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# MMRC

Center Medical Mycology Research

## ANNUAL REPORT OF MEDICAL MYCOLOGY RESEARCH CENTER, CHIBA UNIVERSITY 2021

千葉大学　真菌医学研究センター　報告

# 25

令和3年

# Content
# 目　　次

# Preface for Annual Report for 2021

Major challenges facing a super-aging society include rising numbers of immunocompromised hosts and patients with pneumonia, particularly those with chronic obstructive pulmonary disease (COPD). Moreover, the dramatic increase in worldwide trade, which has led to the spread of severe fungal infectious diseases, is being recognized as a key issue concerning the aging population. In addition, the increased incidence of pulmonary aspergillosis in patients with COVID-19 has become a new issue since last year. The Medical Mycology Research Center (MMRC) at Chiba University has become increasingly important because it serves as a research organization and promotes educational activities to raise public awareness.

Under these circumstances, MMRC serves as a hub for research on infectious diseases, immunity, and pathogens at Chiba University, and also acts as a joint usage and research center for topics such as infectious diseases, immunity, and information life sciences centered on pathogenic fungi. In 2016, we were recertified by the Minister of Education, Culture, Sports, Science and Technology, and we are actively engaged in joint usage, joint research, and educational activities with universities, medical institutions, and companies nationwide. The MMRC group centered on the field of clinical infectious diseases was adopted by the Japan Agency for Medical Research and Development (AMED) for the Science and Technology Research Partnership for Sustainable Development (SATREPS), and since 2016, it has collaborated with the Faculty of Medicine of Campinas University, Brazil. In the SATREPS project we were able to make great strides in elucidating the mechanisms of infectious diseases caused by drug-resistant fungi in the field. In addition, as a National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology, MMRC has been engaged in activities such as cell culture collection, preservation, genome analysis, and the distribution of pathogenic fungi and actinomycetes to researchers. In parallel with these projects, we are promoting basic, product development, and clinical research by independent research group leaders (referred to as primary investigators, or "PIs"), as well as collaborative joint research, including young researcher exchanges, with domestic and overseas research groups. In 2014, the clinical infectious disease research group opened an outpatient clinic at Chiba University Hospital that specializes in fungal diseases; this was the first such clinic in Japan. In 2015, we established a BSL-3 facility specializing in highly pathogenic fungi, and in 2018 we also launched a germ-free animal facility to strengthen our ability to respond to future research issues related to fungal infections. Incidentally, the only BSL-3 facility on campus has been supporting SARS-CoV-2–related PCR testing by the Division of Laboratory Medicine, Chiba University Hospital, since the outbreak of the coronavirus pandemic in 2020. We are also participating in the "Research Institute of Disaster Medicine," which launched in 2021, and will start research and treatment of infectious diseases associated with large-scale disasters and pandemics such as COVID-19.

Accordingly, we envision MMRC as the leading scientific research institution in Japan devoted to excellence in microbiology and immunology, clinical fungal infectious disease research, and the provision of key resources for research on pathogenic fungi and actinomycetes, with the ultimate goal of advancing the field of medical mycology and infectious diseases.

January, 2022

Chihiro Sasakawa PhD
Director of MMRC

# はじめに

我が国はすでに超高齢社会に突入し，高度医療や生活習慣病に起因した日和見感染症，慢性閉塞性肺疾患（COPD）をはじめとする呼吸器病における真菌・細菌感染症は増加の一途を辿り，また経済のグローバリゼーションに伴う輸入真菌症など，真菌症をはじめとするさまざまな感染症の脅威に直面しています．さらに昨年来のCOVID-19感染症患者に合併する肺アスペルギルス症も新たな課題となっています．

このような状況で，本センターは，千葉大学の感染症・免疫・病原体研究のハブとして，さらには病原真菌を中心とする感染症・免疫・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平成28年度に文部科学大臣より再認定を受け，全国の大学，医療機関，企業などと緊密に連携して，共同利用・共同研究，教育活動を積極的に行っています．本センターの臨床感染症分野を中心としたグループは，日本医療開発機構（AMED）による地球規模課題対応国際科学技術協力プログラム（SATREPS）に採択され，平成28年度からブラジル・カンピーナス大学医学部と連携し，現地における薬剤耐性真菌による感染症の実態解明で大きな成果をあげる事が出来ました．また本センターでは，文部科学省のナショナルバイオリソースプロジェクト（NBRP）として，病原真菌や放線菌の収集・保存・ゲノム解析・分与等の活動を行っています．一方でこれらの事業と平行して，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，さらに国内はもとより海外の研究拠点と，若手交流を含む緊密な共同研究を推進しています．平成26年以来，臨床感染症研究分野が，附属病院において我が国初の真菌症専門外来を開設しました．また，平成27年には高度病原真菌に特化したBSL-3施設を整備し，平成30年には無菌動物施設を立ち上げ，今後待ち受ける真菌感染症のあらゆる研究課題に対応すべく研究機能を強化しました．因みにBSL-3施設は，昨年のコロナパンデミックの発生以来，大学病院検査部のSARS-CoV-2のPCR検査業務支援に活用されました．本センターは，令和３年度に新設された，「災害治療学研究所」にも参画し，大規模災害やCOVID-19のようなパンデミックに随伴する感染症の研究と治療にむけた取組も始めます．

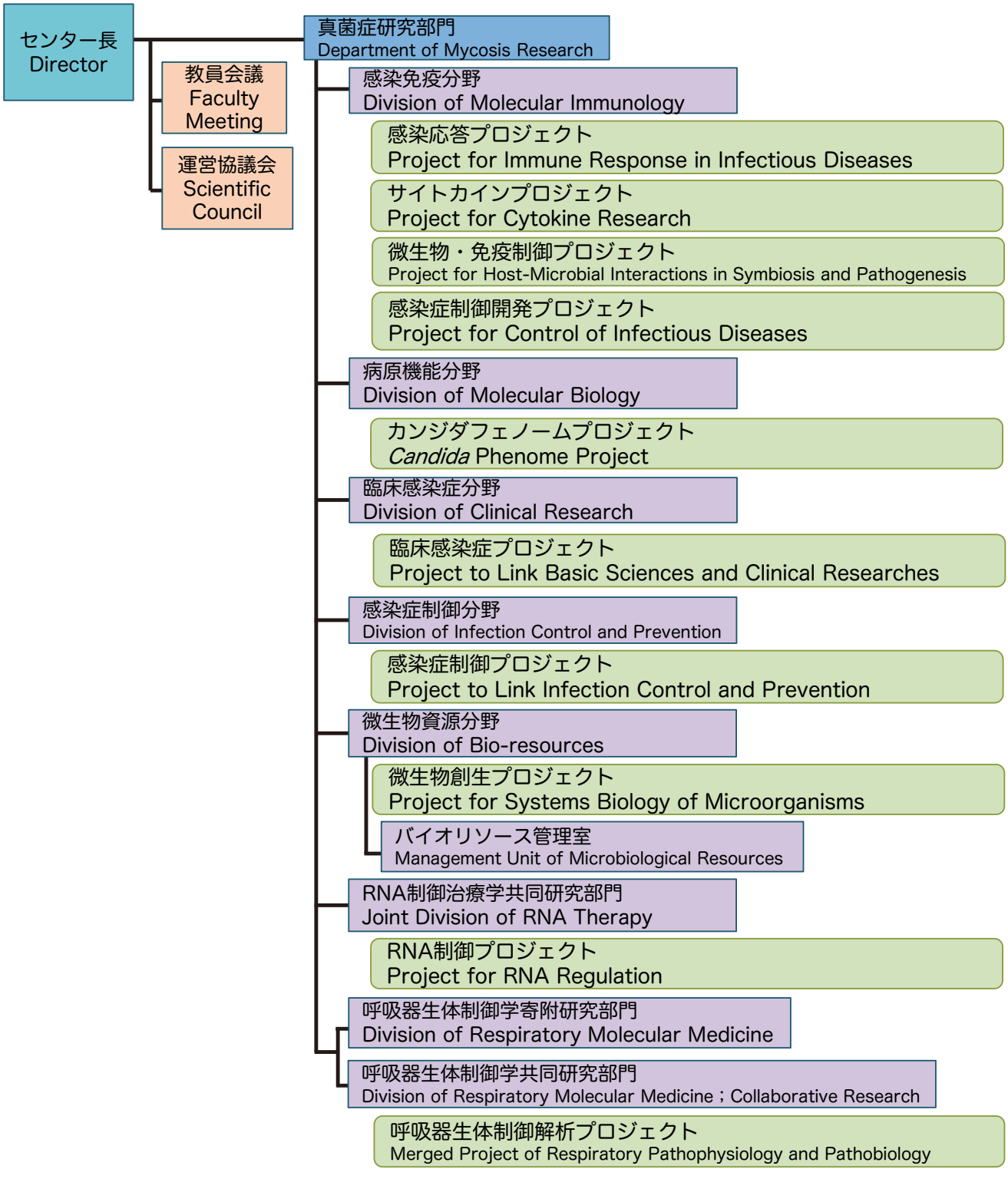
以上のように，本センターでは，「共同利用・共同研究拠点およびバイオリソース中核拠点」，「感染症・免疫基盤研究」，「感染症臨床研究」，「若手育成」の４つを柱として，今後も我が国の真菌医学及び感染症研究の発展に先導的な役割を果たす所存です．

令和４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．**



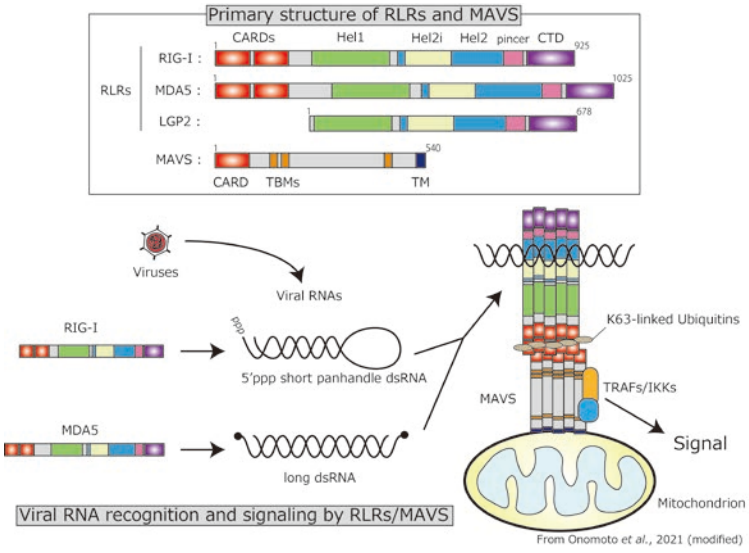
| | | | |
|---|---|---|---|
| Professor | Mitsutoshi Yoneyama | 教　　授 | 米山　光俊 |
| Assistant Professor | Koji Onomoto | 助　　教 | 尾野本浩司 |
| Research Assistant Professor | Kazuhide Onoguchi | 特任助教 | 小野口和英(-2021.5) |
| Research Technician | Yuna Aoki | 技術職員 | 青木　友那 |
| Research Promotion Technician | Miyuki Takizawa | 技術補佐員 | 滝沢みゆき |


### 1. Regulation of RIG-I-like receptor-mediated signaling: Interaction between host and viral factors.


Onomoto K, Onoguchi K, Yoneyama M

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


Retinoic acid-inducible gene I（RIG-I）-like receptors（RLRs）are RNA sensor molecules that play essential roles in innate antiviral immunity. Among the three RLRs encoded by the human genome, RIG-I and melanoma differentiation-associated gene 5, which contain N-terminal caspase recruitment domains, are activated upon the detection of viral RNAs in the cytoplasm of virus-infected cells. Activated RLRs induce downstream signaling via their interactions with mitochondrial antiviral signaling proteins and activate the production of type I and III interferons and inflammatory cytokines. Recent studies have shown that RLR-mediated signaling is regulated by interactions with endogenous RNAs and host proteins, such as those involved in stress responses and posttranslational modifications. Since RLR-mediated cytokine production is also involved in the regulation of acquired immunity, the deregulation of RLR-mediated signaling is associated with autoimmune and autoinflammatory disorders. Moreover, RLR-mediated signaling might be involved in the aberrant cytokine production observed in coronavirus disease 2019. Since the discovery of RLRs in 2004, significant progress has been made in understanding the mechanisms underlying the activation and regulation of RLR-mediated signaling pathways. Here, we review the recent advances in the understanding of regulated RNA recognition and signal activation by RLRs, focusing on the interactions between various host and viral factors.




**2. Functional analysis of RNA binding proteins（RBPs）that are responsible for induction of anti-viral innate immunity.**

Onomoto K, Aoki Y, Ban M, Sakai M, and Yoneyama M

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

We demonstrated that viral infection induces RLRs to accumulate in cytoplasmic granular-like structure, antiviral stress granule（avSG）. We further revealed that avSG plays a critical role as a platform for initiating RIG-I-mediated type I interferon production. We are analyzing several RBPs that play a role in regulating both RIG-I-mediated signal activation and avSG formation. We are also trying to identify novel RBPs involved in antiviral innate immune responses. In addition, we are analyzing molecular interaction between host factors and viral proteins in response to SARS-CoV-2 infection using the BSL3 facility of MMRC.

**3. Molecular interaction between anti-viral innate immune responses and endoplasmic reticulum（ER）stress responses.**

Onoguchi K, Suzuki Y, and Yoneyama M

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

We are interested in how the ER stress-induced response communicates with RLR-mediated signaling in the virus-infected cells. We have identified a novel molecule involved in the activation of both signaling pathways and are analyzing how these two signaling cascades interact to regulate antiviral immunity.

**Publications**

1）Onomoto K, Onoguchi K, Yoneyama M. Regulation of RIG-I-like receptor-mediated signaling: Interaction between host and viral factors. Cell Mol Immunol, 18:539–555, 2021

2）Hayashi Y, Suzuki H, Nakajima W, Uehara I, Tanimura A, Himeda T, Koike S, Katsuno T, Kitajiri S, Koyanagi N, Kawaguchi Y, Onomoto K, Kato H, Yoneyama M, Fujita T, Tanaka N. Virus-infection in cochlear supporting cells induces audiosensory receptor hair cell death by TRAIL-induced necroptosis. PLoS One, 16:e026044318, 2021

# 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 |
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| 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.


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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. TARM1 contributes to development of arthritis by activating dendritic cells through recognition of collagens


Rikio Yabe[1, 2], Soo-Hyun Chung[1], Masanori A Murayama[1], Sachiko Kubo[1], Kenji Shimizu[1], Yukiko Akahori[2], Takumi Maruhashi[1], Akimasa Seno[1], Tomonori Kaifu[1], Shinobu

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TARM1 is a member of the leukocyte immunoglobulin-like receptor family and stimulates macrophages and neutrophils in vitro by associating with FcRγ. However, the function of this molecule in the regulation of the immune system is unclear. Here, we show that Tarm1 expression is elevated in the joints of rheumatoid arthritis mouse models, and the development of collagen-induced arthritis (CIA) is suppressed in Tarm1$^{-/-}$ mice. T cell priming against type 2 collagen is suppressed in Tarm1$^{-/-}$ mice and antigen-presenting ability of GM-CSF-induced dendritic cells (GM-DCs) from Tarm1$^{-/-}$ mouse bone marrow cells is impaired. We show that type 2 collagen is a functional ligand for TARM1 on GM-DCs and promotes DC maturation. Furthermore, soluble TARM1-Fc and TARM1-Flag inhibit DC maturation and administration of TARM1-Fc blocks the progression of CIA in mice. These results indicate that TARM1 is an important stimulating factor of dendritic cell maturation and could be a good target for the treatment of autoimmune diseases.

