした結果，数種の化合物に阻害活性を見出した．スクリーニング株以外での化合物活性についても調べるため，院内アウトブレイクに関わる臨床分離株を用いたところ，スクリーニング株と同様に化合物による活性抑制を見出した．また，得られた新規天然物候補化合物のin vivo効果検証に用いるための細菌感染マウスモデルでの抗菌薬投与解析を行っている．

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”

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. 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．また当部門ではBSL3施設を管理し，感染症災害に対する診断・予防・治療につながる先端基礎研究と人材育成を実施しています．

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, drug susceptibility testing methods, MALDI-TOF MS rapid identification methods, strain preservation methods, infectious disease methods, etc.

病原真菌講習会は，病原真菌・放線菌の基本的取り扱いの知識と技術を習得するために，本センターが実習を中心にして実施し，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，薬剤感受性試験法，MALDI-TOF MS迅速同定法，菌株保存法，感染症法など

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

miRaX Therapeutics K.K.

ミラックスセラピューティクス株式会社

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

1. Development of nucleic acid drugs using miRNA inhibition technology

The miRNA inhibition technology developed by the founders has already been licensed out as a research reagent in many countries and 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 liver diseases.

2. Development of NF-κB inhibitor

Since the transcription factor NF-κB is constitutively activated in inflammatory diseases and many types of cancers and is a promising therapeutic target. However, the molecules targeted by most of the currently available NF-κB inhibitors are located at the cross-road of signal transduction pathway. Therefore, their biological effects are inevitably 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-B.

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, すでに研究用試薬として世界各国で販売されており, 阻害効果の強さや持続の長さで, 高い評価を得ています. この技術を医薬品に応用し, 肝疾患に対する核酸医薬品を生み出すことを目標としています.

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NF-κB阻害剤の多くの標的分子は, シグナル伝達経路の交差点に位置していて, その生物学的効果は広範囲に及んでしまう欠点があります. そこで我々はNF-κBとSWI/SNF複合体をつなぐアダプタータンパク質として転写活性化を担うd4ファミリータンパク質(DPF1, DPF2, DPF3a/b)に着目しました. これまでに, 低分子化合物のスクリーニングを行い, これらのアダプタータンパク質に結合する化合物の同定に成功しており, これらの化合物のNF-κBの阻害活性能の検証を進めています.

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

2021 Fiscal Year Cooperative Research Program Report

令和３年度 共同利用・共同研究報告

研究課題　’21－１

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

我々は近年，慢性アスペルギルス症の特徴の１つであるアスペルギローマに近似した病態を実験的にマウスの皮下に再現したモデルの作成に成功した．本モデルを使用することで，アスペルギローマにおけるアスペルギルスの振る舞いの解析，および生体反応の解析が可能となった．

2021年度は，まず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試みたが，満足の行く結果が得られなかった．そこで組織侵襲に関与する二次代謝産物そのものをターゲットとした計画に変更し，千葉大学真菌医学研究センター臨床感染症分野より分与いただいたgliA（グリオトキシン産生クラスター遺伝子）欠損*Aspergillus fumigatus*, laeA（二次代謝産物産生調節因子）欠損*A. fumigatus*を用い，アスペルギローマ内における*A. fumigatus*の二次代謝産物生合成遺伝子発現解析および二次代謝産物解析を行った．RNA-seq解析により，マウスに留置していない菌球をコントロールとして遺伝子発現量を比較すると，留置７日後の菌球では，コントロールに比して複数の二次代謝産物の生合成遺伝子が高発現であり，特にgliotoxin生合成遺伝子発現が著明に増加していた．これらの結果により，アスペルギローマにおける*A. fumigatus*の二次代謝産物の生合成活性化が示唆された．さらに，菌球内の二次代謝産物を解析すると，野生株のアスペルギローマにおいて，Gliotoxin, Fumagilin, Pseurotin Aの産生が認められたが，Δ laeA株では認めず，*A. fumigatus*が生体内のアスペルギローマで二次代謝産物を産生していることを明らかに出来た．さらに，サイトカイン等の生体反応や，病理学的なアウトカムとの関連性について，解析を継続中である．

研究課題　’21－２

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．

当院肺アスペルギルス症の患者から得られた下気道検体で真菌培養陽性となり形態学的にAspergillus属と診断された126検体で形態学的診断と遺伝子学的診断の菌種名が（狭義で）一致したのは70.6%（89/126），形態学的診断と広義の菌種の菌種名（Complexレベル）での一致は95.2%（120/126）であった．関連種の割合がA. niger complexでは96.8％で，*A. fumigatus*の1.2％に比較し高かった．*A. fumigatus* complexにおいて関連種の割合が少なく，形態学的診断が臨床に与える影響は少ないことが推測された．一方A. niger complexにおいては関連種の割合が多く，更にその関連種はアゾールに低感受性のA. tubingensisの割合が高く，形態学的診断のみでは不適切な抗真菌薬治療になる可能性があると考えられた．

また当院でAspergillus培養陽性の慢性肺アスペル症（CPA）116例の菌種別のアスペルギルス抗体陽性率を検討したところ，fumigatusでは84.8％に対しnon-fumigatusでは37.9％と低く，non-fumigatusが原因のCPAの診断においては注意が必要と考えられた．

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研究課題　’21－３

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

本年度は，計画通りパーシスター全身感染症克服法を開発するために，既存の抗生剤に抵抗性の黄色ブドウ球菌感染症動物モデルを新規に確立した．実験系では，感染直後に抗生剤を投与した場合の効果に比較して，感染後期では，同様の投与量では，抗生剤治療を回避して，生体内で生き残る細菌の集団が出現することが明らかとなり，難治化してしまうモデルが確立できた．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，このモデルを確立することにより，AMED-CRESTの研究課題から新規に見いだされる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，評価が可能となった．