**Publications**

1) Yabe R, Chung SH, Murayama MA, Kubo S, Shimizu K, Akahori Y, Maruhashi T, Seno A, Kaifu T, Saijo S, Iwakura Y. TARM1 contributes to development of arthritis by activating dendritic cells through recognition of collagens. Nat Commun. 12(1): 94. 2021

2) Yamaguchi K, Kanno E, Tanno H, Sasaki A, Kitai Y, Miura T, Takagi N, Shoji M, Kasamatsu J, Sato K, Sato Y, Niiyama M, Goto Y, Ishii K, Imai Y, Saijo S, Iwakura Y, Tachi M, Kawakami K. Distinct Roles for Dectin-1 and Dectin-2 in Skin Wound Healing and Neutrophilic Inflammatory Responses. J Invest Dermatol. 141(1): 164-176. e8. 2021

3) Miyachi H, Wakabayashi S, Sugihira T, Aoyama R, Saijo S, Koguchi-Yoshioka H, Fujimoto M, Núñez G, Matsue H, Nakamura Y. Keratinocyte IL-36 Receptor/MyD88 Signaling Mediates Malassezia-Induced IL-17-Dependent Skin Inflammation. J Infect Dis. 223(10): 1753-1765. 2021

4) Kitai Y, Sato K, Tanno D, Yuan X, Umeki A, Kasamatsu J, Kanno E, Tanno H, Hara H, Yamasaki S, Saijo S, Iwakura Y, Ishii K, Kawakami K. Role of Dectin-2 in the Phagocytosis of Cryptococcus neoformans by Dendritic Cells. Infect Immun. 89(10): e0033021. 2021

5) Yamamoto H, Tomiyama C, Sato K, Kasamatsu J, Takano K, Umeki A, Nakahata N, Miyasaka T, Kanno E, Tanno H, Yamasaki S, Saijo S, Iwakura Y, Ishii K, Kawakami K. Dectin-2-mediated initiation of immune responses caused by influenza virus hemagglutinin. Biomed Res. 42(2): 53-66. 2021

# 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 infections and cancers 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，宿主と腸内細菌間の共生因子であり腸管上皮細胞が発現するα1,2-フコースによる腸内細菌との共生機構を明らかにし，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，特に感染症や代謝疾患の治療法の開発を目的としている．**



| | | | |
|---|---|---|---|
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| Research Promotion Technician | Kaori Nishiyabu | 技術補佐員 | 西藪　香織 |


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


Akira Haku[1], Bei Bei Bi[1], Yoshiyuki Goto[1, 2]

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

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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.

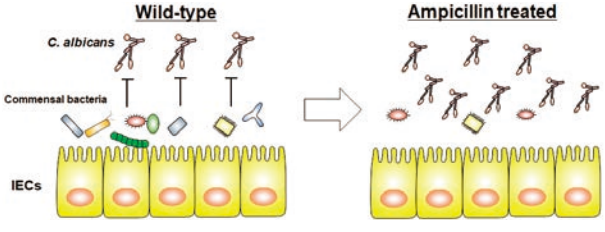


Fig 1. Commensal bacteria prevent the colonization of *C. albicans* in the gut

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

Bei Bei Bi[1], Yoshiyuki Goto[1, 2]

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

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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.

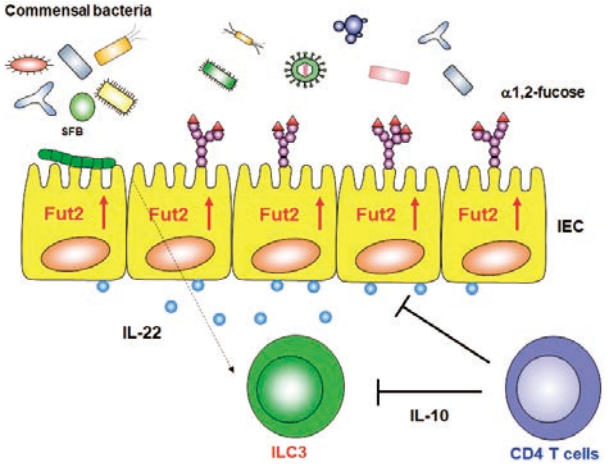


Fig 2. The inductive and regulatory mechanism of epithelial α1, 2-fucose

**Publications**

1）Dainichi T, Kabashima K, Ivanov II, Goto Y. Editorial: Regulation of Immunity by Non-Immune Cells. Front Immunol. 12: 770847. 2021

2）Shimizu K, Seiki I, Goto Y, Murata T. Measurement of the Intestinal pH in Mice under Various Conditions Reveals Alkalization Induced by Antibiotics. Antibiotics. 10: 180. 2021

# 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, in which bacteria can survive in a harsh environment. By repeating the cycle of proliferation and dormancy, bacteria evolve to develop antimicrobial resistance. The interaction between bacteria and host immunity also exerts a similar mechanism, leading to the establishment of persistent or latent infections. This project aims to elucidate the molecular mechanism of bacterial dormancy by analyzing systemic diseases and persistent infection caused by facultative intracellular bacteria, and to find novel compounds that can control the dormancy of bacteria.**

**細菌感染症で用いられる抗菌薬を細菌に曝露すると休眠状態となり，過酷な環境でも生存することができる．細菌は増殖と休眠を繰り返す間にゲノム変化を引き起こすと，薬耐性などのゲノム変化が生じる．更に，感染で生じる宿主免疫との相互作用でも同様の機構が発揮され，持続・潜伏感染となり感染症の克服を難しくする．本プロジェクトでは，細胞内寄生性を有する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探索を目的としている．**



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. ATP-dependent Lon protease regulates awakening from ciprofloxacin-induced persistence


Naoki Maekawa[1], Kengo Itadera[2], Junichi Ishihara[2], Satsuki Kajiya[3], Daiki Tanaka[4], Tetsushi Sekiguchi[4], Shuichi Shoji[3], Masami Ishibashi[1], Hiroki Takahashi[2], Akiko Takaya[1, 2]

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Quinolones such as ciprofloxacin are broad-spectrum antibiotics, which are used for the treatment of different infectious diseases associated with Enterobacteriaceae. However, the wide use as well as overuse of quinolones against diverse infections has led to the increased emergence of quinolone-resistant bacterial strains. The emergence of resistant bacteria is thought to be related to the antibiotic-induced persistence. Here, we focused on how ATP-dependent Lon protease regulates the ciprofloxacin-induced persistence. After treatment with high dose of ciprofloxacin, 1% of wild-type cells were observed at 24 hours. In contrast, only few colonies of Lon-deficient cells were detected at 3 hours and no colonies were observed at 24 hours. Single-cell imaging, however, showed that the number of remained cells after treatment with ciprofloxacin were not influenced by Lon-depletion. Lon-deficient cells treated with ciprofloxacin were able to divide in fresh medium, but the cell shape became significantly smaller than the strain untreated. Furthermore, expression of *lon* in the *lon*-unexpressed cells at 24 hours after treatment with ciprofloxacin led to detect the number of

colonies similar to the wild-type. These findings together, it is suggested that Lon protease could regulate awakening from ciprofloxacin-induced persistence rather than the formation of persisters.

## 2. TRAIL-resistance-overcoming activity compounds from the leaves of *Murraya exotica*

Kritamorn J[1], Takaya A[1,2], Hara Y[1], Sadhu SK[3], Ahmed F[4], Ishibashi M[1]

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Fractionation of the leaf extract from *Murraya exotica* led to the successful isolation of 12 compounds (1-12) with TRAIL-resistance-over-coming activity. Xanthinosin (1), 11α, 13-dihydroxanthinin (2), 11β, 13-dihydroxanthinosin (3), 4α, 11α, 13-trihydroxanthuminol (4), desacetylxanthanol (5), and lasidiol *p*-methoxybenzoate (6) were sesquiterpenes isolated from this plant for the first time, and 3 was isolated from natural sources for the first time. Among them, compounds1 and 5 showed strong TRAIL-resistance-overcoming activity, but their mechanisms have already been revealed. Furthermore, dihydroxanthinin (2), 1, 5-dicaffeoylquinic acid (7), and (-) loliolide (8), which belong to different phytochemical groups, were investigated for their effects on increasing apoptosis induction to overcome TRAIL resistance using Western blot analysis. The results demonstrated that 2, 7, and 8 promoted TRAIL-induced apoptosis by increasing the expression of several proapoptotic markers, including cleaved caspases −3 and −8, and suppressing anti-apoptotic protein Bcl-2.

**Publications**

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# *Candida glabrata* Phenome Project

# 知花ＰＩ（カンジダ・グラブラータフェノーム）プロジェクト


## 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．**



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### Rapid Freezing using Sandwich Freezing Device for Good Ultrastructural Preservation of Biological Specimens in Electron Microscopy


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Chemical fixation has been used for observing the ultrastructure of cells and tissues. However, this method does not adequately preserve the ultrastructure of cells; artifacts and extraction of cell contents are usually observed. Rapid freezing is a better alternative for the preservation of cell structure. Sandwich freezing of living yeast or bacteria followed by freeze-substitution has been used for observing the exquisite natural ultrastructure of cells. Recently, sandwich freezing of glutaraldehyde-fixed cultured cells or human tissues has also been used to reveal the ultrastructure of cells and tissues.

These studies have thus far been carried out with a handmade sandwich freezing device, and applications to studies in other laboratories have been limited. A new sandwich freezing device has recently been fabricated and is now commercially available. The present paper shows how to use the sandwich freezing device for rapid freezing of biological specimens, including bacteria, yeast, cultured cells, isolated cells, animal and human tissues, and viruses. Also shown is the preparation of specimens for ultrathin sectioning after rapid freezing and procedures for freeze-substitution, resin embedding, trimming of blocks, cutting of ultrathin sections, recovering of sections, staining, and covering of grids with support films.

**Publications**


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2）Pedro P, Catarina C, Mónica G, Diana P, Takahashi-Nakaguchi A*, Chibana H, Arsenio F, Miguel T: Role of CgTpo4 in polyamine and antimicrobial peptide resistance: determining virulence in *Candida glabrata*. International Journal of Molecular Sciences, 2021.
3）Cavalheiro M, Pereira D, Formosa-Dague C, Leitão C, Pais P, Ndlovu E, Viana R, Pimenta AI, Santos R, Takahashi-Nakaguchi A, Okamoto M, Ola M, Chibana H, Fialho AM, Butler G, Dague E, Teixeira MC: From the first touch to biofilm establishment by the human pathogen *Candida glabrata*: a genome-wide to nanoscale view. Commun Biol. 4(1) 2021.
4）Xinyue C, Iwatani S, Kitamoto T, Chibana H, Kajiwara S: The lack of SNARE protein homologue Syn8 influences biofilm formation of Candida glabrata. Frontiers in Cell and Developmental Biology, 2021.

# Project of Clinical Investigation

## 亀井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. 400 cases in 2021). 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, the development of their diagnostic and therapeutic methods, and the defence mechanism of infection of intractable fungal diseases.**

**A collaborative study with Sao Paulo State University of Campinas, Brazil (SATREPS), which has started in 2016, has made the last topic as its primary target.**

**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. 2021年の全国の医療機関からの依頼件数は, COVID-19感染の大流行にもかかわらず, 400件あまりに達した. この診療サポートにより全国の医療機関によるネットワークが形成され, 菌株を含めた検体や貴重な臨床情報の収集と研究に役立つとともに, 多くの共同研究を生む母体ともなっている. 診療活動としては, 全国から寄せられる真菌症のコンサルテーションに対応する一方で, 附属病院に真菌症専門外来を設け, 全国からの患者の診療を行なうなど精力的に臨床活動を行っている. 研究面では国立感染症研究所をはじめ帯広畜産大, 理科大, NHO東京病院, 慶応大学病院, 東海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. 中でも三大真菌症の一つであるアスペルギルス症については, 耐性株の疫学と耐性機構や感染機構の研究を進め, 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, カンジダ症については主要2菌種の耐性機構, 疫学など研究を, クリプトコッカス症についてはカドミウムに対する反応性の研究を行ない, 多くの論文を発表した. さらに, 国際連携による共同研究も盛んに行ない, 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）を精力的に遂行している.**



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**1. Prevalence of Antifungal Resistance, Genetic Basis of Acquired Azole and Echinocandin Resistance, and Genotyping of *Candida krusei* recovered from an International Collection. Antimicrob Agents Chemother, in press.**

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 non-clinical 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* hotspot 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 non-clinical 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.

**2. Genetic Basis of Azole and Echinocandin Resistance in Clinical *Candida glabrata* in Japan. Antimicrob Agents Chemother 64(9): e00783-20, 2020.**

Khalifa HO, Arai T, Majima H, Watanabe A, Kamei K

Infections caused by *Candida glabrata* have caused worldwide concern, especially when they are associated with increasing echinocandin and azole resistance. In this study, we analyzed the molecular mechanisms of azole and echinocandin resistance in *C. glabrata* isolates obtained from hospitalized patients in Japan from 1997 to 2019. All isolates were checked phenotypically for resistance and genotypically for mutations in *PDR1, ERG11*, hot spot 1 (HS1), HS2, and HS3 of *FKS1*, and HS1 and HS2 of *FKS2*, and all isolates were genotyped by multilocus sequence typing (MLST). Interestingly, 32.6% of the isolates were resistant to caspofungin, and 4.7% were resistant to micafungin. The isolates showed low rates of resistance to azoles, ranging from 2.3% to 9.3%, and only 4.7% of the isolates were non-wild type for flucytosine susceptibility. For the first time in Japan, 4.7% of the isolates were identified as multidrug-resistant strains. Nonsynonymous mutations in *PDR1*, including two novel mutations associated with azole resistance, were identified in 39.5% of the isolates, and a single nonsynonymous mutation was identified in *ERG11* Nine isolates from the same patient harbored nonsynonymous mutations in HS1 of *FKS2*, and a single isolate harbored a single nonsynonymous mutation in HS1 of *FKS1* MLST genotyping revealed 13 different sequence types (STs), with 3 new STs, and ST7 was the most prevalent among the

patients (35%) and was associated with high resistance rates. Our results are of crucial clinical concern, since understanding the molecular mechanisms underlying fungal resistance is imperative for guiding specific therapy for efficient patient treatment and promoting strategies to prevent epidemic spread.

**3. Genetic system underlying responses of *Cryptococcus neoformans* to cadmium.**
**Curr Genet 68(1): 125-141, 2022.**

Toh-e A, Ohkusu M, Ishiwada N, Watanabe A, Kamei K

*Cryptococcus neoformans*, basidiomycetous pathogenic yeast, is basically an environmental fungus and, therefore, challenged by ever changing environments. In this study, we focused on how *C. neoformans* responds to stress caused by cadmium that is one of high-risk pollutants. By tracking phenotypes of the resistance or sensitivity to cadmium, we undertook forward and reverse genetic studies to identify genes involved in cadmium metabolism in *C. neoformans*. We found that the main route of $Cd^{2+}$ influx is through $Mn^{2+}$ ion transporter, Smf1, which is an ortholog of Nramp (natural resistance-associated macrophage protein 1) of mouse. We found that serotype A strains are generally more resistant to cadmium than serotype D strains and that cadmium resistance of H99, a representative of serotype A strains, was found to be due to a partial defect in *SMF1*. We found that calcium channel has a subsidiary role for cadmium uptake. We also showed that Pca1 (P-type-ATPase) functions as an extrusion pump for cadmium. We examined the effects of some metals on cadmium toxicity and suggested (i) that $Ca^{2+}$ and $Zn^{2+}$ could exert their protective function against $Cd^{2+}$ via restoring cadmium-inhibited cellular processes and (ii) that $Mg^{2+}$ and $Mn^{2+}$ could have antagonistic roles in an unknown Smf1-independent $Cd^{2+}$ uptake system. We proposed a model for $Cd^{2+}$-response of *C. neoformans*, which will serve as a platform for understanding how this organism copes with the toxic metal.

**4. Azole and Echinocandin Resistance Mechanisms and Genotyping of *Candida tropicalis* in Japan: Cross-Boundary Dissemination and Animal-Human Transmission of *C. tropicalis* Infection. Clin Microbiol Infect 28(2): 302. e5-302. e8, 2022**

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.

**5. Genetic differences between Japan and other countries in *cyp51A* polymorphisms of *Aspergillus fumigatus*. Mycoses 64(11):1354-1365, 2021.**

Majima H, Arai T, Kusuya Y, Takahashi H, Watanabe A, Miyazaki Y, Kamei K

**Background:** Mutations in *cyp51A* gene are known as main mechanisms of azole resistance in *Aspergillus fumigatus*, whereas azole-susceptible strains also carry *cyp51A* mutations (polymorphisms). The polymorphisms found in Europe mainly consist of two combinations of mutations, that is combinations of five single-nucleotide polymorphisms (SNPs) of *cyp51A*, referred to as *cyp51A*-5SNPs, and combinations of three SNPs of *cyp51A*, referred to as *cyp51A*-3SNPs. Few studies have compared the distributions of *cyp51A* polymorphisms between different regions.
**Objectives:** The aim of this study was to investigate the regional differences of *cyp51A* polymorphisms.
**Methods:** We compared the proportions of *cyp51A* polymorphisms in clinical and environmental strains isolated in various countries, and analysed the strains phylogenetically using short tandem repeats (STRs) and whole-genome sequence (WGS).
**Results:** Among the Japanese strains, 15 out of 98 (15.3%) clinical strains and 8 out of 95 (8.4%) environmental strains had *cyp51A* polymorphisms. A mutation of *cyp51A*$^{N248K}$ was the most prevalent polymorphism in both clinical (n = 14, 14.3%) and environmental strains (n = 3, 3.2%). Only one environmental strain harboured *cyp51A*-5SNPs, which was reported to be the most prevalent in Europe. For phylogenetic analyses using STRs and WGS, 183 and 134 strains, respectively, were employed. They showed that most of the strains with *cyp51A*$^{N248K}$ clustered in the clades different from those of the strains with *cyp51A*-5SNPs and *cyp51A*-3SNPs as well as from those with $TR_{34}$ /L98H mutations.
**Conclusions:** This study suggests that there are genetic differences between *cyp51A* polymorphisms of *A. fumigatus* in Japan and Europe.

**6. Hmg1 mutations in *Aspergillus fumigatus* and their contribution to triazole susceptibility. Med Mycol 59(10):980-984, 2021.**

Arai T, Umeyama T, Majima H, Inukai T, Watanabe A, Miyazaki Y, Kamei K

Triazole-resistant *Aspergillus fumigatus* is a global health concern. In general, each triazole resistance pattern caused by the specified amino acid substitution of Cyp51A has a typical pattern depending on the mutation site. We evaluated the contribution of both Cyp51A and Hmg1 mutations to atypical triazole resistance in *A. fumigatus*. We used clinical triazole-resistant *A. fumigatus* strains collected in Japan and investigated the sequences of *cyp51A* and *hmg1* genes. To delineate the association between the *hmg1* mutation and atypical triazole resistance, the mutant *hmg1* alleles in clinical multi-azole resistant strains were replaced with the wild-type *hmg1* allele by CRISPR/Cas9 system. In our study, the combination of Cyp51A mutation and Hmg1 mutation was shown to additively contribute to triazole resistance. We also demonstrated that the triazole resistance conferred by the Hmg1 mutation showed a different pattern depending on the mutation site, similar to the Cyp51A mutation. Our results indicate that focusing on the phenotypes of multiple genes is essential to clarify the overall picture of the triazole resistance mechanism of *A. fumigatus*.

**7. Evaluation of Surveyor Nuclease for rapid identification of *FKS* genes mutations in *Candida glabrata*. J Infect Chemother 27(6):834-839, 2021.**

Khalifa HO, Arai T, Majima H, Watanabe A, Kamei K

**Introduction:** Infections with *Candida glabrata* have recently gained worldwide attention owing to its association with long hospitalizations and high mortality rates. This problem is highlighted when the infection is associated with echinocandin resistance, which is used for first-line therapy. Echinocandin resistance is exclusively attributed to functional mutations in *FKS* genes, and especially in hot spot (HS) regions. Unfortunately, few studies have focused on the rapid

identification of *FKS* mutations associated with echinocandin resistance in *C. glabrata*. This study was intended to evaluate and validate the use of Surveyor nuclease assay (SN) for detection of *FKS* gene mutations.

**Methods:** SN was evaluated against three segments of *FKS1* and *FKS2* genes including whole gene, regions including all HSs, and the region including only HS1.

**Results:** Our results showed that SN results are basically dependent on the type of gene as well as the segment type. Interestingly, SN can detect mutations in the region containing HS1 in both *FKS1* and *FKS2* genes. Furthermore, SN can detect mutations in the segment containing all HS regions for *FKS1* but not *FKS2*. SN was unable to detect mutations in the whole *FKS1* and *FKS2* genes.

**Conclusions:** As far as we know, this is the first study to validate SN for rapid identification of *FKS* gene mutations. This assay could be used as a sample for rapid identification of mutations associated with HS1 region in *FKS* genes, which have a predominant role for echinocandin resistance induction in *C. glabrata*.

### 8. In Vitro Characterization of Twenty-One Antifungal Combinations against Echinocandin-Resistant and -Susceptible *Candida glabrata*. J Fungi (Basel) 7(2): 108, 2021.

Khalifa HO, Majima H, Watanabe A, Kamei K

This study was designed to analyze the interaction of 21 antifungal combinations consisting of seven major antifungal agents against 11 echinocandin- susceptible and six-resistant *C. glabrata* isolates. The combinations were divided into five major groups and were evaluated by checkerboard, disc diffusion, and time-killing assays. Synergy based on the fractional inhibitory concentration index of ≤0.50 was observed in 17.65-29.41% of the cases for caspofungin combinations with azoles or amphotericin B. Amphotericin B combination with azoles induced synergistic interaction in a range of 11.76-29.41%. Azole combinations and 5-flucytosine combinations with azoles or amphotericin B did not show synergistic interactions. None of the 21 combinations showed antagonistic interactions. Interestingly, 90% of the detected synergism was among the echinocandin-resistant isolates. Disk diffusion assays showed that the inhibition zones produced by antifungal combinations were equal to or greater than those produced by single drugs. The time-killing assay showed the synergistic action of caspofungin combination with fluconazole, voriconazole, and posaconazole, and the amphotericin B-5-flucytosine combination. Furthermore, for the first time, this assay confirmed the fungicidal activity of caspofungin-voriconazole and amphotericin B-5-flucytosine combinations. The combination interactions ranged from synergism to indifference and, most importantly, no antagonism was reported and most of the synergistic action was among echinocandin-resistant isolates.

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# Project for Infection Control and Prevention

## 石和田ＰＩ（感染症制御）プロジェクト


**Summary（研究概要）**

**Our research focuses on epidemiology and pathogenesis of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Streptococcus agalactiae*. We organize several clinical researches for development of diagnostic and therapeutic methods of intractable respiratory infectious diseases collaborating with clinicians and care for the patients in Chiba University Hospital. We also recently conduct the research on risk education for vaccination and rubella elimination.**

**インフルエンザ菌，肺炎球菌，B群レンサ球菌（GBS）の病原性解析ならびにインフルエンザ菌感染症と肺炎球菌感染症，GBS感染症の疫学研究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株の病原因子の解析を行い，新たな予防法の開発を目指す．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も行っている．さらに，ワクチンのリスク教育，風疹排除に関する研究にも取り組んでいる．**



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|---|---|---|---|
| Professor | Naruhiko Ishiwada | 教　　授 | 石和田稔彦 |
| Research Assistant Professor | Noriko Takeuchi | 特任助教 | 竹内　典子 |
| Research Technician | Misako Ohkusu | 技術職員 | 大楠美佐子 |
| Adjunct Research Technician | Mihoko Ohhata | 非常勤技術職員 | 大畑美穂子 |


### 1. Epidemiology of hospitalised paediatric community-acquired pneumonia and bacterial pneumonia following the introduction of 13-valent pneumococcal conjugate vaccine in the national immunisation programme in Japan


Takeuchi N[1], Naito S[2], Ohkusu M[1], Abe K[3], Shizuno K[4], Takahashi Y[5], Omata Y[5], Nakazawa T[5], Takeshita K[2], Hishiki H[2], Hoshino T[6], Sato Y[7], Ishiwada N[1]

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Abstract

Studies on community-acquired pneumonia (CAP) and pneumococcal pneumonia (PP) related to the 13-valent pneumococcal conjugate vaccine (PCV13) introduction in Asia are scarce. This study aimed to investigate the epidemiological and microbiological determinants of hospitalised CAP and PP after PCV13 was introduced in Japan. This observational hospital-based surveillance study included children aged ⩽15 years, admitted to hospitals in and around Chiba City, Japan. Participants had bacterial pneumonia based on a positive blood or sputum culture for

bacterial pathogens. Serotype and antibiotic-susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates from patients with bacterial pneumonia were assessed. The CAP hospitalisation rate per 1000 child-years was 17.7, 14.3 and 9.7 in children aged <5 years and 1.18, 2.64 and 0.69 in children aged 5-15 years in 2008, 2012 and 2018, respectively. There was a 45% and 41% reduction in CAP hospitalisation rates, between the pre-PCV7 and PCV13 periods, respectively. Significant reductions occurred in the proportion of CAP due to PP and PCV13 serotypes. Conversely, no change occurred in the proportion of CAP caused by H. influenzae. The incidence of hospitalised CAP in children aged ⩽15 years was significantly reduced after the introduction of PCV13 in Japan. Continuous surveillance is necessary to detect emerging PP serotypes.

**2. Pneumococcal serotype-specific IgG and opsonophagocytic activity in young Japanese patients with asplenia.**

Takeshita K[1,2], Takeuchi N[2], Takahashi Y[3], Fukasawa C[4], Hishiki H[1], Hoshino T[4], Ishiwada N[2], Shimojo N[5]

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Abstract

Patients with asplenia are at high risks of severe infections caused by encapsulated bacteria, particularly *Streptococcus pneumoniae*. Thirteen-valent pneumococcal conjugate vaccine (PCV13) and 23-valent pneumococcal polysaccharide vaccine (PPSV23) are recommended for invasive pneumococcal disease prevention; however, little is known about the immunity to pneumococci in young patients with asplenia. We measured pneumococcal serotype-specific IgG (Pn-IgG) levels and pneumococcal opsonophagocytic activity (Pn-OPA) against some PCV13-contained serotypes (1, 3, 5, 6A, 7 F, 19A) in 23 young patients with asplenia using surplus serum samples. In this study, 5 and 13 patients had received PCV13 during routine immunizations and PPSV23, respectively; however, >5 years had passed since the last dose in most cases. The geometric mean concentrations (GMCs) of Pn-IgG in all study patients were not under the cutoff level against six serotypes, but they were lower than the those of age-matched healthy controls, as we have previously published. The patients who had received only PPSV23 had significantly lower GMCs against four serotypes (serotypes 1, 6A, 7 F, and 19A) than that of the patients who had received at least one PCV13 vaccination. The patients who had received only PPSV23 also had significantly lower geometric mean titers (GMTs) of Pn-OPA against all three serotypes we measured (serotypes 3, 5, and 19A) than that of the patients who had received at least one PCV13 vaccination. Our findings are useful data that can indicate insufficient immunity in young patients with asplenia against some PCV13 pneumococci serotypes and suggest the need for appropriate vaccinations in the post-PCV13 era.

**3. Population-based study of a free rubella-specific antibody testing and immunization campaign in Chiba city in response to the 2018-2019 nationwide rubella outbreak in Japan.**

Takeshita K[1], Takeuchi N[1], Ohkusu M[1], Ohata M[1], Suehiro M[2], Maejima H[2], Abe H[3], Ohta F[3], Ohama Y[3], Tamai K[3], Haraki M[3], Ishiwada N[1]

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[3] Chiba City Medical Association, Chiba, Japan.

Abstract

Japan has not been able to eliminate rubella; as a result, the large rubella epidemic has occurred. Considering the complicated history of the vaccine policy in Japan, some

susceptible populations became infected with rubella, resulting in an outbreak. We conducted a large serosurveillance against rubella in Chiba city after initiating free rubella-specific antibody testing and an immunization campaign during 2018-2019. The total number of rubella specific antibody tests that was conducted in the nationwide campaign and Chiba city original campaign was 8277 and 6104, respectively. The proportion of participants with an antibody titer of ≤1:16 using the hemagglutination inhibition (HI) test was higher in those in their 20-30s. On the contrary, the proportion of participants with an antibody titer of <1:8 using the HI test was higher in men in their 40-50s. This discrepancy possibly reflects the complicated history of the vaccine policy. The number of participants in the nationwide immunization campaign in this city was 1517, whereas that in the Chiba city campaign was 3607. The Chiba city campaign was effective against women in their 20-30s (child-bearing generation); however, the nationwide campaign was not sufficiently effective against men in their 40-50s because many workers were did not visit medical facilities to receive the measles-rubella vaccine.

**4. The effects of health education on health science teachers' intention to recommend adolescent HPV vaccine for female students in Japan.**

Ishiwada N[1], Suzuki C[2], Hasebe S[2], Tsuchiya A[2], Takeuchi N[1], Hishiki H[3], Sato Y[4], Sugita K[2]

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Abstract

The Japanese government suspended proactive recommendation of human papillomavirus (HPV) vaccination due to several reports of adverse events related to it in 2013. After that, the immunization rate of HPV vaccine quickly declined in Japan. Health science teachers (HSTs) are qualified and licensed teachers in charge of health care and health education for students in Japanese schools. HSTs have not recommended HPV vaccination to female students, since active governmental recommendation for HPV vaccination was halted for 5 y. We conducted a primary survey targeting HSTs (N = 39) and university students taking the HST training course (N = 123). In each group, awareness regarding HPV vaccine and disease burden was evaluated and factors related to and barriers to HPV vaccine recommendation were identified. The primary survey found that many HSTs and university students recognized their insufficient knowledge regarding the HPV vaccine. Based on the primary survey's results, infectious disease specialists, collaborating with Japanese HSTs, developed educational slide sets on HPV vaccine. A secondary survey was conducted before and after the lecture with HSTs (N = 162), where we evaluated their intelligibility and intention to recommend HPV vaccination for female students. In the post-lecture, secondary survey, the number of HSTs who recommended the HPV vaccine to their students had statistically increased from 76 before the lecture, to 103 ($p < .05$). An educational lecture using appropriate materials improved HSTs' vaccine confidence and intention to recommend the HPV vaccine to their students, verifying the study's hypothesis.

**5. Emergence of *Haemophilus influenzae* with low susceptibility to quinolones isolated from pediatric patients in Japan.**

Takeuchi N[1], Ohkusu M[1], Hoshino T[2], Yamamoto S[2], Segawa S[3], Murata S[3], Ishiwada N[1]

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[2] Division of Infectious Diseases, Chiba Children's Hospital, Chiba, Japan.
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Abstract

In 2010, oral fluoroquinolone tosufloxacin (TFX) granules were released as the first oral respiratory quinolone for children in Japan. To investigate the recent trend of *H. influenzae* strains with low susceptibility to quinolones in children, we analyzed the gene sequences of quinolone resistance-determining regions (QRDRs) of gyrA, gyrB, parC, and parE of 23 clinical isolates from 15 patients aged <15 years with an MIC of ≥0.5 μg/mL for TFX from 2010 to 2018. Amino acid substitutions were observed in both GyrA and ParC in 13 strains (81%, 13/16), except two strains with a TFX MIC of 0.5 μg/mL with amino acid substitution in only GyrA and one strain with a TFX MIC of 1 μg/mL with no amino acid substitution. Four ST422 strains were observed in 2018, the detection age range was wide (0-7 years), and the residential city was varied. A total of 3/15 patients had a clear history of TFX treatment. Even for the strain with an MIC of 0.5 μg/mL for TFX, it is highly possible that it harbors a mutation in gyrA, which is the first step toward quinolone resistance, and it may also harbor mutations in both gyrA and parC. Furthermore, several specific sequence type quinolone-resistant *H. influenzae* strains, particularly ST422, may be widespread among children in Japan. It is necessary to investigate changes in resistance both at the MIC and gene levels. The continuous monitoring of strains and the use of antimicrobial drugs in treatment should be carefully observed.

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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 | 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 |


### 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

Kengo Itadera, 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**


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# Management of Unit of Microbiological Resources

## バイオリソース管理室


**Summary（研究概要）**

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

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



| | | | |
|---|---|---|---|
| Associate Professor | Takashi Yaguchi | 准教授 | 矢口　貴志 |
| Assistant Professor | Sayaka Ban | 助教 | 伴　さやか |
| Research Technician | Junko Ito | 技術職員 | 伊藤　純子 |
| JSPS Post Doctoral Fellow | Vit Hubka | 学振外国人特別研究員 | ヴィト・フブカ |
| Research Promotion Technician | Kaomi Higuchi | 技術補佐員 | 樋口芳緒美 |
| Research Promotion Technician | Akiko Kota | 技術補佐員 | 甲田　暁子 |


### 1. Isolation and characterization of the polyhexamethylene biguanide hydrochloride-resistant fungus, *Purpureocillium lilacinum*.


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

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We aimed to examine the presence of microorganisms highly resistant to polyhexamethylene biguanide hydrochloride（PHMB）—a widely used antimicrobial—with a goal to address public health concerns pertaining to it and devise strategies to prevent the development of resistance. We isolated a fungus from a 20% aqueous solution of PHMB and examined its morphology and drug resistance profile. Based on the sequence of the internal transcribed spacer region of ribosomal DNA, the fungus was identified as *Purpureocillium lilacinum*. Although the *P. lilacinum* type and resistant strains showed similar morphology, the latter had extremely low PHMB susceptibility and was able to grow in 20% aqueous solution of PHMB, which eliminated the type strain. The minimum inhibitory concentration（MIC）of PHMB for the resistant strain was significantly higher than that of the type strain and other pathogenic filamentous fungi and yeasts. Furthermore, we sequenced the genome of the isolate to predict PHMB resistance-related genes. Despite its high resistance to PHMB, no genes homologous to fungal PHMB-resistant genes were detected in the genome of the resistant strain. In summary, *P. lilacinum* was found to be significantly more resistant to PHMB than previously reported, via an unidentified mechanism of drug resistance.