また，COVID-19関連の黄色ブドウ球菌２次感染による肺炎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，パーシスター発生や，抗生剤耐性獲得に関わっていることを見出した．

研究課題　’21－４

Analysis of immunological mechanism for latent infection with *Cryptococcus neoformans* and its reactivation using a murine model

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潜在性クリプトコックス感染と内因性再燃の動物モデルの作成と免疫学的機序の解析

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研究成果

近年，クリプトコックス症は潜在性感染から内因性再燃により発症すると考えられるようになった．しかし，この仮説の真偽を検証した報告はない．申請者らは，本真菌に対するＴ細胞応答を効率的に解析する目的で，その主要なＴ細胞抗原であるchitin deacetylase 2（Cda2）をMHCクラスⅡ拘束性に認識するＴ細胞受容体を高発現するトランスジェニックマウス（CnT-II）を樹立することに成功した．（*J. Immunol.* 205: 686-698, 2020）本研究では，このマウスを用いることで潜在性クリプトコックス感染（LCNI）モデルを作成するとともに，肺内における真菌特異的エフェクターＴ（Teff）細胞やメモリー Ｔ（Tm）細胞の解析を実施した．さらに，LCNIモデルに免疫抑制剤を投与することで内因性再燃モデルの作成を試みた．

CnT-IIマウスの肺内にクリプトコックスB3501株を感染させると，肺内生菌数は徐々に減少し，感染２，３ヶ月後に10^2～10^3CFU/マウス程度で安定した．フローサイトメトリー解析では，肺内のCD4陽性Tm細胞，Teff細胞，エフェクターTm（Tem）細胞が増加するとともに，Teff細胞やTm細胞において細胞内IFN-γの発現が観察された．病理学的解析では，肺内に肉芽腫が多数形成され，その中で真菌を貪食するマクロファージや多核巨細胞とともに，その周囲に集積するＴ細胞と濾胞様構造を形成するＢ細胞が観察された．これらの細胞からは，肉芽腫の形成維持に重要なTNF-αやIFN-γ，MCP-1の発現が確認された．次に，LCNIマウスに臨床で用いられる免疫抑制剤の一つであるタクロリムス（FK506）を投与したが，残念ながら肺内生菌数の再増加として観察される内因性再燃は認められなかった．一方，クリプトコックスをタクロリムスとともにin vitroで培養するとその増殖が有意に抑制された．

本研究において，CnT-Ⅱマウスにクリプトコックスを感染させることで潜在性感染モデルの作製に成功するとともに，肺に形成された肉芽腫内のマクロファージや多核巨細胞に潜伏する真菌周囲にTm細胞やTeff細胞の集積が観察され肉芽腫の形成維持に関与している可能性が示唆された．タクロリムス投与では内因性再燃モデルの作成には至らず，今後は他の免疫抑制剤についても検討する必要があるものと考えられた．

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研究課題　'21－5

Molecular mechanism of microRNA-mediated human defense system induced by RNA viral infection

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RNAウイルス感染によるmicroRNAを介したヒトの生体防御の分子機構解明

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研究成果

哺乳類細胞にウイルスが感染すると，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を誘導して細胞を防御する．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ことが知られている．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．受入教員の米山教授は，ウイルスセンサータンパク質であるRIG-I like receptors（RLRs）としてRIG-I, MDA5, LGP2といった3つの因子を見出している．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．我々はこれまでの研究により，ウイルス感染により発現誘導されたLGP2は，RNAサイレンシングの主要因子であるTRBPと相互作用することで，TRBPによって生合成されるはずであったmicroRNAの生合成を阻害することを見出した．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見出した．本研究では，ウイルス感染と同様のインターフェロン応答を示す，合成核酸であるpoly（I: C）を用いてLGP2とTRBPの相互作用を介したmicroRNAの制御による遺伝子発現ネットワークの全貌の解明を目指した．その結果，LGP2-TRBP相互作用によって生合成過程が抑制されるmicroRNA群は特徴的な二次構造をもつことを明らかにした．すなわち，塩基対合確率（Base pairing probability）の高いsiRNAはTRBPと結合しやすいことを明らかにした．さらに，それらが発現制御するmRNA群をウェブツールによる予測とRNAシークエンシングによる予測を組みあわせることによって特定し，microRNA-mRNAネットワークの詳細を明らかにした．poly（I: C）によって駆動するmicroRNAを介した遺伝子発現制御ネットワークは，極めて複雑であるにもかかわらず，最終的にはアポトーシス経路に収束するという

興味深い結果が得られた．本成果は現在論文投稿準備中である．

研究課題　’21－6

Transcription regulation of antifungal drug resistance and biofilm formation in Candida glabrata: aiming improved diagnosis and therapeu

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研究成果

To understand the molecular basis of azole drug resistance in *C. glabrata*, this collaborative study focused on *CgMar1*（Multiple Azole Resistance 1）, a transcription factor encoded by ORF CAGL0B03421g, to investigate its role in azole resistance and its association with biofilm formation. The results of RNA sequencing showed that the biofilms were associated with azole resistance. First, RNA sequencing revealed that *CgMar1* regulates 337 genes under fluconazole stress, including genes related to lipid biosynthetic pathways. Among these, *CgMar1* and its target, *CgRSB1*, encoding a sphingoid long-base efflux transporter, were found to contribute to plasma membrane sphingolipid uptake and membrane permeability, reducing fluconazole accumulation *CgMar1* was found to be associated with the promoter of *CgRSB1* and bound, and a promoter containing two CCCCTCC consensus was found to be required for *CgRSB1* activation during fluconazole stress. This suggests that the pathway regulating azole sensitivity in *C. glabrata* is due to the neofunctionalization of Hap1-like transcription factors. However, the *Δ cgmar1* strain showed no change in biofilm formation in RPMI medium compared to the parental wild strain. These results were summarized in the following paper.