### 2. Itraconazole resistance of *Trichophyton rubrum* mediated by the ABC transporter TruMDR2.


Yamada T[1,2], Yaguchi T[3], Tamura T[4], Pich C[5], Salamin K[5],

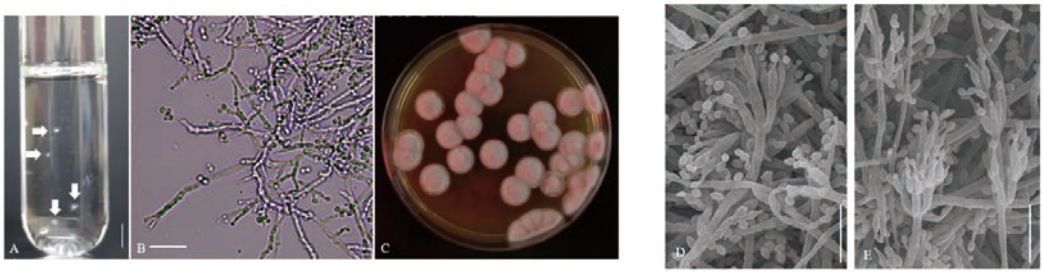

FIG. 1. (A) White floating masses in a 20% aqueous solution of polyhexamethylene biguanide hydrochloride (PHMB). Scale bar = 5 mm. White arrows indicate the floating masses that were observed after leaving the PHMB in polyethylene containers to stand for more than 1 month. (B) Mycelia and spores of the white floating masses observed in a 20% solution of PHMB. Scale bar = 20 μm (light microscope). (C) Colonies formed from mycelium of the floating masses inoculated on potato dextrose agar at 22.5℃ for 6 days. (D, E) Scanning electron micrographs of Purpureocillium lilacinum grown on potato dextrose agar at 25℃ for 14 days. IFM 63780 (D), IFM 47467$^{T}$ (E). Scale bar = 10 μm.

Feuermann M[6], Monod M[5]

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[5] Department of Dermatology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
[6] Swiss-Prot group, SIB Swiss Institute of Bioinformatics, Geneva, Switzerland

**Background:** Dermatophytes showing reduced sensitivity to antifungal agents have emerged in several countries. One terbinafine resistant strain of *Trichophyton rubrum*, TIMM20092, also showed reduced sensitivity to itraconazole (ITC) and voriconazole (VRC). The expression of two genes (TruMDR2 and TruMDR3) encoding multidrug transporters of the ABC family was found to be highly up-regulated in this strain. Deletion of TruMDR3 in TIMM20092 abolished its resistance to VRC but only slightly reduced its resistance to ITC.

**Objectives:** We examined the potential of *T. rubrum* to develop resistance to ITC by analysing the mechanism of ITC resistance in TIMM20092.

**Methods:** The deletion of TruMDR2 by gene replacement was

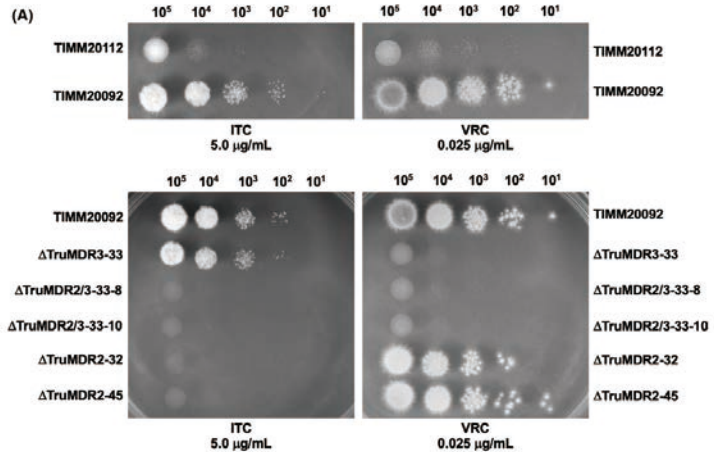


FIG. 2. Antifungal susceptibility of *T. rubrum* strain TIMM20092, one TruMDR3 single mutant (ΔTruMDR3-33), two TruMDR2 single mutants (ΔTruMDR2-32 and ΔTruMDR2-45) and two TruMDR2/TruMDR3 double mutants (ΔTruMDR2/3-33-8 and ΔTruMDR2/3-33-10) to VRC and ITC. (A) Serial dilution drug susceptibility assays for VRC and ITC. *T. rubrum* spores were spotted at different dilutions on SDA plates as described in the Materials and Methods section. The plates were incubated at 30℃ for 6 days.

performed in TIMM20092 and one TruMDR3-lacking mutant (ΔTruMDR3) previously generated from TIMM20092. TruMDR2 single and TruMDR2/TruMDR3 double mutants (ΔTruMDR2 and ΔTruMDR2/3) were successfully obtained, respectively.

**Results:** The suppression of TruMDR2 was shown to abolish resistance to ITC in TIMM20092 and the TruMDR3-lacking mutant, strongly suggesting that TruMDR2 is a major contributor to ITC resistance in TIMM20092.

**Conclusions:** Our study highlights the possible role of the ABC transporter TruMDR2 in ITC resistance of *T. rubrum*.

### 3. The ubiquitous distribution of azole-resistant *Aspergillus fumigatus*-related species in outdoor environments in Japan.

Watanabe K[1], Yaguchi T[2], Hirose D[1]

[1] School of Pharmacy, Nihon University, Chiba, Japan.
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*Aspergillus fumigatus*-related species are responsible for causing aspergillosis, which is a fatal infectious disease. Recently, there has been a series of reports of *A. fumigatus*-related species that are resistant to azole drugs used in clinical practice for the treatment of fungal infections. Some of these species have been isolated from outdoor environments. Testing the drug susceptibility of the strains from outdoor environments is important. In this study, we isolated and cultured 72 strains of *A. fumigatus*-related species from the outdoor environment in Japan. The isolates identified *via* morphological observation and molecular phylogenetic analysis were *A. felis*, *A. lentulus*, *A. pseudoviridinutans*, *A. udagawae*, and *A. wyomingensis*. The results of the drug susceptibility testing revealed that *A. felis* (6 of 14 strains) and *A. pseudoviridinutans* (13 of 17 strains) were resistant to itraconazole (ITCZ), with minimum inhibitory concentrations (MICs) higher than 4 mg/L. The voriconazole (VRCZ)-resistant strains with MIC higher than 4 mg/L were *A. felis* (14 of 14), *A. lentulus* (4 of 4), *A. pseudoviridinutans* (15 of 17), *A. udagawae* (23 of 34), *A. wyomingensis* (1 of 3), and *A. pseudoviridinutans* (1 of 3). Among them, *A. felis* (1 of 14) and *A. pseudoviridinutans* (7 of 17) demonstrated MICs higher than 8 mg/L for ITCZ and VRCZ. These results indicate that *A. fumigatus*-related species resistant to ITCZ and VRCZ are distributed in outdoor environments in Japan.

### 4. An exploratory MALDI-TOF MS library based on SARAMIS superspectra for rapid identification of *Aspergillus* section *Nigri*

Ban S[1, 2], Kasaishi R[2], Kamijo T[2], Noritake C[2], Kawasaki H[2].

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[2] Biological Resource Center, National Institute of Technology and Evaluation, Chiba, Japan.

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) is a broadly used technique for identification and typing of microorganisms. However, its application to filamentous fungi has been delayed. The objective of this study was to establish a data library for rapid identification of the genus *Aspergillus* sect. *Nigri* using MALDI-TOF MS. With respect to sample preparation, we compared the utility of using mature mycelia, including conidial structures, to accumulate a wider range of proteins versus the conventional method relying on young hyphae. Mass spectral datasets obtained for 61 strains of 17 species were subjected to cluster analysis and compared with a phylogenetic tree based on calmodulin gene sequences. Specific and frequent mass spectral peaks corresponding to each phylogenetic group were selected (superspectra for the SARAMIS system). Fifteen superspectra representing nine species were ultimately created. The percentage of correct identification for 217 spectra was improved from 36.41% to 86.64% using the revised library. Additionally, 2.76% of the spectra were assigned to candidates that comprised several related species, including the correct species.

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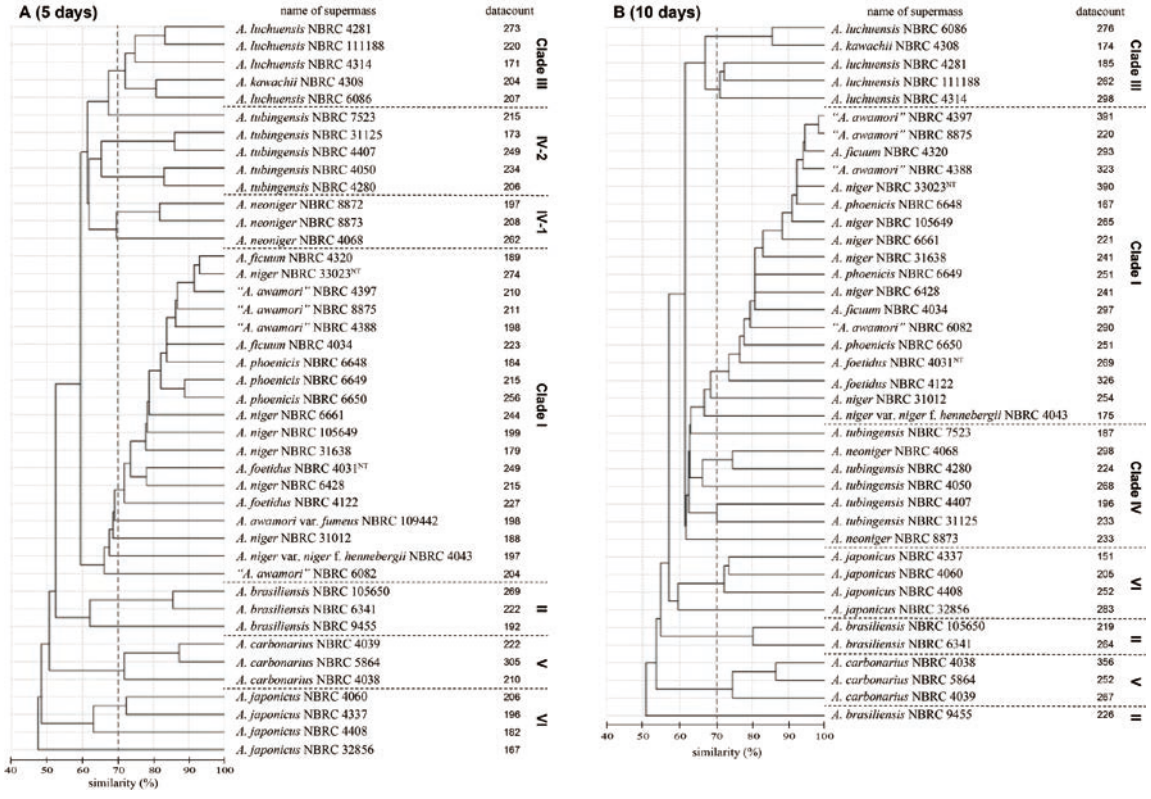


FIG. 3. Dendrogram of *Aspergillus* sect. *Nigri* constructed from the aggregated mass spectra per strain by the SARAMIS using single-linkage agglomerative cluster analysis. A: 5 d and B: 10 d incubation on PDA. The scale bar below indicates the similarity (%). Broken horizonal line shows separation of phylogenetic clades. Although the branching order was different, the clusters in the two dendrograms were roughly equivalent to each other and to those in the phylogenetic tree.

Makita Y. Isolation of Nocobactin NAs as notch signal inhibitors from *Nocardia farcinica*, a possibility of invasive evolution. J Antibiot. 74: 255-259, 2021.

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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制御ネットワークを形成する主要な因子として，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に注目する．そして発現の異常亢進により遺伝子制御ネットワークの乱れの原因となるmiRNAを制御する方法論の開発を行い，がんを初めとしたヒト疾患の制圧への基盤研究を展開する．**



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|---|---|---|---|
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| Research Assistant Professor | Kazuyoshi Kobayashi | 特任助教 | 小林　和善 |
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| Research Promotion Technician | Naomi Aikawa | 技術補佐員 | 相川　尚美 |
| Visiting Professor | Hideo Iba | 客員教授 | 伊庭　英夫 |


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


Takeshi Haraguchi, Kazuyoshi Kobayashi and Hideo Iba

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


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

For innovative therapy for broad spectrum of tumors, we now target miR-21, which is expressed in almost all the epithelial tumors at very high levels and has been shown to be strong causative of cancer through inhibition of many important tumor suppressor genes simultaneously. Since miR-21 is one of the most abundant miRNA species in cancer cells, both high dosage of Super-S-TuD21 (targeting miR-21) and efficient DDS would be required. However, high dosage of Super-S-TuD encapsulated by COATSOME-X was

toxic to nude mice. We therefore used COATSOME-Y instead, which shows 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.

# Merged Project of Respiratory Pathophysiology and Pathobiology

## 巽・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 into consideration other than pathogens that cause infection. To control intractable infections, elucidation of respiratory biological control mechanisms could be essential in regard with treatment strategy aimed for recovery and regeneration from lung injury. Two major topics have been set up since this merged project of respiratory pathophysiology and pathobiology was started.**

**1）search for new treatment seeds on the basis of combining deep clinical phenotyping and omics analysis.**

**2）search for mechanistic functions to overcome respiratory infection.**

**呼吸器内科臨床で遭遇する難治性感染症は，感染を生じる病原体の問題以外に，生体構造が形態的／機能的に障害を受けている個体（難病）に発症することが問題となる．健常人に発症した新型コロナウイルス感染症は，重篤な肺障害を受けた後でも驚異的な回復をすることを経験する．しかし，基礎病態のある個体に発症した新型コロナウイルス感染症では後遺症が残る可能性が高い感触がある．難治性感染症の制御には，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．**

**今年度の主なテーマ（Research Focus）は下記の２点であるが，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）呼吸器生体制御に関する病態解明および新規治療開発に関する研究**

**２）呼吸器感染症を呼吸器疾患生体制御の観点から研究**



| | | | |
|---|---|---|---|
| Research Professor | Koichiro Tatsumi | 特任教授 | 巽　浩一郎 |
| Research Professor | Jiro Terada | 特任教授 | 寺田　二郎 |
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| Research Assistant Professor | Hiroyuki Tajima | 特任助教 | 田島　寛之 |


### 1. Selective targeting of KRAS-driven lung tumorigenesis via unresolved ER stress. JCI Insight. 2021; 6: e137876.


Shimomura I, Watanabe N, Yamamoto T, Kumazaki M, Tada Y, Tatsumi K, Ochiya T, Yamamoto Y


Lung cancer with oncogenic KRAS makes up a significant proportion of lung cancers and is accompanied by a poor prognosis. Recent advances in understanding the molecular pathogenesis of lung cancer with oncogenic KRAS have enabled the development of drugs, yet mutated KRAS remains undruggable. We performed small-molecule library screening and identified verteporfin, a yes-associated protein 1（YAP1）inhibitor; verteporfin treatment markedly reduced cell viability in KRAS-mutant lung cancer cells in vitro and suppressed KRAS-driven lung tumorigenesis in vivo. Comparative functional analysis of verteporfin treatment and YAP1 knockdown with siRNA revealed that the cytotoxic

effect of verteporfin was at least partially independent of YAP1 inhibition. A whole-transcriptome approach revealed the distinct expression profiles in KRAS-mutant lung cancer cells between verteporfin treatment and YAP1 knockdown and identified the selective involvement of the ER stress pathway in the effects of verteporfin treatment in KRAS-mutant lung cancer, leading to apoptotic cell death. These data provide novel insight to uncover vulnerabilities in KRAS-driven lung tumorigenesis.

**2. Pathophysiological Roles of Stress-Activated Protein Kinases in Pulmonary Fibrosis. Int J Mol Sci. 2021; 22: 6041.**

Kasuya Y, Kim JD, Hatano M, Tatsumi K, Matsuda S

Idiopathic pulmonary fibrosis (IPF) is one of the most symptomatic progressive fibrotic lung diseases, in which patients have an extremely poor prognosis. Therefore, understanding the precise molecular mechanisms underlying pulmonary fibrosis is necessary for the development of new therapeutic options. Stress-activated protein kinases (SAPKs), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (p38) are ubiquitously expressed in various types of cells and activated in response to cellular environmental stresses, including inflammatory and apoptotic stimuli. Type II alveolar epithelial cells, fibroblasts, and macrophages are known to participate in the progression of pulmonary fibrosis. SAPKs can control fibrogenesis by regulating the cellular processes and molecular functions in various types of lung cells (including cells of the epithelium, interstitial connective tissue, blood vessels, and hematopoietic and lymphoid tissue), all aspects of which remain to be elucidated. We recently reported that the stepwise elevation of intrinsic p38 signaling in the lungs is correlated with a worsening severity of bleomycin-induced fibrosis, indicating an importance of this pathway in the progression of pulmonary fibrosis. In addition, a transcriptome analysis of RNA-sequencing data from this unique model demonstrated that several lines of mechanisms are involved in the pathogenesis of pulmonary fibrosis, which provides a basis for further studies. Here, we review the accumulating evidence for the spatial and temporal roles of SAPKs in pulmonary fibrosis.

**3. Multi-institutional prospective cohort study of patients with pulmonary hypertension associated with respiratory diseases. Circ J. 2021; 85: 333-342.**

Tanabe N, Kumamaru H, Tamura Y, Taniguchi H, Emoto N, Yamada Y, Nishiyama O, Tsujino I, Kuraishi H, Nishimura Y, Kimura H, Inoue Y, Morio Y, Nakatsumi Y, Satoh T, Hanaoka M, Kusaka K, Sumitani M, Handa T, Sakao S, Kimura T, Kondoh Y, Nakayama K, Tanaka K, Ohira H, Nishimura M, Miyata H, Tatsumi K

Background: There is limited evidence for pulmonary arterial hypertension (PAH)-targeted therapy in patients with pulmonary hypertension associated with respiratory disease (R-PH). Therefore, we conducted a multicenter prospective study of patients with R-PH to examine real-world characteristics of responders by evaluating demographics, treatment backgrounds, and prognosis. Methods and Results: Among the 281 patients with R-PH included in this study, there was a treatment-naïve cohort of 183 patients with normal pulmonary arterial wedge pressure and 1 of 4 major diseases (chronic obstructive pulmonary diseases, interstitial pneumonia [IP], IP with connective tissue disease, or combined pulmonary fibrosis with emphysema); 43% of patients had mild ventilatory impairment (MVI), whereas 52% had a severe form of PH. 68% received PAH-targeted therapies (mainly phosphodiesterase-5 inhibitors). Among patients with MVI, those treated initially (i.e., within 2 months of the first right heart catheterization) had better survival than patients not treated initially (3-year survival 70.6% vs. 34.2%; P=0.01); there was no significant difference in survival in the group with severe ventilatory impairment (49.6% vs. 32.1%; P=0.38). Responders to PAH-targeted therapy were more prevalent in the group with MVI.
Conclusions: This first Japanese registry of R-PH showed that a high proportion of patients with MVI (PAH phenotype) had better survival if they received initial treatment with PAH-targeted therapies. Responders were predominant in the group with MVI.

**4. Cell tracking suggests pathophysiological and therapeutic role of bone marrow cells in Sugen5416/hypoxia rat model of pulmonary arterial hypertension. Can J Cardiol. 2021; 37: 913-923.**

Miwa H, Sakao S, Sanada TJ, Suzuki H, Hata A, Shiina Y, Kobayashi T, Kato F, Nishimura R, Tanabe N, Voelkel N, Yoshino I, Tatsumi K

Background: The mechanism of vascular remodelling in pulmonary arterial hypertension (PAH) remains unclear. Hence, defining the origin of cells constituting intractable vascular lesions in PAH is expected to facilitate therapeutic progress. Herein, we aimed to evaluate the origin of intractable vascular lesions in PAH rodent models via bone marrow (BM) and orthotopic lung transplantation (LT).
Methods: To trace BM-derived cells, we prepared chimeric rats transplanted with BM cells from green fluorescent protein (GFP) transgenic rats. Male rats were transplanted with lungs obtained from female rats and vice versa. Pulmonary hypertension was induced in the transplanted rats via Sugen5416 treatment and subsequent chronic hypoxia (Su/Hx).
Results: In the chimeric Su/Hx models, GFP-positive cells were observed in the pulmonary vascular area. Moreover, the right ventricular systolic pressure was significantly lower compared with wild-type Su/Hx rats without BM transplantation (P = 0.009). PAH suppression was also observed in rats that received allograft transplanted BM transplantation. In male rats that received LT and Su/Hx, BM-derived cells carrying the Y chromosome were also detected in neointimal occlusive lesions of the transplanted lungs received from female rats.
Conclusions: BM-derived cells participate in pulmonary vascular remodelling in the Su/Hx rat model, whereas BM transplantation may contribute to suppression of development of PAH.

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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「病原真核微生物」

In FY2002, the Ministry of Education, Culture, Sports, Science and Technology (MEXT) implemented the National BioResource Project (NBRP) to construct the framework for systematic collection, preservation, and distribution of bioresources, with a focus on those that required strategic development by the national government. After the reviewing the NBRP every five years, in FY2017, the forth 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.

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生物資源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，2017年度より第４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．

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．

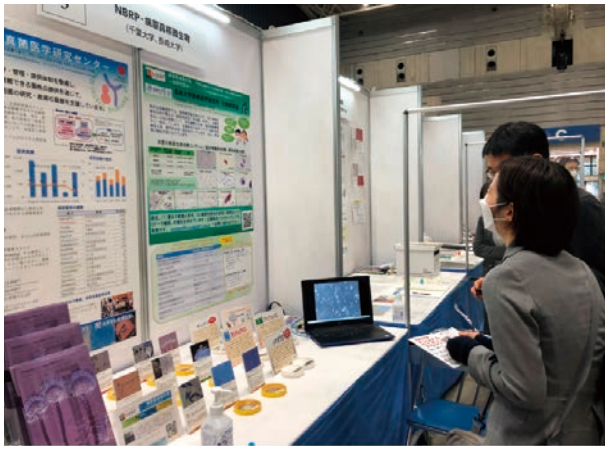

FIG.1. Exhibition at the 45th Annual Meeting of the Molecular Biology Society of Japan.

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

| Number of strains | FY2017 | FY2018 | FY2019 | FY2020 | FY2021* |
|---|---|---|---|---|---|
| Collection | 626 | 563 | 579 | 886 | 650 |
| Preservation | 24, 604 | 24, 459 | 24, 899 | 25, 785 | 26, 435 |
| Provision | 1, 429 | 980 | 1, 319 | 222 | 1, 246 |

*: to 31th Dec., 2021

# Cooperative Research of Priority Areas with NEKKEN, Nagasaki University

## Project for Morphological, Physiological and Molecular Biological Analysis of Pathogenic Fungi and Actinomycetes Collected in Africa and Vietnam.

# 長崎大学熱帯医学研究拠点特定領域共同研究

## 「熱帯地域，特にアフリカおよびベトナムで発生している真菌症・放射菌症の原因菌の収集と形態学的，生理学的，分子生物学的解析」プロジェクト

Under assistance of Kenya Research Station, Inst. NEKKEN, Nagasaki univ., we are analyzing toxins contaminating major local grains (maze, wheat) and milks, and also producer fungi. We found the local foods are contaminated by the toxins at concentrations far above the international standards. The result has been announced in newspapers, and received large attention.

Fumonisins are produced by Fusarium, a pathogen of wheat, and while wheat contamination has been investigated worldwide, there is little research on corn, a staple food in Kenya. In this study, we isolated the fungus and measured fumonisin production from corn in Kenya.

These studies should contribute significantly to the future of health care and quality of life for the people of Japan as well as Kenya in the face of globalization, global warming and deteriorating environmental and food conditions.

長崎大学熱帯医学研究所ケニア拠点の助力を得て，ケニアを中心に上記プロジェクトを展開している．現在までにケニア各地の主要穀物（コーン，小麦）やミルクなどを汚染するカビ毒（発がん性アフラトキシン他）とその生産菌の解析を進め，現地の食糧の多くが，世界の安全基準値を大きく上回るカビ毒で汚染されていることを明らかにしてきた．その結果は，現地のマスコミにも取り上げられた．

また，フモニシンは小麦の病原菌である*Fusarium*が産生し，小麦汚染については世界的に調査されているが，ケニアの主食であるコーンに関する研究はほとんどない．そこで，ケニアのコーンからカビを分離するとともにフモニシン産生量を測定した．

これらの研究は地球のグローバル化，温暖化，環境・食糧事情の悪化が進む中で，ケニアばかりでなく日本の人々の医療やQOLの維持にも，将来大きく貢献するはずである．

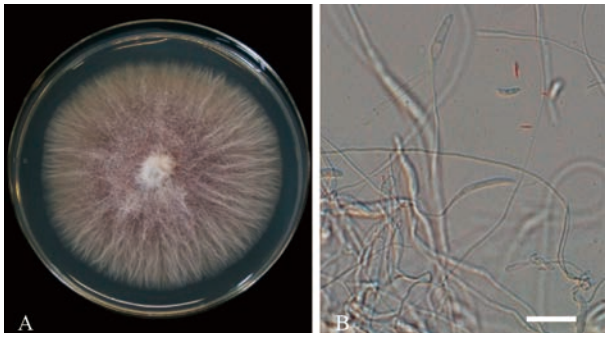


FIG.1. *Fusarium verticillioides* causing corn *Fusarium* head blight. (A) Colony, PDA, 25℃, 7 days. (B) Conidiogeneses and macroconidia. Scale = 10 μm.

TABLE 1. *Fusarium* sprcies isolated from corn in Kenya.

| | Region | | |
|---|---|---|---|
| *Fusarium* species | Rift Valley (n = 11) | Lower Eastern (n = 41) | Total (n = 52) |
| *F. andiyazi* | 5 (45.5%) | 4 ( 9.8%) | 9 (17.3%) |
| *F. verticillioides* | 6 (54.5%) | 36 (87.8%) | 42 (80.8%) |
| *F. temperatum* | 0 (0%) | 1 (2.4%) | 1 (1.9%) |

# 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

This project aims to cope with the intractable fungal disease, aspergillosis particularly in elderly and neonates, by investigating the epidemiology, exacerbating factors and obstacles for treatment such as drug resistance. Fungal infections in neonates have been an unexplored field of investigation, and is another target of this project.

The development of azole-resistance among *Aspergillus fumigatus* has become a growing serious threat. Mutation in Cyp51A is known to be one of the critical mechanisms of azole resistance in *A. fumigatus*. We had already reported a novel mechanism of azole-resistance (Hmg1 mutation) previously (2018). This year, we analyzed the Hmg1 mutation in detail using the CRISPR/Cas9 system and reported it as an article in Medical Mycology (2021). In addition, we investigated cyp51A gene polymorphisms of the fungal strains isolated in Japan and overseas, compared them with other countries (particularly Europe, where resistant strains are frequently isolated), and reported in Mycoses (2021). Furthermore, we also investigates the azole-resistance caused by an amino-acid substitution in other protein participating in the lipid metabolism, and will present these results in an international conference.

The investigation into the mechanism of the development of aspergillosis is critical to prevent its exacerbation. To this end, we have been analyzing proteins in the fungal cell wall as candidates for exacerbation. This year, we clarified how the fungal cell wall proteins interact with the host's innate immune system (in preparation for submission).

Through collaboration with Keio University and some other major domestic hospitals, in the cases of fungal infections caused by non-*fumigatus Aspergillus*, the sensitivity of serum aspergillus antibody testing is reduced compared with the ones caused by *A. fumigatus*, and reported in Journal of Clinical Microbiology (2021). We are carrying out further analysis on the detail of the resistant isolates.

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 utilize 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 useful for the appropriate treatment of this severe case. In this year, we analyzed another *Aspergillus fumigatus* strain isolated from neonatal invasive deep-seated mycosis. We compared above two strains using STRs analysis and the two strains were of different background. Environmental fungal survey in neonatal intensive care unit is needed for preventing deep-seated mycosis in extremely premature and low birth weight infant. Above study results gave us the important information for the establishment of diagnosis, and treatment for aspergillosis and the other mycoses in neonate patients.

先進各国と同様に我が国で最も死亡者が多い深在性真菌症（内臓真菌症）はアスペルギルス症であり，特に我が国では高齢化や慢性閉塞性肺疾患（COPD）等の慢性肺疾患に好発する慢性肺アスペルギルス症の頻度が高いことから，本疾患の研究は社会的意義が大きい．更には，現在世界中を混乱に陥れているCOVID-19感染に多くのアスペルギルス症の合併（CAPA: COVID-Associated Pulmonary Aspergillosis）がみられたことから，アスペルギルス症の重要性は世界的に再認識されている．本プロジェクトはこれらの疾患の疫学，増悪因子，重要な難治化因子である薬剤耐性（とく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により，新規診断法，治療法，予防法の開発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出来ている．今年はCOVID-19の流行にもかかわらず，検査依頼400件あまりに達した．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ている．令和３年度は我々が報告した本菌の新規耐性メカニズム（Hmg1変異）について，CRISPR/Cas9システムを用いて詳細な解析を行い論文として報告した（Med Mycol誌にて発表）．また，我が国及び海外の分離株を対象に遺伝子多型の調査を行い，諸外国（特に耐性株が多い欧州）との比較を行いその知見について報告を行った（Mycoses誌にて発表）．また脂質代謝に関与する別のタンパクの変異によるアゾール薬耐性化について解析を進め，成果を国際学会において発表予定である．一方，難治化克服のための基礎研究として，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存在を示唆してきたが，今年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ることが出来た（論文準備中）．

また，慶應大学病院等との国内共同疫学研究では，*A. fumigatus*以外の*Aspergillus* spp. による感染で血清を用いた抗体検査の感度が低下することを明らかにし，論文として報告を行った（J Clin Microbiol誌にて発表）．現在収集した薬剤耐性菌株についてさらに詳細な解析を進めている．

新生児領域における研究に関しては，これまで新生児深在性真菌感染症発症状況全国調査を実施し，国内の実態を明らかにした．また，乳児の胃液の真菌培養による糸状菌感染症診断を試みたところ，真菌に易感染性を示すことで知られる慢性肉芽腫症の乳児肺炎症例から*Rasamsonia piperina*の分離に成功し，論文を公表した．また，新生児の*Aspergillus 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出来た症例を経験し．論文公表した．本年は，別の新生児侵襲性アスペルギルス感染症症例から分離された*Aspergillus fumigatus*株と上記髄膜炎・脳室炎症例の分離株についてSTRs（microsatellite）解析を実施したが，異なる株と判断された．いずれの症例も，超早産・超低出生体重児で，皮膚症状を初発症状としており，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うことを計画している．これらの検討結果は，新生児アスペルギルス症の診断・治療法策定において極めて重要な情報を提供する．

# 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.

Due to the COVID-19 pandemic worldwide, we are actively conducting research meetings and sharing experimental procedures using remote video systems.

We have already 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 are developing resistance gene detection methods based on LAMP（Loop-Mediated Isothermal Amplification）. Using several resistant strains, we partially established the detection method by LAMP and published as an article.

Besides, we examined clinical and environmental isolates of Candida krusei and C. tropicalis, whose antifungal resistance is one of the clinically facing problems worldwide. We have reported their antifungal mechanisms and phylogenetic analysis.

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. Several medical institutions in Brazil have participated in a multi-center database of mycosis cases, and more than 250 cases

have already been enrolled.

Besides, using this consortium, a bio-resource bank for fungal strain has been established, and we have already started 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．

本研究では，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す．

今般のCOVID-19の世界的な蔓延のため，遠隔ビデオシステムを用いた研究ミーティングや実験手技共有を積極的に行っている．

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すでに我々は見出している．すなわち，抗真菌薬耐性のメカニズムは国や地域によって異なる可能性が示唆され，耐性遺伝子検出法を開発するうえで国情を考慮すべきであることが確認された．

以上のようなブラジルの状況を踏まえ，耐性遺伝子検出法の開発を開始している．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論文として報告した．また抗真菌薬耐性を有する菌種として臨床的に問題となっている *Candida krusei* および *C. tropicalis*

について，系統解析および耐性メカニズムの探索を行い，論文発表を行った．

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，多施設共同で真菌症の症例が集積されつつあり，これまでに250症例を超える症例数が登録されている．

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を開始している．

# 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」

Antibiotics have helped control various bacterial infections but have also promoted bacterial evolution to acquire resistant genes, leading to the emergence of multidrug-resistant bacteria worldwide. Therefore, a novel therapeutic approach requires both the inhibition of bacterial growth and the deceleration of bacterial evolution.

Bacterial evolution is triggered when bacteria survive in lethal environments, such as in the presence of antibiotics and host immune responses. Bacteria survive with minimal cellular activity in harsh environments and re-grow after stress removal, during which they epigenetically memorize stresses and improve their tolerance to such environments. Bacterial cells with increased tolerance, called persisters, continue to infect the host for an extended period. While surviving continuous exposure to stress, bacteria gain genetic mutations and exogenous genes, resulting in the generation of descendants exhibiting new phenotypes. We believe that understanding bacterial tolerance is essential for the discover of next-generation antimicrobial therapeutics.

One of the hurdles in antibiotics development is the quality of the compound library. We will overcome this hurdle by using an original, unconventional natural product library. Natural products are attracting attention as novel resources for drug discovery.

This project aims to find novel screening targets for infection-controlling compounds, based on the molecular mechanism of bacterial persistence infection, and identify novel privileged molecular structures for antimicrobial drug discovery. Our multi-faceted project will enable a novel and unique discovery of next-generation therapeutics to overcome bacterial tolerance and resistance.