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研究課題　’21－7

Search for anti-fungal seeds using genetically engineered *Candida glabrata* library

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Aspergillus属菌の二次代謝に影響を及ぼすマイコウイルスの探索と解析

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研究成果

本共同研究は令和元年度より千葉大学 共同利用・共同研究のテーマとして開始された．またAMED BINDS支援課題としても進めている．深在性真菌症の適応薬は，核酸合成を阻害するピリミジン系，エルゴステロールと結合するポリエン系，エルゴステロール合成酵素の阻害剤であるアゾール系，細胞壁合成阻害剤であるエキノキャンディン系の４系統しかなく，いずれも副作用や耐性菌の出現が問題になっており，新規の作用機序をもつ抗真菌薬の開発が必要である．そこで，本研究では，大村智記念研究所が保有する化合物ライブラリーを用い

たスクリーニングによって得られた候補化合物の薬剤標的分子を同定し，新規抗真菌薬の創出をめざしている．

令和元年度には，600種類の天然化合物コレクション（大村ライブラリー）に対して*Candida glabrata*の薬剤高感受性株を用いて生育阻害活性を指標に一次スクリーニングを実施した結果，80サンプルに生育阻害活性が確認され，二次スクリーニングへと移行した．二次スクリーニングでは，48種類の病原性真菌について，生育阻害活性を測定し，それぞれの菌種についてMICを決定した．さらに，培養細胞を用いた呼吸阻害活性を測定した．これらの結果より，８サンプルが真菌特異的に阻害活性を有しており，抗真菌薬シーズ候補として選抜した．

令和２年度には，抗真菌薬シーズ候補として選抜した８種の化合物について*Candida glabrata*遺伝子組み替え体ライブラリーを用いた標的分子探索を実施し，２種類について特異的な候補遺伝子を抽出することができた．

令和３年度には，抗真菌薬シーズ候補として選抜した８種の化合物について*Candida glabrata*感染マウスモデルにおけるin vivo治療実験を実施するために評価系の構築を行った．*C. glabrata*は病原性が低いためマウスの生存率を得るためには，免疫抑制剤（シクロフォスファミド）の投与が必要であるが，投与量の調整が難しいため詳細な調整を行った．その結果，シクロフォスファミドを菌体接種前に175mg/kg ２回投与しながら３回目の投与を決定する方法を取った．これにより，マウスのロットによる感受性の相違への対応が可能となった．並行して感染実験に必要な量を確保するために候補化合物の発酵と抽出を実施した．また令和２年度に引き続き有望な抗真菌薬シーズの標的分子について検証実験を進めている．

研究課題　’21－８

Rewiring of the regulatory circuitry underlying the expression of key fitness attributes in major fungal pathogens of humans

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病原真菌における重要な適応特性の発現を支える制御回路の再解析

研究成果

本プロジェクトは，Candida glabrataとCandida albicans栄養素とストレスの適応を制御する制御回路の再構築を目的にしており，まずは本研究の発展のために英国の大規模な助成金であるWellcome Trustの獲得に向けて，予備実験と綿密なメール会議を繰り返すことによって申請書の作成が主な共同研究となった．予備実験については，前年度は千葉大学真菌医学研究センターにおいて，組換え体コレクションの中から1,000株を用いてハイスループット解析の条件検討を進めた．多数の組み換え体を混合した状態で各株の優占率を測定する既知の方法を構築した．令和３年度は同手法を用いて，マウスにC. glabrataの遺伝子組み換え体1,000株を同時に感染させ１週間後に臓器を回収し各菌株の占有率を測定する実験系を構築した．この手法を基盤としたWellcome Trustへの研究提案書を申請した．研究提案書の審査は第三ステージまで達したが，最終審査で不採択となった．今後，更に予備実験を積み重ね再提案することになった．

研究課題　’21－９

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

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

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研究成果

2019年末に出現した新型コロナウイルスによる急性呼吸器感染症（COVID-19）は，瞬く間に世界中に拡大し，今尚猛威を奮っている．病原体は１本鎖RNAをゲノムとするウイルスSARS-CoV-2で，変異を繰り返しながら新たな流行の波を起こしている．最近mRNAワクチンが開発され，大きな予防効果が示されたものの，感染やワクチン接種によって成立した免疫から逃避するように新しい変異株が出現するため，常に流行株の状況を監視する必要がある．治療薬については，幾つかの薬剤が臨床の現場で使われ重症化阻止に貢献している．しかし，完全な治療法として定着している薬剤は未だない．そこで本研究では，SARS-CoV-2の分離と遺伝子解析法を確立すると共に新規治療薬探索に向けた基礎研究として様々な化合物の抗ウイルス活性を評価することを目的とした．

先ずVERO-E6/TMPRSS2細胞を用いたウイルス分離法を確立し，千葉大学附属病院の入院患者鼻腔拭い液を出発材料として当地で流行した種々の変異株（R1系統株，α変異株，δ変異株，オミクロン変異株など）計20株の分離に成功した．遺伝子解析については，特にSpike遺伝子領域を中心に感度の良いOneStep-RT-PCR法を確立し，国立感染研から分与された標準６株（β，γ変異株など）を含めて計40株以上の解析を行った．

治療薬探索としては，既知のレムデシビルやナファモスタット（各々10 μM）に加え，クロロキン，酢酸アビラテロン，ダクラタスビル，イベルメクチンのいずれかを添加（5 μM）すると，クロロキンでは10^3 $TCID_{50}$まで，他の３種類の薬剤では10^5 $TCID_{50}$までの高ウイルス量に対して抑制効果があることが判明した．単剤ではなく複数の抗ウイルス剤併用による治療という新しいアプローチの可能性が示されたものと考える．

研究課題　’21－10

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

ASP2397は，アステラス社の研究グループによって真菌アクレニウム・ペルシシヌムから単離・構造決定された新規シデロフォア型抗真菌薬である．理研グループは，環状アミノ酸構造の側鎖に関する構造活性相関研究を進め，ASP2397に匹敵する抗真菌活性を有する新規誘導体合成に成功した．新規ASP2397誘導体について薬効薬理評価を進めたところ，*in vitro*抗菌活性試験ではいくつかの菌種において，*in vitro*での活性がASP2397を上回り，マウス感染モデルを用いた薬効試験においてASP2397と同等以上の効果が示されていた．本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．その結果，*C. glabrata*に対してASP2397と比較し誘導体が約２倍の強い活性（MIC: 0.03 μg/ml）が確認された．さらに*C. auris*に対してはAPS2397がMIC ＞50 μg/mlに対して

新規誘導体ではMIC: 1 – 4 μg/mlの新規活性が示された. *C. auris*については, CDCやAMEDで最も注目すべき薬剤耐性菌としてリストアップされており〈https://id3catalyst.jp/apid/list.html〉本誘導体の期待は大きい. 現在, マウスを用いて新規誘導体の*in vivo*での薬効評価実験を進めている. 研究成果としてこれまでの実験結果をもとにPCT特許を出願し, 企業連携体制の構築を進めている.

研究課題　’21－11

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

白癬菌Trichophyton rubrum TIMM20092は, 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）に低感受性を示す. 申請者らは, 本株のITC・VRC低感受性化の要因の解析を進める過程で, 細胞外へのアゾールの排出との関連が示唆される6個の薬剤排出トランスポーター（ポンプ）を見出した. そこで, 遺伝子工学的手法を用いて, これらの分子が細胞外に排出する薬剤（基質）の解析を実施したところ, major facilitator superfamilyに属するMFS1にはフルコナゾール（トリアゾール）に加え, クロラムフェニコールとその誘導体の排出機能があることがわかり, 白癬菌の元々の性質であるクロラムフェニコール耐性の一端をMFS1が担っていたことが判明した.

Trichophyton indotineaeは, 数年前に新種として提案された人好性白癬菌である. 申請者らは, 日本国外で分離された多数のT. indotineaeを対象に, ITCとVRCに対する感受性調査を実施し, 薬剤低感受性が認められる複数の株を見出した. そこで, これらの株に共通する薬剤低感受性化の要因を解析し, 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遺伝子に繰り返し重複が起こり, ゲノム中でタンデムリピート化したことによりCYP51Bが過剰発現したことが主な原因であると結論づけた. 病原真菌ではこのような薬剤低感受性機構の報告がなく, 新規メカニズムの発見となった.

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研究課題　’21－12

Comprehensive analysis of effect of mycovirus on mycotoxin production

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マイコウイルスによるカビ毒産生に対する影響の体系的解析

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研究成果

マイコウイルスは糸状菌宿主に持続的に感染し，時として生理機能に影響を及ぼすことが報告されている．特に近年は，糸状菌二次代謝に対するマイコウイルスの影響に注目が集まっており，2020年には植物病原菌イネいもち病菌の毒素の一つであるテヌアゾン酸の生産が，マイコウイルスの感染により促進されることを当グループが発見した．糸状菌の毒素産生に対するウイルスの影響は，より広範な菌株においても起こる可能性が考えられ，体系的解析が必要となる．そこで本研究では，食品汚染の原因菌となる３種のカビ毒生産菌を対象として，ウイルス感染株のスクリーニングを実施した．

千葉大学真菌医学研究センター所蔵のA. flavus（72株），A. parasiticus（20株），A. ochraceus（38株）を対象に，RNAウイルス感染の指標となるdsRNAを電気泳動により検出することでウイルス探索を実施した．その結果，それぞれ８，５，25株のウイルス感染株が見つかった．続いて，A. flavusの感染ウイルスに着目し，そのゲノム配列をFLDS法により明らかにし，感染ウイルス種を同定した．さらに，抗ウイルス化合物を利用してウイルスの脱離を試み，フリー化株を得た．ウイルス感染株とウイルスフリー化株のセットにおいて，生育，胞子形成能，二次代謝プロファイルを比較した．元来，アフラトキシンの生産能を持たない株が多数含まれており，カビ毒生産への影響について確定的なデータは得られなかった．その一方で，培養抽出物のHPLC解析において，ウイルス感染の有無による差異が認められたセットが存在し，マイコウイルスが宿主糸状菌の二次代謝に影響を及ぼすことが再確認された．

研究課題　’21－13

Antibacterial/antimicrobial activity analysis of newly developed macrolide antibiotics

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新規マクロライド系抗菌剤の抗真菌活性ならびに抗細菌活性研究

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研究成果

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

2021年度は，過去に実施した薬剤感受性測定結果と構造活性相関解析結果に基づき，候補化合物の絞り込みを行い，合成法をより簡略化し短工程で供給可能とした新規ユーシェアリライド類縁体の製造を行い，in vitro活性

試験の実施ならびにin vivo用大量合成法の確立を試みた．その結果，検討したユーシェアリライド類縁体（EU-TON, EU-TON-OH, EU-N-2）のうち，EU-TONにおいて，*Candida albicans*と*Aspergillus fumigatus*に対する抗真菌活性が認められた．

研究課題　’21－14

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．2020年度までに，*ATG32*の転写調節に必要なプロモーター領域の同定，その領域に結合する転写因子候補遺伝子の同定，候補タンパク質の遺伝子破壊株を作製，解析を行った結果，エキソリボヌクレアーゼ*XRN1*が鉄依存的リプレッサーとして働くことが示唆された．また，ウエスタンブロットおよびクロマチン免疫沈降アッセイを行った結果，*XRN1*は鉄依存的に*ATG32*上流領域に結合していることが示唆された．2021年度は*XRN1*の遺伝子破壊株と野生株について鉄欠乏による発現変動を比較するため，RNA-seq解析を行った．*XRN1*破壊株では野生株と比較してミトコンドリア関連遺伝子の発現が特に大きく（ミトコンドリア関連の218遺伝子が４倍以上に増加）増加していることが明らかになり，*XRN1*は*ATG32*だけではなく，複数遺伝子の発現調節に関与していることが明らかとなった．また，ウエ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局在タンパク質の量が増加しており，ミトコンドリア染色試薬を用いた顕微鏡観察の結果からもミトコンドリア量の増加が示唆された．