抗菌薬開発の成功は，多くの細菌感染症制御を可能としたが，多剤耐性菌など新たな難治性感染症の要因をも生み出した．現在，既存抗菌薬では制御不能な難治性感染症の克服が喫緊の課題となっている．

細菌進化は，過酷なストレス環境での生存機構と密接に関わる．感染時，細菌は，増殖と細胞活動が最小限の休眠状態を繰り返す．この間ストレスを記憶，寛容性を向上させ，持続的な宿主内生存を可能とする．同時に，抗菌薬投与，免疫応答等の環境変化に耐えながら，ゲノムアレンジを引き起こし，新たな表現型を示す細菌へと変容する．従って，過酷な環境で働く細菌の制御因子は，新規感染症薬の標的となる．

以上を背景に，本研究では「抗菌薬・免疫応答依存的な細菌持続感染症の分子機構解明」と「難治性感染症制御のための次世代型感染症創薬研究」を両輪とし，パーシスターを制御する次世代型抗感染症薬の創出への貢献を目指している．

# Leading Research Promotion Program, Institute for Global Prominet Research

## Advanced Research of Infection and Immunity Based on Integrative Understanding of Host-Microbe Interactions

# 千葉大学グローバルプロミネント研究基幹・リーディング研究育成プログラム

## 「“超個体”の統合的理解に基づく次世代型「感染制御学」研究推進拠点」

The research group, composed of the researchers in MMRC, School of Medicine, Faculty of Pharmaceutical Sciences, and University Hospital, was selected as the Leading Research Promotion Program of Chiba University. The members focus on the understanding of molecular interactions between hosts and microbes, especially commensal fungi and bacteria, using the model assay systems targeting the skin, respiratory, and digestive organs. The members aim to reveal the molecular machinery underlying the disruption of homeostatic balance in the hosts by invasive pathogens, which induce infectious diseases. The findings obtained from the project will help to create innovative achievements in therapeutics of infectious diseases and lead to the improvement of human health.

千葉大学では，学内での研究の核となる新たな研究グループの創出を目指す「グローバルプロミネント研究基幹」を設置しており，当センター教員が中心となった研究プロジェクト「“超個体”の統合的理解に基づく次世代型「感染制御学」研究推進拠点」が，リーディング研究育成プログラムに採択され，活動を実施している．本プログラムでは，感染免疫分野の教員が中心となり，医学研究院，薬学研究院，附属病院の研究者と連携し，共生微生物と宿主である個体の免疫システムとの相互作用，そこへ侵入する病原体による恒常性の破綻と感染症の発症機序などについての基礎研究を，皮膚，呼吸器，消化器など各種器官でのモデル実験系を用いて解析し，そこから得られる成果を統合的に理解することで，感染症・免疫制御の分子メカニズムを明らかにする次世代型の「感染制御学」を創出し，我々の健康維持と感染症などの克服へつながる新規イノベーション創生を目指している．

# 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 diseases with unmet medical needs.

**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.

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，治療法の確立されていない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，NF-κBの阻害剤の開発を進めています．

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

# 2020 Fiscal Year Cooperative Research Program Report

# 令和２年度 共同利用・共同研究報告

## 研究課題　’20－１

### Analysis of microRNA-mediated human defense system induce by viral infection

Kumiko Ui-Tei
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Mitsutoshi Yoneyama, Koji Onomoto
（Medical Mycology Research Center, Chiba University）
Tomoko Takahashi
（Graduate School of Science and Engineering, Saitama University）

### ウイルス感染で誘発されるmicroRNAによるヒトの新しい生体防御機構の解明

程久美子
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米山光俊・尾野本浩司
（千葉大学真菌医学研究センター）
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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　’20－２

### Development of a novel vaccine for preventing infectious diseases caused by *Cryptococcus neoformans*

Kazuyoshi Kawakami, Jun Kasamatsu
（Tohoku University Graduate School of Medicine）
Katsuhiko Kamei, Susumu Kawamoto
（Medical Mycology Research Center, Chiba University）

## クリプトコックス感染症の発症予防を目指した新規ワクチンの開発

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### 研究成果

クリプトコックス症は，エイズ，糖尿病，膠原病，腎疾患，血液悪性疾患など免疫低下を伴う基礎疾患や免疫抑制剤の使用により発症する真菌感染症である．本感染症は免疫不全を背景として発症するため，難治化しやすく臨床上問題となっている．特に，クリプトコックス髄膜炎は，世界的に結核に次いでエイズ死因の第２位とされている．このため，ワクチンによる発症予防法の開発が期待されているが，現時点では臨床で使用可能なワクチンは存在しない．

本研究では，クリプトコックスの主要なT細胞抗原として知られるchitin deacetylase 2（Cda2）を直径100nmの酸化鉄ナノ粒子に結合させ（Cda2-NP），C57BL/6マウスの気道内に投与することで抗原特異的な気道粘膜免疫の誘導効果について解析した．Cda2単独では気管支肺胞洗浄液中のCda2特異的IgGがほとんど検出できなかったが，Cda2-NPでは顕著なIgG及びIgAの産生が観察された．しかし，IgG産生のほとんどがTh2型のIgG1であったため，アジュバントとしてPoly（I:C）を併用することでTh1型のCda2特異的なIgG2cの顕著な産生が検出された．併せて，所属リンパ節でCda2特異的なTh1細胞の分化誘導が検出されたが，感染部位である肺そのものではIFN-γ産生の増強は観察されなかった．また，千葉大学真菌医学研究センターにて分子生物学的に解析を行ったクリプトコックス株を用いた感染実験では，Cda2-NPによる感染防御効果は認められなかった．

以上の結果から，Cda2-NP/Poly（I:C）の気道投与によって顕著な抗原特異的粘膜免疫の誘導が観察されたが，クリプトコックス感染防御に必要なTh1細胞の効率的な誘導には至らなかった．今後は，所属リンパ節から実効臓器へのTh1細胞のマイグレーションがみられなかった原因を探るとともに，他のアジュバントへの変更等，効率的な感染防御効果の誘導へ向けた開発を進めていきたい．

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## 研究課題　’20－３

## Comprehensive screening of inhibitory receptors recognizing pathogenic fungi

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## 臨床分離真菌を認識する免疫抑制型受容体の網羅的探索

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### 研究成果

免疫抑制型受容体いわゆる免疫チェックポイント分子は，がん治療の分野において画期的な治療標的として注目を集めている．病原体感染の分野でも免疫チェックポイント分子の存在が報告されており，治療標的としての可能性が検討されている．しかしながら，真菌を認識する免疫抑制型受容体はほとんど知られていない．そこで本共同研究では，千葉大学真菌医学センターが保有する病原性真菌株および佐賀大学医学部附属病院検査部にて分離された真菌株から，免疫抑制型受容体が認識する真菌の探索を行なった．免疫抑制型受容体レポーター細胞（リガンド認識をGFP発現によりモニターできる細胞株）47種類を用いて探索を行なった結果，アスペルギルス属（*A. fumigatus*, *A. flavus*, *A. nidulans*）と白癬菌属（*T. rubrum*, *T. mentagrophytes*, *T. tonsurans*）を認識する免疫抑制型受容体を複数見出した．ヒト単球系細胞株U937細胞に，これらの免疫抑制型受容体を過剰発現させると，アスペルギルス属や白癬菌属に対する免疫応答が減弱したことから，免疫チェックポイント分子として機能し得ることが明らかとなった．

現在は，免疫抑制型受容体が認識するリガンド分子の同定と，免疫抑制型受容体に対する中和抗体による治療効果の検討を行なっている．前者に関しては，クロロホルムメタノール抽出物にリガンド活性が認められたことから脂溶性リガンドを想定して，TLCによるリガンド分子の単離精製を進めている．後者に関しては，中和抗体による免疫応答の増強効果を*in vitro*で確認済みであり，*in vivo*における治療効果を検討するための条件検討を進めている．

## 研究課題　’20－４

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

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### 研究成果

This is the second term of our project, and we have published a paper as we did last year. The abstract of the paper has been attached below.

*Candida glabrata* adheres to human epithelial mucosa and forms biofilm to cause persistent infections. In this project, Single-cell Force Spectroscopy（SCFS）was used to glimpse at the adhesive properties of *C. glabrata* as it interacts with clinically relevant surfaces, the first step towards biofilm formation. Following a genetic screening, RNA-sequencing revealed that half of the entire transcriptome of *C. glabrata* is remodeled upon biofilm formation, around 40% of which under the control of the transcription factors CgEfg1 and CgTec1. Using SCFS, it was possible to observe that CgEfg1, but not CgTec1, is necessary for the initial interaction of C. glabrata cells with both abiotic surfaces and epithelial cells, while both transcription factors orchestrate biofilm maturation. Overall, this study characterizes the network of transcription factors controlling massive transcriptional remodelling occurring from the initial cell-surface interaction to mature biofilm formation. The gathered knowledge is also expected to guide the delineation of strategies to surpass the widespread of multidrug resistance among fungal pathogens with implications in Human Health and Biotechnology.

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## 研究課題　’20－５

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

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

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### 研究成果

深在性真菌症の中でも*Aspergillus fumigatus*を主要病原菌とするアスペルギルス症は増加傾向にあり，予後が非常に悪い．近年，アスペルギルスのバイオフィルム形成がアスペルギルス感染に関与することが示唆されている．特にアスペルギローマの菌糸塊に見られる菌糸周囲には厚い細胞外マトリクスが観察されている．このようなバイオフィルムを形成する状態では，いくつかの抗真菌薬に対する感受性が低下する現象が示され，難治性の原因の１つになっていると考えられる．しかしながら，バイオフィルム形成，および，それによる抗真菌薬耐性の詳細な分子メカニズムは不明な点が多い．本研究では，バイオフィルム形成に関わる新規遺伝子を同定し，抗真菌薬耐性との関連性を明らかにすることを目的とする．2020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，プラスミドライブラリの作製を行った．

昨年度まで，CRISPR/Cas9ゲノム編集技術を*A. fumigatus*で応用し，次世代シーケンサーと組み合わせたCRISPRスクリーニングを行うことによって，血清存在下の生育に必須と予想される遺伝子の取得を試みていた．本年度は，CRISPRライブラリに挿入する変異導入標的配列として，Af293株のゲノム配列にコードされていると予想される全9,840遺伝子のうち9,807遺伝子について設計し，negative controlとしてゲノム配列上に存在しない配列と合わせて，10,403種類のpooled oligo DNAを合成した．合成したpooled oligo DNAをCRISPR/Cas9ベクターにクローニングし，大腸菌NEB10-beta株に形質転換することによりCRISPRライブラリを作製した．pooled oligo DNAの導入効率を調べるために，CRISPRライブラリのガイドRNA配列の周辺をPCRにより増幅し，イルミナシークエンサーMiSeqもしくはナノポアシークエンサーMinIONによるアンプリコン解析を行った．最終的に，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，設計した配列の99.7%を網羅できていることを確認できた．今後，作製したCRISPRライブラリを*A. fumigatus*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．

## 研究課題　’20－６

## 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．

2020年度は，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，*A. fumigatus*の二次代謝産物がアスペルギローマの病態に与える影響の解析を進めた．我々は，in vitroで作成した菌球を健常マウスの皮下空洞に直接留置すると，１週間後には組織侵襲を起こすことを見出していることを利用し，laeA欠損株とgliA欠損株の組織侵襲度を親株と比較した．その結果，laeA欠損株では親株と比較し明らかに組織侵襲度が低下することを見出し，種々の二次代謝産物が組織侵襲に必要である可能性が示唆された．gliA欠損株では親株の組織侵襲度が低かったため，親株との侵襲度の差異は認めなかった．

さらに，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GFP発現*A. fumigatus*によるアスペルギローマモデルマウスを作成し，長崎大学に設置されている発光in vivoイメージングシステム IVIS Lumina Ⅱによるアスペルギローマの非侵襲的検出条件を検討し，体内に留置した菌球を体外から観察することに成功した．さらに適切な観察条件の検討を継続中である．

## 研究課題　’20－７

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

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**研究成果**

本プロジェクトは，知花博士が作製したユニークな組換え体コレクションを活用して，*Candida glabrata*と*Candida albicans*栄養素とストレスの適応を制御する調節回路の進化的な再配線を明らかにする．初年度１年間は，今後の研究発展を目的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について検討した．予備実験については，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として，ゲノム内に組み込まれている96種類のsignature tag sequenceを用いてマイクロアレイ法やNGSによって実施される．しかし，本コレクションにはsignature tag sequenceが組み込まれていないため新たな方法を構築する必要があった．

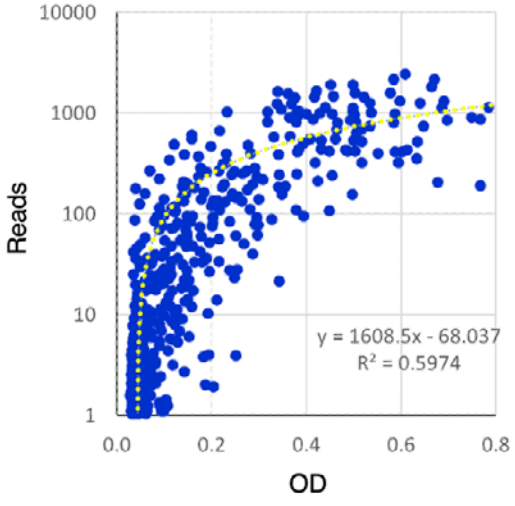

図. ODとリード数の相関

96wellプレートを用いて菌株を個別に培養し，OD600を測定後，全菌体を混合しDNAを回収しイルミナ次世代シークエンサーHiseqを用いて各菌株に由来する形質転換マーカー挿入周辺領域のシークエンスリード数を測定した．その結果，各菌株のODとリード数の間にはR2＝0.6の相関関係（図）で，各菌株の菌数がリード数として反映されることが確認された．さらに既存の方法とは異なり混合可能な株数が96以内に限定されないため，約4,000株の同時解析が可能か否か検討中である．

## 研究課題　’20－８

## Analysis of mycovirus affecting secondary metabolism in *Aspergillus* species

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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

マイコウイルスは糸状菌宿主に持続的に感染し，生理機能に大きな影響を及ぼすことが近年報告されている．糸状菌は天然化合物の探索源，物質生産宿主としての機能性に優れ，種々の産業で利用されていることから，本研究では糸状菌二次代謝に対するマイコウイルスの影響を体系的に理解することを目的とした．

これまでに，155株の病原菌 *Aspergillus fumigatus* を対象とした探索において19株のウイルス感染を見出している．さらに解析対象を広げて，代表的なカビ毒生産菌である *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. terreus* の計194株を，千葉大学真菌医学研究センターから分譲していただき探索を進めた．マイコウイルスの検出は，各株の培養菌体からdsRNAを抽出し，アガロースゲル電気泳動により判定した．その結果，RNAウイルス由来と考えられるバンドを示した株は *A. flavus*：13.9%（10/72株），*A. parasiticus*：65.8%（25/38株），*A. ochraceus*：25%（5/20株），*A. terreus*：20.3%（13/64株）となった．これまで，複数の種に渡るマイコウイルスの大規模探索は世界的にも例がない．本研究により，*Aspergillus* 属菌において，マイコウイルスが一定の割合で広く感染している実態が明らかになった．今後は，ゲノム解析により感染ウイルスの種を決定，ウイルスを除去したウイルスフリー株を作製し，ウイルスの有無による宿主の二次代謝の違いを比較する．

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## 研究課題　’20－９

## Establishment of an assay to evaluate trichothecene toxicity in vitro

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## in vitro でのトリコテセン類毒性評価法の確立

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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．しかし，動物を用いた方法においては，個体差やその手法の煩雑さなどの問題があり，培養細胞も由来となった動物種や組織等により，また培養細胞株で，その結果が異なるという問題点もあ

る.

本研究ではこのような不安定性等を取り除くために, in vitroにおけるトリコテセン類の評価方法の確立を目指した. 本研究ではトリコテセン類が示す主要な毒性機構であるタンパク質合成阻害に焦点を絞り, 迅速・簡便に化合物同士の比較が可能な手法を検討した. その結果, 市販のキットを用いて0.5mLPCRチューブでの反応と引き続く酵素活性測定による迅速・簡便な方法を確立した. 難水溶性を示すトリコテセン類も存在するため, さらに検討を進め, 本法がアセトニトリルに溶解したトリコテセン類でも利用が可能であることを明らかとした. 本研究で開発した方法はトリコテセン類の毒性評価に広く応用できると期待する.

## 研究課題　'20－10

## 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

申請者らが見出した臨床分離白癬菌株*Trichophyton rubrum* TIMM20092は, テルビナフィン（以下, TBF）に加え, アゾール系抗真菌薬であるイトラコナゾール（以下, ITC）およびボリコナゾール（以下, VRC）に低感受性を示す. 申請者らは, 分子生物学的アプローチのもと, 本株のITC・VRC低感受性化の要因の解析を進める過程で, アゾールの排出機能を示す6個のトランスポーター(MDR1, MDR2, MDR3, MDR5, MFS1およびMFS2)を見出した. そして, ABC型トランスポーターであるMDR3がTIMM20092で過剰発現していることを突き止めた. しかしながら, ミュータントを利用した遺伝学的解析の結果, VRC低感受化の主要因はMDR3の過剰発現であるものの, ITC低感受性化には別のトランスポーターが関与していることが強く示唆された. そこで, 先の6個の薬剤排出トランスポーターのうち, ITC排出機能を有する可能性が見出されたMDR2, MDR5およびMFS1について, 再びミュータントを利用した遺伝学的解析を行った結果, MDR3と同じABC型トランスポーターであるMDR2の過剰発現がTIMM20092におけるITC低感受性化の主要因であることが判明した.

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## 研究課題　'20－11

## Identification of transcriptional regulation of *CgATG32* in *Candida glabrata*

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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. 2019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を行い, その領域に結合する転写因子候補遺伝子を複数同定した. 2020年度は, 各候補タンパク質の遺伝子破壊株を作製し, *ATG32*発現解析を行った. その結果, エキソリボヌクレアーゼ*XRN1*の遺伝子破壊株において, 鉄存在下での*ATG32*発現抑制が解除されることを見出し, *ATG32*発現調節において*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. HAタグを付加した*XRN1*発現株を作製し, 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. 現在, *XRN1*のRNase活性と*ATG32*発現調節の関係について調べている.

## 研究課題　'20－12

## 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

アスペルギルス症は患者数が多く, 予後も不良であることから, 世界で最も重要な真菌感染症の一つと考えられる. 近年, 本疾患の主力薬剤であるアゾール薬にたいして, 原因菌である*Aspergillus fumigatus* が耐性を示すようになってきており, 新しい抗真菌薬の開発が望まれている. アゾール薬は真菌細胞膜の主成分であるergosterolの合成を阻害することにより作用を発揮するため, CYP51遺伝子の突然変異がアゾール耐性に関与することが明らかになってきているが, ergosterol合成経路と耐性との関連については未解決の問題が多く残されている. そこで本研究では, これまで詳細に研究されていないCYP51の下流の遺伝子の機能を分析し, 新規抗真菌薬の開発の端緒となることを目指す.

2020年は当初, COVID-19のpandemicにより中国, 日本とも研究活動に大きな支障が生じたため, 研究の遂行が困難な状態が続いた. 後半より徐々に活動が可能となったため研究を開始し, ergosterol生合成の下流域で働く酵素の解析をするにあたって生合成の速度決定酵素として機能するHMG-CoA還元酵素の解析を行った. この理由として, Hmg1変異を伴うトリアゾール耐性*A. fumigatus* の報告が相次いでおり, 現在, Hmg1変異によるトリアゾール耐性*A. fumigatus* は国際的な注目を集めていることが挙げられる. *A. fumigatus*のHMG-CoA還元酵素は, Hmg1とHmg2の２つが存在する事が知られているが, 今回, この２つisozymeの細胞生存における役割を検討した.

*hmg1*と*hmg2*の発現量を解析した結果, 発現量に顕著な差があり*hmg1*が優位に発現していることがわかった. そこで*hmg1*のpromoterを条件付き発現promoterに置換した形質転換体を作成し, Hmg1が生存に必須であるか検証した結果, *A. fumigatus*の細胞生存にHmg1が必須であることが確認された.（投稿準備中）.

## 研究課題　'20－13

## 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2021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388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104名120株について，*A. fumigatus*のアゾール耐性株は全体では8.3％であったがアゾール使用例での耐性率は21.3％に上昇していることを確認した．

また今年度から検討を追加した担子菌については，2014年７月から2019年８月の間に，東京病院における呼吸器検体から同定された18検体の担子菌の同定と，臨床所見との関連性を検討した．症例は17例で，喀痰から８検体，気管支鏡検体から２検体検出され，菌種はスエヒロタケ４検体，ヤケイロタケ２検体，シイサルノコシカケ，アラゲカワラタケ，カワラタケ，ウスバタケが１検体ずつ同定された．全ての症例が肺に基礎疾患（陳旧性肺結核２例，肺非結核性抗酸菌症５例，気管支拡張症２例，間質性肺炎２例，気管支喘息１例）を有していた．スエヒロタケの症例でアレルギー性気管支肺真菌症が１例，慢性肺真菌症疑いを１例認め，その他の症例はcolonizationと考えられた．

### 発表論文

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## 研究課題　’20－14

## Development of molecular diagnosis of antifungal resistant fungi

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### 研究成果

Infections due to triazole resistant *Aspergillus fumigatus* are increasingly reported worldwide and are associated with treatment failure and mortality. The principal class of azole resistant isolates is characterized by the presence of tandem repeats within the promoter region of the *cyp51A* gene. We developed e a Loop-mediated isothermal amplification（LAMP）assay method to detect the tandem repeat insertion in the *cyp51A* gene promoter region based on novel LAMP primer sets. Our method is specific, rapid, and also provides crucial insights to enable development of novel antifungal therapeutic strategies against severe fungal infections due to *A. fumigatus* with tandem repeats.

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## 研究課題　’20－15

## 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，TFRXのMIC≥0.5μg/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．16株中13株（81％）でGyrAとParCの両方に変異が認められ，うち10株はMIC 1μg/mL以上を示したが，3株はMIC 0.5μg/mLと低値であった．

MLST解析では，2018年分離株のうち4株がST422と同一であったが，年齢や居住地は異なり，関連性は認めなかった．本研究の結果，MIC 0.5μg/mLの株であっても，複数の変異を有する株があり，また，特定のST株が複数分離されていることから，インフルエンザ菌のキノロン薬耐性状況について，監視していく必要性が示唆された．

### 発表論文

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## 研究課題　’20－16

## Functional analysis of RNA-binding proteins in antiviral innate immunity

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## 抗ウイルス自然免疫誘導におけるRNA結合タンパク質の機能解析

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### 研究成果

本研究では，高等脊椎動物における抗ウイルス自然免疫において重要な役割を担うウイルス感染センサーであるRIG-I-like受容体（RLR）によるウイルスRNA検知の分子機構と生理機能について継続して解析を行ってきた．2020年度の研究では，以前の共同研究で見出していたI型インターフェロン（IFN）遺伝子の発現を増強させる宿主RNA結合タンパク質に焦点をあて，その機能解析を継続実施した．当該分子は，培養細胞での解析においてIFNプロモーター活性を増強することが明らかである一方で，RLRの下流シグナルアダプター分子（IPS-1/MAVS）や転写因子（IRF-3/7）の活性化を直接

誘導しないこと，また当該分子が，ウイルス感染によって誘導される細胞内ストレス顆粒（SG）にRLRやウイルスRNAと共局在することから，RLRと協調してウイルス核酸認識に関与している可能性が示唆された．一方，CRISPR/Cas9によるゲノム編集により作出していたノックアウトマウスについてさらに詳細な解析を実施し，A型インフルエンザウイルス（IAV）を経鼻感染させた場合に，肺における抗ウイルス活性が顕著に減弱すると共に，IAV感染に対するマウスの生存が抑制されたことから，この分子が抗ウイルス応答の誘導に重要な役割を担うことが動物個体レベルでも確認された．

## 研究課題　’20－17

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

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## 無莢模型インフルエンザ菌による侵襲性感染症の病態解析

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### 研究成果

2020年度は呼吸器感染症由来の無莢膜型インフルエンザ菌（non typeable *Haemophilus influenzae*：NTHi）と千葉大学真菌医学研究センターから分与して頂いた侵襲性感染症由来の菌株を用いてバイオフィルムの産生能について検討した．まず対象菌株の分子疫学的背景として莢膜に関する*bexA*, *bexB*遺伝子，および*Cap*（a-f）遺伝子の解析を行った．上記遺伝子の保有はなく，無莢膜型インフルエンザ菌であることを確認した．これらの菌株を用いてバイオフィルム産生能の解析をPlate assayを用いて行った．呼吸器感染症由来のNTHiと比較し，侵襲性感染症由来のNTHiはバイオフィルムを多く形成していた．バイオフィルム内の状況を確認するために呼吸器由来のNTHi 1株を侵襲性感染症由来のNTHi 3株を対象としてDrip flow assayを行った．Cover slip上に形成されたバイオフィルムのaverage thickness, biomassを比較するとともにLIVE/DEAD stainで染色しバイオフィルム内の菌の分布および生菌の割合を評価した．侵襲性感染症由来NTHiはaverage thickness, biomassともに呼吸器由来NTHiと比較して高値であり，バイオフィルム内に含まれる菌の密度も高く，死菌よりも生菌が著しく多く分布していた．通常，バイオフィルム内はquorum sensing機構によりautoinducerを介して菌量は制御されるが，侵襲性感染症由来NTHiは通常とは異なる制御機構でバイオフィルムの制御を行っている可能性が考えられ，またバイオフィルム内で菌同士が接着している可能性も考えられるため接着因子に関しても解析を行っていく予定である．

## 研究課題　’20－18

## Elucidation of antifungal resistance mechanisms in *Candida auris*

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## *Candida auris*の抗真菌薬耐性機序の解明

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### 研究成果

近年，抗真菌薬に耐性を示す*Candida auris*のアウトブレイクが世界各国で問題となっている．抗真菌薬耐性*C. auris*株による侵襲性カンジダ症は致死率が高く，新たな治療戦略の開発が必要である．*C. auris*のキャンディン系薬耐性機序として，薬剤標的であるFKS遺伝子のS639F変異が知られているが，当該アミノ酸変異を有していない*C. auris*耐性株も臨床分離されている．本研究では，新規薬剤耐性遺伝子あるいは遺伝子変異を同定し，キャンディン系薬への耐性機序を明らかにすることを目的とした．

これまでに我々は，S639F変異を持たない*C. auris*臨床分離株から，薬剤耐性に関与すると予想される新規遺伝子変異を複数同定している．これらの変異遺伝子の影響を調べるため，ゲノム編集技術CRISPR-Cas9を*C. auris*で応用し，感受性株に変異遺伝子を挿入することに成功した．得られた変異挿入株をキャンディン系薬存在下で培養すると耐性を示したことから，当該遺伝子変異が薬剤耐性に関与していることが示唆された（未発表）．他の遺伝子変異も同様の耐性を示すか，引き続き確認を進めている．

## 研究課題　’20－19

## Development of novel therapeutic approach for skin persister infections

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## 皮膚を場としたパーシスター感染症克服法の開発

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### 研究成果

我々はこれまでの研究により，健常乳児期の皮膚では黄色ブドウ球菌（*Staphylococcus aureus*）のクオラムセンシング領域に変異が誘導され，排除される一方で，アトピー性皮膚炎（Atopic dermatitis, 以下AD）の皮膚では機能が維持され，皮膚炎のつながっている可能性を見出し，報告した[論文1)]．検体は，千葉大学小児科が出生コホート研究で採取した*S. aureus*菌株約270株を全ゲノムシークエンス行い，生後１ヶ月から６ヶ月に同一クローンが生着し続けた場合に，健常皮膚と，AD発症皮膚での特徴的なゲノム変化が病原微生物に誘導されるかを検討した．その結果，健常皮膚でのみ，*S. aureus*のゲノムに生着中にAgrクオラムセンシング領域に変異が誘導されるという現象を見出した．この変異は機能喪失型の変異であり，実際に健常皮膚では，生後１ヶ月に比べ，６ヶ月で*S. aureus*の生着数は減少した．マウスモデルで，野生型及び，Agr欠損株を用いて検討したところ，Agrクオラムセンシングの発現が，皮膚の生着には必須であり，ADにおける皮膚炎の発症に関与していることが示された．

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## 研究課題　’20－20

## Characterization and ecological survey of phomopsin-producing fungi

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## カビ毒ホモプシン産生菌の機能解析および生態学的研究

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### 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た．昨年度の研究において設計した，ホモプシン類合成遺伝子を検出可能なPCRプライマーを用いて当該遺伝子の検出を試みたところ，真菌*Beauveria bassiana*および*Colletotrichum gloeosporioides*においてDNA増幅が確認された．これらの微生物はホモプシン類産生能を有することが予想されたため，培養液から化合物を抽出し，LC-MSにより分析した．昨年度の研究において確立した分析手法により，*B. bassiana*においてホモプシン類と推定される化合物が検出された．一方，北海道に自生するマメ科植物のルピナスからホモプシン類産生菌の分離を試みたが，現在までのところ見つかっていない．また，これらのルピナス試料について成分抽出を行い，LC-MS分析に供したが，ホモプシン類は検出されていない．今後は，*B. bassiana*が産生するホモプシン類の構造の決定を試みるとともに，マメ科植物におけるホモプシン類産生菌およびホモプシン類の存在について，継続的に調査する．

## 研究課題　’20－21

## Analysis of environmental fitness through high-throughput phenotyping of *Aspergillus fumigatus*

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### 研究成果

病原真菌*Aspergillus fumigatus*は，肺アスペルギルス症の主要な原因菌である．本菌の感染は，特定の病原因子ではなく，多数の因子から決定される環境適応能が感染成立と密接に関わっていることが明らかとされてきたが，いまだその全容は明らかとなっていない．

我々は，菌株の有する表現型，特に宿主肺環境で晒される環境ストレスに本菌がどのように応答するのかに着

目して，それと密接に関わる因子の探索を進めている．本年度は，低酸素応答が長期的に獲得されうるかどうかを低酸素下での長期培養によって試験し，低酸素ストレス耐性が向上した株を取得できた．作出した株について，カイコ感染モデルで病原性を評価したところ，病原性が向上していたことから，低酸素適応能と病原性が密接に関連していることが示唆された．その他の表現型についても変化の有無を調べることで，長期培養によってどのような環境因子に対する応答が変化しうるかの評価を進めることを計画している．

当初計画していた相互訪問による表現型解析の実施は，COVID-19の影響により見合わせた．

## 研究課題　’20－22

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

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## *Candida glabrata* 遺伝子組み替え体ライブラリーを用いた抗真菌薬シーズの探索

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### 研究成果

令和元年度より千葉大学真菌研究センターとの共同研究を開始し，初年度分を報告する．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，本研究計画では，北里大学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）に対してカンジダ・グラブラータ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．現在，標的分子について検証実験を進めている．

## 研究課題　’20－23

## Development of antifungal drugs from natural chemical compound library

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## 天然化合物ライブラリーを用いた抗真菌薬の開発研究

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### 研究成果

本邦において分離された天然化合物資源の中から様々な薬剤シーズを開発することは重要な研究課題である．重篤な真菌感染症が世界的に問題となっている現状を踏まえ，微生物化学研究所でも抗真菌薬開発の重要性を認識していたが，抗真菌薬開発においては，常に副作

用の問題が存在し，専門的な研究手技や知識が必要とされるため，抗真菌薬を専門とする担当研究部が存在せず着手することができなかった．そこで，千葉大学真菌医学研究センター知花准教授と共同研究を進めスクリーニングを進めている．2020年度は，2,400種類の天然化合物について*Candida glabrata*の高感度薬剤感受性株Δ pdr1を用いて生育阻害活性物質の一次スクリーニングを終了した．その結果，48種類の化合物について生育阻害活性が認められた．これらの化合物について*Candida albicans, Candida auris, Aspergillus fumigatus, Cryptococcus neoformans*等の主要な病原真菌に対して生育阻害活性が確認された．次にこれらの化合物について，マウスの培養細胞を用いて呼吸阻害活性を指標とした細胞毒性を測定したところ，６種類の化合物に細胞毒性が確認されず，抗真菌薬「シーズ候補」とした．現在，これら６種類の化合物について，マウスを用いたin vivoでの効果を調査中である．