研究課題　’21－15

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

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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

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研究成果

近年我々が見出した分泌性環状ペプチド生合成因子は，ほぼ全てのカビ・キノコに広く多様に保存されており，小胞体への移行シグナルペプチドと高い繰り返し配列を持つ．その糸状菌子嚢果形成への影響を解析するため，糸状菌*Aspergillus nidulans*および*Neosartorya fischeri*について，千葉大学真菌医学研究センターが保有する株から，子嚢果形成度の異なるものを昨年度の各２株に加えてさらに各２株ずつ選抜し，ゲノム解析を行った．結果，*A. nidulans*における３つの当該因子のうち１つについて，子嚢果形成度と配列繰り返し回数が相関していることを見出した．そこで，本因子について，子嚢果形成条件下で遺伝子発現を調べたところ，子嚢果形成度に相関して遺伝子発現が上昇していた．さらに，本因子を子嚢果形成度の高い系列株から破壊したところ，限定的結果ではあるが，子嚢果が形成されなくなったことから，本因子が子嚢果形成に関与することが示唆された．

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研究課題　’21－16

Establishment of an assay to evaluate water-insoluble trichothecene toxicity in vitro

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難水溶性トリコテセン類のin vitroでの毒性評価法の確立

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研究成果

トリコテセン類は，食の安全を脅かすカビ毒として知られている．ムギ類で赤カビ病を引き起こす病原菌がトリコテセンの一種であるデオキシニバレノールを産生することがよく知られている．日本でも，天候不順等による赤カビ病発生およびそれに伴う小麦の質的低下がデオキシニバレノール汚染につながることから，非常に重要視をされるカビ毒である．トリコテセン類は200種以上の化合物群であることも一つの特徴である．これらの毒性を評価・比較する方法として，動物や培養細胞を用いた方法が報告されている．2020年度の研究では水溶性のトリコテセンであるデオキシニバレノールを用いて，0.5mLPCRチューブでの無細胞系のタンパク質合成システムを用いて，翻訳反応の阻害活性を迅速・簡便に測定する方法を確立した．一方で，難水溶性トリコテセン類については有機溶媒に溶解する必要があり，2020年度に確立した方法で同様に測定ができるか，不明であった．そこで，有機溶媒に溶解したトリコテセン類を用いて，無細胞系のタンパク質合成システムによる毒性評価が可能であるかどうかを検討した．

本研究ではアセトニトリルに溶解したデオキシニバレノールと難水溶性トリコテセンであるT-2トキシンおよびHT-2トキシンをこれまでに確立した方法に適用し

た. その結果, アセトニトリルに溶解したトリコテセン類でも本法が利用可能であることを明らかとした. アセトニトリルに溶解したトリコテセン類が利用できることは本法の応用範囲を大幅に広げるものである. なお, これらの成果は2021年にToxins誌にて報告した.

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研究課題　’21－17

Analysis of antimicrobial susceptibility, drug resistance, and pathogenic genes of major pathogenic bacteria derived from pediatric clinical specimens.

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小児臨床検体由来の主要病原細菌の抗菌薬感受性と薬剤耐性, および病原遺伝子に関する検討

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研究成果

小児用キノロン製剤（TFLX）のインフルエンザ菌臨床分離株に与える影響を調べる目的で, 千葉県こども病院において2010年～2018年の期間に, 小児から分離されたインフルエンザ菌のうち, TFLXのMIC ≧0.5 μg/mLの16株について, キノロン耐性決定領域（GyrA, GyrB, ParC, ParE）変異の有無について検討を行った. その結果, キノロン薬の使用の有無にかかわらずキノロン低感受性株であってもキノロン耐性決定領域の１か所および２か所の変異がみられる株が多くみられており, 同一のsequence typeが存在することが判明し, 耐性変異株が水平伝播していることが示唆された.

黄色ブドウ球菌については, 県内複数施設からの小児の臨床由来株の収集を行っており, 各種トキシンの有無について解析を行い, 発表を予定している.

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Emergence of *Haemophilus influenzae* with low susceptibility to quinolones isolated from pediatric patients in Japan.
J Infect Chemother. 2021 Mar 1: S1341-321X(21)00062-3. doi: 10.1016/j.jiac.2021.02.022.

研究課題　’21－18

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. This study is

act as baseline data of clinical profiles of pediatric IPD in Dr. Sardjito General Hospital in Yogyakarta, Due to COVID-19 pandemic, we postponed the study. We applied for continuing ethical clearance for this study again, and will continue to recruit the patient June 2022. We will improve our bacterial culture system. During vacuum period, we searched secondary data from medical record of pediatric patients who have the positive culture of S. pneumoniae. We prepared the manuscript entitled "Pediatric invasive pneumococcal disease in Yogyakarta, Indonesia: A case series", and submitted it in Global Pediatric Health.
eaay4068. 2020.

研究課題　'21－19

Screening of the antifungal substance from the plant extract library

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植物エキスライブラリーを用いた抗真菌活性物質のスクリーニング

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研究成果

国立研究開発法人 医薬基盤・健康・栄養研究所は，国民の健康の保持及び増進に関する調査，研究，国民の栄養その他国民の食生活に関する調査及び研究等を行うことにより，公衆衛生の向上及び増進を図り，国民保健の向上に資することを事業目的としている．薬用植物資源研究センターは，薬用植物に関する国内唯一の総合研究センターとして，野生植物を中心とする植物や生薬，コケ類のエキスを約10,000種類保存したエキスライブラリーを保有しており，様々な疾患治療薬の探索源としても用いられてきたが，これまで抗真菌活性物質の探索源として体系的に使用された実績はなかった．そこで今回，真菌を用いて網羅的なスクリーニングを行った．一次スクリーニングとして，実験的取り扱いが簡便で薬剤耐性の問題が生じている*Candida glabrata*の液体培地中で濁度を指標にして生育阻害活性を測定した．その結果，10,000種類のエキスサンプルのうち233サンプルで阻害活性が確認された．今後，ヒト培養細胞に対する生育阻害活性や，その他の病原真菌に対する生育阻害活性が確認されたサンプルについては，分画精製を進め物質を同定する予定である．

研究課題　'21－20

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た．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）．

これまでの解析の結果，千葉市における国事業の実施状況は約40,000人（対象者の約30％）が抗体検査を受け，8,000人（抗体陰性者の約90％）がワクチン接種を受けている状況である．抗体検査，ワクチン接種共，土曜日に医療機関を受診している人が多かった．一方，千葉市事業に関しては，現在までこの事業を利用し，約9,500人が抗体検査を受け，約5,600人がワクチン接種を受けていた．千葉市事業には，産婦人科が多く関与していた．

これらの結果は毎月１回ニュースレターとして千葉市および千葉市医師会にフィードバックしている．また，中間報告を論文公表し，第25回日本ワクチン学会学術集会において発表した．共同研究の成果は，千葉市の作成した市民向け風疹対策広報用ポスターに紹介されている．

MRワクチン５期定期接種の延長が決まったこともあり，千葉大学・千葉市・千葉市医師会の３者で今後の市民への啓発方針に協議を行うことを予定している．

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Hum Vaccin Immunother. 2021; 17(6): 1779-1784. ’20－22

研究課題　’21－21

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．2021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の*A. fumigatus*への形質転換効率を検討した．

昨年度は，１つのライブラリ中に10,371種類の配列が含まれていることが確認できたCRISPRライブラリを大腸菌で構築できた．次の段階として，*A. fumigatus*に形質転換する必要があるが，通常のprotoplast-PEG法による形質転換では効率が低く，スクリーニングに必要なライブラリスケールを得るために，より効率の高い形質転換方法が求められた．その方法の候補として，エレクトロポレーション法について様々な条件検討を行った．使用機種として，ThermoFisher社のNeon systemおよびBTX社のECM600を用いた．電気パルスの形状・電圧・時間等の様々な条件検討を行っており，より良い条件を確立すべく検討を継続中である．今後，作製したCRISPRライブラリを*A. fumigatus*に高効率に形質転換し，CRISPRスクリーニング法を確立することにより，血清刺激に応答するシグナル伝達機構の解明を目指す．

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研究課題　’21－22

Search for new antifungal drug seeds from unutilized biological resources

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未利用生物資源を利用した新たな抗真菌薬シーズの獲得

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研究成果

本研究では，海綿動物や渦鞭毛藻など，十分に研究が行われていない未利用生物資源を素材として，深在性真菌症の原因真菌に対して抗真菌活性を示す新たな生物活性天然物の探索を行った．

その結果，*Agelas*属，*Dysidea*属，*Hippospongia*属，および*Pseudoseratina*属と*Suberea*属の海綿動物からそれぞれ単離した１個の新規ジテルペノイド，１個の新規ポリヒドロキシステロール，５個の新規メロテルペノイド，３個の新規ブロモチロシンアルカロイド，ならびに３種の*Amphidinium*属渦鞭毛藻から単離した２個の新規マクロリドおよび１個の長鎖ポリケチドについて，各種スペクトルおよび計算化学により得られたデータに基づいて構造解析を行い，平面構造および一部の立体化学を明らかにした．

これらのうち，*Dysidea*属の海綿動物から単離したポリヒドロキシステロールは，４種の真菌（*Candida albicans, Trichophyton mentagrophytes, Aspergillus niger, Cryptococcus neoformans*）に対してIC_{50} 8～16 μg/mLで抗真菌活性を，３種の細菌（*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis*）に対してMIC ４～16 μg/mLで抗細菌活性を示すことが明らかになっている．今後，他の化合物についても抗菌活性を評価する予定である．

研究課題　’21－23

Editing by CRISPR-Cas9 of ergosterol biosynthesis *in Aspergillus fumigatus*: Effects of sterol composition on fungal growth and development

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ゲノム編集を用いた*Aspergillus fumigatus*におけるerogsterol生合成遺伝子の機能解析

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研究成果

Mutations in cyp51A are considered important as azole resistance mechanism in *A. fumigatus*. On the other hand, the impact of a loss of function of Cyp51A on azole sensitivity has not been discussed in depth. In this study, we identified environmental isolates with an insertional mutation（insertion of four GTGG sequences between L464 and G465）and a clinical isolate with a nonsense mutation（change of the sequence of G395 to a stop codon）in cyp51A, respectively. The MICs（in μg/ml）of each isolate were fluconazole（FLCZ）32, itraconazole（ITCZ）0.25, and voriconazole（VRCZ）0.25 for the insertion mutant strain and FLCZ 32, ITCZ 0.25, and VRCZ 0.5 for the nonsense mutant strain. To verify the correlation between loss of Cyp51A function and MIC, we transformed the laboratory strain AfS35 using the CRISPR/Cas9 system. The increase of azole susceptibility was also observed in the transformants with the cyp51A region in each strain, with FLCZ 32, ITCZ 0.25, and VRCZ 0.25 in the mutant insertion strain and FLCZ 32, ITCZ 0.25, and VRCZ 0.25 in the nonsense mutant strain. These results are similar to the previously reported increase of the azole susceptibility in Δcyp51A strains, suggesting that loss of Cyp51A function contributes to the increased azole susceptibility.