## 研究課題　’20－24

## Effect of gut microbe on *Salmonella* systemic infection

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## サルモネラ全身感染制御における腸内細菌叢の影響

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### 研究成果

サルモネラは，経口から感染後，腸管上皮細胞から侵入し全身感染を発症させる．腸管上皮細胞からの侵入には，サルモネラが発現する*Salmonella* pathogenicity island 1（SPI1）とべん毛の２つの３型分泌システムの機能発現が寄与する．又，宿主腸内細菌叢によって形成される腸内環境が細菌侵入に影響する．我々はこれまでに，サルモネラ分子シャペロンであるDnaJがSPI1およびべん毛機能発現に関わることを見出していたが，その制御機構は不明であった．DnaJへの基質の受け渡しに，リボソームに近接する分子シャペロンTigが一部寄与する．そこで今回，サルモネラ全身感染発症におけるTigの寄与を調べたところ，Tigは全身感染発症に寄与することを見出した．しかしながら，Tig欠損株のSPI1，べん毛機能発現は野生株と同レベルであった．そこで，DnaJ・Tig2重欠損株を構築し性状を調べたところ，顕著な細胞侵入能低下と細胞分裂阻害が観察された．液体培地で繰り返し培養したところ，細胞分裂が回復する株を得た．細胞分裂が阻害されたDnaJ・Tig2重欠損株のSPI1及びべん毛発現はDnaJ欠損株よりも減少したものの，細胞分裂が回復したDnaJ・Tig2重欠損株ではSPI1・べん毛の機能が回復した．DnaJ欠損株でもわずかに細胞分裂阻害がみられるが，これは環境ストレス依存的な変化が寄与することが示唆された．以上より，サルモネラは腸内環境ストレスに応答して変化するタンパク質活性をTig-DnaJ分子シャペロンネットワークにより適切に制御することで，細胞分裂と細胞侵入に関わる病原因子の発現をコントロールし，全身感染発症を可能にすると考えられる．

## 研究課題　’20－25

## Study on the relativeness of simultaneous growth among mite and fungi in house

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## 室内に分布するダニおよび真菌の増殖に関する研究

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### 研究成果

住宅室内では，アレルゲンとなる真菌およびダニ類によってしばしば同時に高度に汚染されることから，これらの増殖は互いに関連している可能性がある．そこで，両者の増殖の関連性に関するエビデンスを取得することを目的とした検討を行った．

これまでの成果から，ダニ類は複数菌種のカビよりも酵母である*Candida albicans*に対して走性が強く，共培養した際に増殖効率が高いことが示されている．そこで2020年度は，ダニは*C. albicans*のみを好むのか，又はその他の酵母類も好むのかを明らかにする目的で，ハウスダストの真菌叢を優占的に構成する酵母である*Malassezia furfur*を選定し，2019年度に用いた手法に改良を加え，走性を確認するための実験を行った．

実験手法としてはJIS L1920「繊維製品の防ダニ性能試験方法」で定める「侵入阻止法」を応用した．即ち，標準布（JIS L0803 綿3-1号）を小シャーレ内に敷き，その中央に4種の真菌（表参照）各々の凍結粉末0.05 gを置き試験区とした．陰性対照区として綿布のみ，陽性対照区としてダニ類の飼料として用いる酵母粉末を用いた．これを大シャーレ中央に設置し，小シャーレ周囲にヤケヒョウヒダニ約10,000頭を撒き，暗条件・26℃で24h静置した．その後，小シャーレ内のダニを計数した．この操作を3回ずつ実施し，試験区÷陰性対照区の比を求めた．

結果，今回の真菌種の中では*M. furfur*において最も高い試験区/陰性対照区の比を示し，当該酵母はダニに対する誘引効果を持つこと，さらに2019・2020年度の研究成果を総合すると，ダニは酵母類に誘引されることが示唆された．

今後は，今回の手法を用いて，住宅で検出されやすい又はアレルギー性の高い真菌種を増やし，継続的に調査する．

## 研究課題　’20−26

## Functional analysis of the enzymes involved in galactomannan biosynthesis in *Aspergillus fumigatus*

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## *Aspergillus fumigatus*のガラクトマンナン生合成酵素の機能解明

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### 研究成果

真菌の細胞壁とその生合成酵素は，新規抗真菌剤のターゲットとして期待されている．我々は，細胞壁他棟の中でもガラクトマンナンという多糖の生合成に着目して研究を進めてきた．今年度は，本共同研究において同定および機能解明を行った真菌型ガラクトマンナンのコアマンナン生合成を担う，α-(1→2)-マンノース転移酵素（CmsA/Ktr4; Onoue T. *et. al.*, *Sci. Rep.*, 2020）の立体構

造を1.95Åの分解能でX線結晶構造解析により明らかにした．また，CmsA/Ktr4と$Mn^{2+}$/GDP複合体の立体構造も1.90Åの分解能で明らかにすることができた．CmsA/Ktr4タンパク質は，ドナー基質であるGDP-マンノースに対する高度に保存された結合ポケットを持つだけでなく，そのN-末端領域とC-末端領域によって形成されるユニークな大きな凹み構造を持ち，アクセプター基質であるマンナン鎖を認識することが期待された．また，これらの結晶構造をもとに，アクセプター基質のモデル構造としてα-Man-(1→6)-α-Man-(1→2)-α-Man-OMeを用いて，ドッキングおよび分子動力学シミュレーションにより作成した酵素-基質複合体の３次元構造モデルを提示した．CmsA/Ktr4は，これまでに知られていたα-(1→2)-マンノース転移酵素とは異なり，その糖鎖認識部位に大きな凹み構造を持つことで比較的長い鎖長の糖鎖を認識していることが示唆された．さらに，その凹み構造と末端のα-Man-(1→6)-α-Man構造との親和性も高いことが示唆された．これらの結果は，医薬品や農薬として利用できる特異的なα-マンナン生合成阻害剤を開発するための基礎情報となると考えている．

### 発表論文

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## 研究課題　’20－27

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

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Mamiko Suehiro
（Chiba City Health Center）
Naruhiko Ishiwada
（Medical Mycology Research Center, Chiba University）

## 千葉市における大学・行政・医師会が連携した風疹対策共同研究

玉井和人
（千葉市医師会）
末廣真美子
（千葉市保健福祉局）
石和田稔彦
（千葉大学真菌医学研究センター）

### 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）．解析を終えた範囲での抗体検査結果においてMRワクチン接種対象基準値未満の人の割合は，国事業で約15%，千葉市事業で約35%であった．年代別に見るとHI法で1：16以下の割合は20歳代男性が最も多く約50%，次いで20歳代女性で約40%であったが，HI法で1：8未満の割合は40歳～50歳代男性が多く約15％であった．また，千葉市事業においては，抗体検査に基づく接種だけでなく，妊婦健診の抗体検査に基づく接種が多いことが明らかになった．さらに，千葉市の事業を利用して抗体検査を受ける男性の多くは土曜日を選択している一方，国の事業を利用して抗体検査を受ける男性は，土曜日だけでなく平日も選択していたが，千葉市外や千葉県外の検査機関を積極的に利用していることが明らかになった．子育て世代の女性に関しては千葉市事業の有効性が示唆されたが，40-50歳代男性に対する国事業のMRワクチン接種は進んでおらず，勤労世代ゆえの受診の困難さが影響している可能性が考えられた．

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## 研究課題　’20－28

## 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．

一方で，ユーシェアリライドの炭化水素を主成分とする大環状構造が活性発現に必須であるか否かは不明であった．そこで大環状構造を有さない新規ユーシェアリライド類縁体（P1-P5）を考案し，種々のグラム陽性菌に対する活性試験を行っていただいた．その結果，単純な脂環式炭化水素あるいは単鎖脂肪酸エステルを有する類縁体（P1, P2）はほとんど活性を示さなかったが，長鎖炭化水素を有する類縁体（P3, P4, P5）の中でP3のみが，肺炎連鎖球菌などのグラム陽性菌に対して高い抗細菌活性を示すことが明らかになった．今後，さらにユーシェアリライド類縁体の炭化水素部位の構造最適化を進めたい．

## 研究課題　’20－29

## Biological function of secretory cyclic peptides widely and diversely produced by fungi

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## 真菌類が広く多様に産生する分泌性環状ペプチド群の生物学的機能解明

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### 研究成果

真菌において近年見出され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いる．これまでの報告や実験結果から，強い微小管形成阻害活性を示すカビ毒としての作用の他，接合因子や宿主免疫系制御といったカビの生態において重要な機

能を示すものが多く含まれ，特に子嚢果形成への関与が示唆された（1）．そこで本年度は，千葉大学真菌医学研究センターが保有する真菌リソースの中から，*Aspergillus nidulans* および *Neosartorya fischeri* それぞれについて子嚢果形成度の異なる２株ずつを選択し，本因子群の数や配列の違いを比較した．４株のうち，*A. nidulans* IFM 60678と *N. fischeri* IFM 65258は基準株でありすでにゲノム情報が公開されているが，その他の２株についてはまだであったため，ゲノム抽出・解析を行った．参照アセンブリの結果，*A. nidulans* の２株間では，大きな欠損・挿入はなかったもののゲノム全体が10カ所程度で大きくリアレンジメントしていた．一方 *N. fischeri* の２株は，ゲノム配列がほぼ相同であった．得られたゲノム配列から本因子群を独自アルゴリズムで抽出したところ，*A. nidulans* で３つ，*N. fischeri* で５つ見出され，それらのアミノ酸配列は対応する２株間で全く同じであった．そこで現在，本因子群を含むすべての遺伝子発現状態の違いを見るため，子嚢果形成プレート上で培養した菌体からRNAを精製し，トランスクリプトーム解析を行っているところである．

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## 研究課題　’20－30

## Distinct roles for Dectin-1 and Dectin-2 in skin wound healing


Emi Kanno, Kazuyoshi Kawakami, Hiromasa Tanno
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## 皮膚創傷治癒過程における Dectin-1と Dectin-2の役割の相違


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### 研究成果

皮膚が損傷後の創傷部位には様々な外因性分子（微生物由来）やダメージに関連した内因性分子が存在し，パターン認識受容体による認識を介した‘炎症反応の制御’が治癒の鍵を握る．真菌は難治性創傷の創面から検出されることが報告されており，創傷治癒との関連が示唆されている．さらに真菌の細胞壁を構成するβ-グルカンを創傷に塗布すると治癒が促進すること，また我々のグループにより，α-マンナンを創傷に塗布すると治癒が遅延することが報告されているが，詳細な炎症誘導メカニズムは明らかではない．そこで，本研究ではβ-グルカン受容体であるDectin-1, α-マンナン受容体であるDectin-2, それぞれの生理的な創傷治癒過程における役割を明らかにすることを目的とし解析を行った．まず，野生型（WT）マウスとDectin-1, およびDectin-2それぞれの欠損（KO）マウスの背部に全厚の創傷を作成し，これらの分子の欠損による影響と，リガンドであるβグルカン，あるいはαマンナンの投与による影響を調べた．その結果，Dectin-1 KOマウスではWTマウスと比較して創傷治癒が遅延すること，逆にDectin-2 KOマウスでは治癒が促進することから，Dectin-1を介したシグナルは創傷治癒を促進すること，Dectin-2のシグナルは創傷治癒を阻害することが明らかとなった．また，Dectin-1は初期段階の好中球集積を誘導することで創傷治癒の促進に寄与するのに対し，Dectin-2は好中球反応の遷延や好中球の細胞外トラップ形成（NETs）形成に関与し，創傷治癒の遅延につながることがわかった．さらに，Dectin-2の欠損は，コラーゲンの沈着やTGF-β1の発現を改善した．これらの結果から，Dectin-1とDectin-2は，好中球反応に対する影響の違いを通じて，創傷治癒に異なる役割を果たしていることが示された．

### 発表論文


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## 研究課題　'20－31

## 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, Indonesia. Bacterial analysis of *S. pneumoniae* isolates from IPD patients is performed in Eijkman Institute in Jakarta, the national reference center of *S. pneumoniae* in Indonesia. Dr. Dodi Safari is responsible for the bacterial analysis in Eijkman Institute in Jakarta. This study will be able to estimate the impact of pneumococcal conjugate vaccine introduction in Indonesia based on the established active surveillance system of pediatric IPD in Japan. The study started from August 2020 because of the influence of COVID-19 epidemic in Indonesia. We collected about 10 strains of *S. pneumoniae* those were sent to Dodi's lab at Eijkman Institute in Jakarta for serotyping and genotyping. We are also writing a short manuscript of a case series of *S. pneumoniae* in children collaborating with Dr. Ishiwada.

# 2021 Scientific Meetings & Seminars

# 2021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　2020年度成果報告会」**
日時：令和３年３月９日（火）～３月11日（木）
場所：オンライン開催

令和３年３月10日（水）
【特別講演】
竹内　理（京都大学大学院医学研究科）
「RNA分解による免疫応答の制御機構」

【合同成果報告会（千葉大学真菌医学研究センター）】
程久美子（東京大学）
「microRNAを介したウイルス応答の制御によるヒトの新しい生体防御機構の解析」

名木　稔（国立感染症研究所）
「Candida glabrataにおけるマイトファジー関連遺伝子ATG32の転写調節因子の同定」

梅村舞子（産業技術総合研究所）
「病原性真菌が産生する新規分泌性環状ペプチド群の生理機能解明」

椎名　勇（東京理科大学）
「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」

【領域３：感染症・免疫共同研究領域】
辻　典子（産業技術総合研究所）
「小腸自然免疫レセプターの機能解析」

小澤　真（鹿児島大学）
「A型インフルエンザウイルス感染動態を反映する新規ポリメラーゼ活性測定法の確立」

浦木隆太（名古屋市立大学）
「制御性T細胞を標的としたインフルエンザ感染症に対する新規治療法の開発」

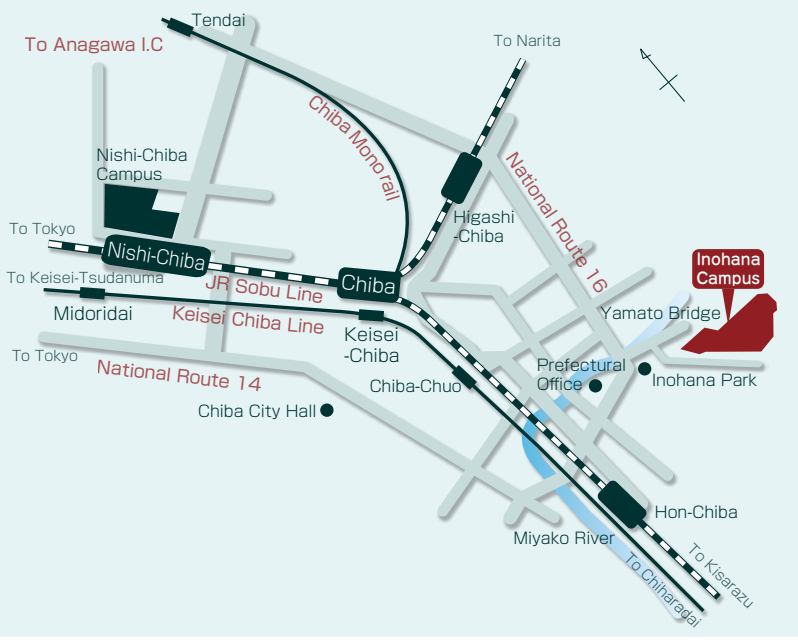
佐々木泉（和歌山県立医科大学）
「コレラ毒素の免疫アジュバント活性を制御する分子基盤の解明」

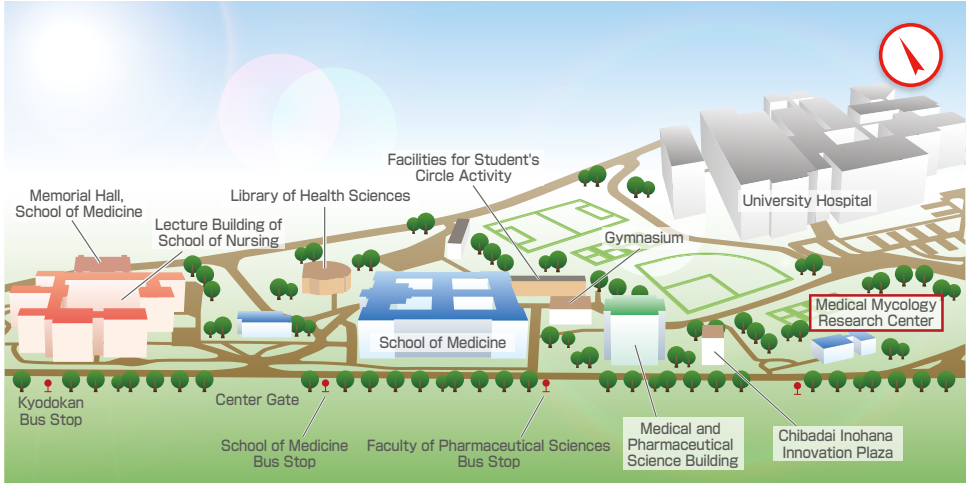
**「真菌医学研究センター セミナー」**
全てオンライン開催

１．令和３年２月９日（火）　16：00～17：00
河岡義裕（東京大学医科学研究所　教授）
「新型コロナウイルス：これまでにわかったこと」

２．令和３年５月６日（木）　16：00～17：00
石井　健（東京大学医科学研究所　教授）
「ワクチン開発研究の新展開；mRNAワクチンと核酸アジュバントの次へ」

３．令和３年10月５日（火）　16：00～17：00
荒瀬　尚（大阪大学免疫学フロンティア研究センター・微生物病研究所　教授）
「新型コロナウイルスに対する感染増強抗体と中和抗体」

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