研究課題　’21－24

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

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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（serotype replacement）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．

2021年度は，本共同研究で解析を行った基礎疾患のない血清型10A sequence type（ST）11189のペニシリン耐性肺炎球菌による髄膜炎例について論文を作成した．

また，胆道閉鎖症に対して生後半年に生体肝移植が施行されシクロスポリンを内服中の３歳児の尿からムコイド型肺炎球菌が分離された症例について，分離菌の解析と罹患前後での血清中の特異抗体価，オプソニン（OPA）活性を測定した．本症例では尿から肺炎球菌のみが分離され，治療反応性と罹患後の特異抗体価とOPA活性の上昇を認めた点を併せて肺炎球菌による尿

路感染症と診断した．臓器移植後で免疫抑制剤内服中であったこと，ワクチンによる特異免疫が誘導されにくい血清型３型肺炎球菌が起炎菌であったことが，本症例でワクチン接種後にも関わらず同菌による尿路感染症を発症した原因と考えられた．本解析結果について学会発表し，論文化を進めている．

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Jpn J Infect Dis. 2021 Sep 22; 74(5): 477-480. doi: 10. 7883/yoken.JJID.2020.841.

研究課題　’21－25

Search for effective anti-fungal seeds against *Aspergillus* in serum

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血清中で有効なAspergillus症治療薬シーズの探索

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研究成果

寒天培地上で*Aspergillus*に対する強力な抗真菌活性を示す化合物が見出されても，血清中で活性が低下または消失する場合がある．そこで，初期スクリーニングで血清培地による拡散法を導入することで疑陽性を排除する．その際に，真菌医学研究センター・バイオリソース管理室で保有する臨床分離*A. fumigatus*およびその関連菌３種を対象に，大村智記念研究所・創薬グループが保有する真核生物寄生菌類を中心とする微生物ライブラリーを用いて新たな抗真菌薬シード化合物の探索を行うことを目的とした．

・１次スクリーニング通過基準：*A. fumigatus* に対し10 mL/discで生育阻害を示す
・２次スクリーニング通過基準：４種の*Aspergillus*に対し10 mL, 50 mL/discで濃度依存的に生育阻害を示す

２次スクリーニングまで通過した菌株から新規化合物３成分を含む全14成分を活性成分として取得した．14成分の骨格は６系統に分類されるため，代表６成分について血清の有無で活性の変化を評価した．その結果，中程度の活性を示したCladobotric acid Eは血清中でもほぼ同等の活性を維持したが，Ophioborin K, F2928-1および(－)-*N*-Hydroxyapiosporamideは活性が完全に消失した．今回取得した中で最も強い活性を示したPhoslactomycin EおよびFは，血清中で活性が低下したものの，依然として強い活性を示す結果となった．血清中で活性を示した３成分はin vivo試験での成果が期待される．

研究課題　’21－26

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

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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

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（千葉大学真菌医学研究センター）

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（nontypeable 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イルム産生能が低かった．この結果をふまえ，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る．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log102.0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．ともに1株ずつの解析であり，今後複数の菌株を用いて解析を行う．今後，バイオフィルムを維持するシステムにおいてauto-inducerとして働いている他のfactorの検討を継続し，侵襲性NTHi感染症の病態解明を行い，予防策に繋げたい．現在，本研究成果について論文を作成中である．

研究課題　’21－27

Characterization and ecological survey of phomopsin-producing fungi

Toshiki Furuya
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（Medical Mycology Research Center, Chiba University）
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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

古屋俊樹
（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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（国立医薬品食品衛生研究所）
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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，*Beauveria bassiana*の培養液中の代謝産物成分を固相カラムにより濃縮し，昨年度までに確立した手法によるLC-MS分析を繰り返したところ，ホモプシン類と推定される化合物由来のイオンシグナルが再現よく検出された．さらに，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が，現在までのところ構造決定には至っていない．引き続き，濃縮・精製法や分析条件等を詳細に検討し，構造決定を試みる．一方，北海道に自生するマメ科植物のルピナスを採取し，これらのルピナス試料について成分抽出を行い，LC-MS分析に供した．その結果，いくつかの試料でホモプシン類の存在を示唆する化合物イオンシグナルが検出された．今後は，これらの試料についてより詳細な解析を進める．

感染症研究グローバルネットワークフォーラム2022

The 9th 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10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11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
萩原大佑（筑波大学生命環境系）
渡辺　哲（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）
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）
米山光俊（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，2022年度で第９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世話人となり，「真菌」をテーマとし，現在世界的に注目されている真菌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．病原真菌の薬剤耐性からその病原性機構まで、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，感染時の宿主との相互作用のみならず，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 28題の発表が，二日目は招待講演９題（日本・カナダ・イギリス・アメリカ・中国）の発表が行われた．二日間で延べ125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：萩原大祐先生（筑波大学）
１．**Dr. Robert A. Cramer（Geisei School of Medicine, USA）**
“*Aspergillus fumigatus* biofilms: Form and Function in Disease”

座長：渡邉　哲先生（千葉大学真菌医学研究センター）
２．**Dr. Donald Sheppard（McGill University, Canada）**
“Understanding the role of galactosaminogalactan in *Aspergillus* virulence: a sticky problem”

座長：知花博治先生（千葉大学真菌医学研究センター）
３．**Dr. Suzanne Noble（UCSF, USA）**
“*Candida albicans* is a fungal pathobiont of mammalian hosts”

【午後の講演】
午後の講演：Afternoon Session

座長：渡邉　哲先生（千葉大学真菌医学研究センター）
４．**萩原大祐先生（筑波大学）**
“Antifungal-resistant strains of *Aspergillus fumigatus* in the agricultural environment”
５．**Hazim O. Khalifa（United Arab Emirates Universit）**
“Genetic Basis and Genotyping of Non-*albicans Candida* in Japan”

座長：後藤義幸先生（千葉大学真菌医学研究センター）

6．**Fabio Seiti Yamada Yoshikawa（Chiba University）**
"Revisiting the role of C-type lectin receptors in the immune response to *Aspergillus fumigatus*"

7．**目黒和行先生（千葉大学）**
"SBNO 2 deficiency, a novel inherited immunodysregulatory syndrome, reveals new molecular mechanisms in host defenses"

座長：高橋弘喜先生（千葉大学真菌医学研究センター）

8．**Xiao-wen Wang（Beijing Hospital, China）**
"New insights on the pathogenicity of Trichophyton mentagrophytes complex"

9．**Darius Armstrong-James（Imperial College London, UK）**
"Phenotypic and clinical correlates of genomic diversity in *Aspergillus fumigatus*"

【閉会の挨拶】

高橋弘喜先生（千葉大学真菌医学研究センター）

2022 Scientific Meetings & Seminars

2022年講演会

「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　2022年度成果報告会」

日時：令和５年３月13日（月）～３月15日（水）
場所：ハイブリッド開催

令和５年３月14日（火）
【特別講演】
高橋大介（慶應義塾大学准教授）
「有機化学を基盤とした人獣共通感染症に対する糖鎖医薬開発への挑戦」

【合同成果報告会（千葉大学真菌医学研究センター）】
後藤憲志（久留米大学）
「無莢模型インフルエンザ菌による侵襲性感染症の病態解析」

平塚知成（理化学研究所）
「シデロフォア型抗真菌薬誘導体の薬効試験」

田代将人（長崎大学）
「アスペルギローマの病態解析 」

松岡悠美（大阪大学）
「パーシスター全身感染症克服法の開発」

【領域３：感染症・免疫共同研究領域】
森田英明（国立成育医療研究センター研究所）
「アレルギー性炎症の抑制に関与する新規自然リンパ球サブセットの探索」

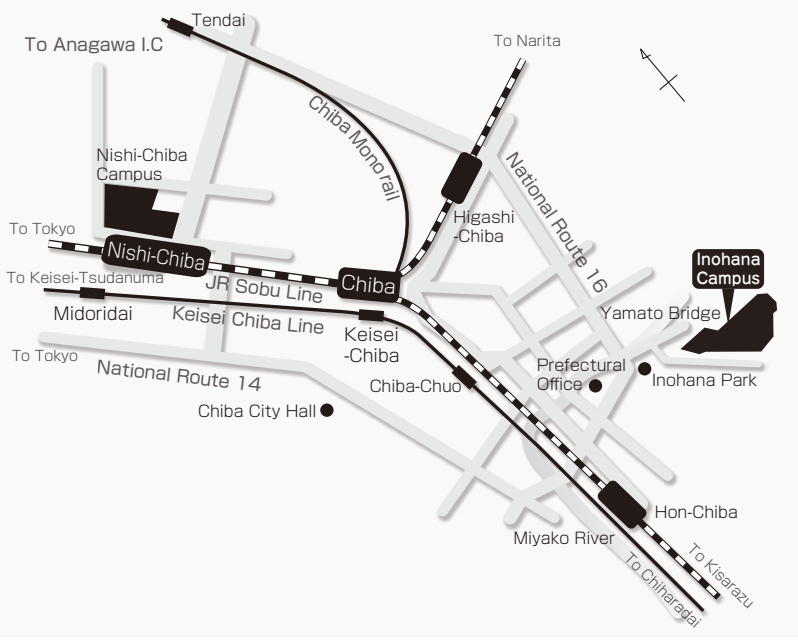
澤　洋文（北海道大学）
「新型コロナウイルスの感染病態解明に向けた研究」

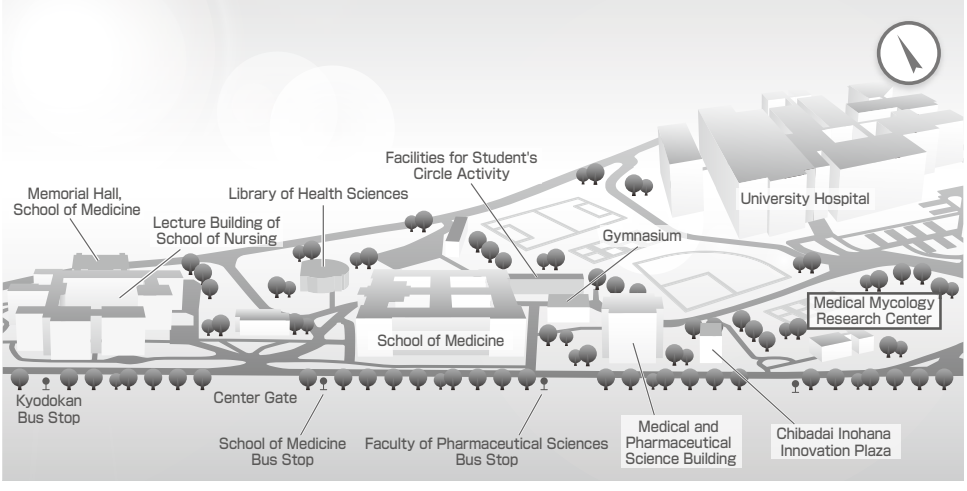
川合　覚（獨協医科大学）
「ボリビアリスザルを用いた熱帯熱マラリアのin vivo および in vitro 実験系の確立」

渡辺登喜子（大阪大学）
「鳥インフルエンザウイルスのリスク評価に資する研究」

「真菌医学研究センター セミナー」
オンライン開催

日時：令和４年４月19日（火）16時～17時
講師：国立感染症研究所
　　治療薬・ワクチン開発研究センター　第十室
　　氣駕恒太朗室長
　　「細菌を翻弄するバクテリオファージ―薬剤耐性菌問題の救世主になり得る」





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