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千葉大学　真菌医学研究センター　報告

20

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はじめに

我が国では超高齢社会の到来により，癌や高度医療に伴う日和見感染症に加え，生活習慣病や呼吸器病の増大とそれに伴う難治性真菌症も増加し，近年真菌感染症の脅威は，社会に広くクローズアップされてきました．また多剤耐性真菌や多剤耐性細菌の増加と拡散は，医療現場においても益々大きな脅威となっています．さらに経済のグローバリゼーションと国際旅客数の増加により，高度病原真菌をはじめとするさまざまな病原微生物による輸入感染症の脅威にも直面しています．このような状況の中で，本センターは，我が国唯一の公的な真菌症の研究・教育機関として，また千葉大学においては，感染症・免疫・病原体の研究プラットホームとして，その使命は以前にも増して重要になっています．本センターは，病原真菌による感染症研究の共同利用・共同研究拠点として文部科学大臣による認定を受け，平成22年より，大学，国公立研究機関，千葉大学関係部局，医療機関，企業と緊密に連携して，共同利用，共同研究，教育活動を積極的に行ってきました．同時に本センターでは，文部科学省によるナショナルバイオリソースプロジェクトにおける「病原微生物」の中核拠点として，病原真菌・放線菌の収集・保存・分与を行うとともに，薬剤耐性遺伝子，病原性遺伝子をはじめとする真菌のゲノム情報解析を行っています．さらにこれらの事業と平行して，独立研究グループリーダー制（PI制度；Principal Investigator）による基盤研究，臨床研究，創薬研究を推進しています．平成26年10月には附属病院において我が国初の真菌症専門外来を開設し，また平成28年度からは，新規抗真菌薬の標的とシーズ化合物の探索研究や，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に採択された本センター教員を中心にした研究グループにより，真菌を含めた微生物叢と宿主免疫の相互作用の研究も開始されました．したがって本センターでは，「共同利用・共同研究，バイオリソース拠点事業」，「感染症・免疫基盤研究」，「臨床・創薬研究」の三つを柱として，今後も我が国の真菌医学の発展に先導的な役割を果たす所存です．

平成29年1月

千葉大学真菌医学研究センター長

笹川千尋

Preface

The advent of a 'super-aging' society in Japan has led to an increasing number of people with opportunistic infections, concomitant with cancer and the need for advanced medical care. In addition, increases in the incidence of lifestyle and respiratory diseases have led to similar increases in intractable mycosis. Consequently, the threat of fungal infections has attracted a great deal of attention in recent years. Moreover, the proliferation of multi-drug resistant fungi and bacteria represents a dire threat in clinical settings. We are also faced with dramatic increases in worldwide trade and international travel, which has increased the risk of importing infectious diseases via transport of pathological microorganisms, including highly pathogenic fungi. The Medical Mycology Research Center (MMRC) at Chiba University is the only public research and educational institution in Japan devoted to fungal diseases, and has become an increasingly important platform for research on infectious diseases, immunity, and pathogens. MMRC has been recognized by the Minister of Education, Culture, Sports, Science and Technology as a Joint Usage / Research Center. Accordingly, since 2010, MMRC has been actively engaged in medical mycology research and related educational activities through partnerships with universities, public institutions, medical institutions, and pharmaceutical companies. MMRC also serves as a key institution in the National BioResource Project (NBRP) recognized by the Ministry of Education, Culture, Sports, Science and Technology, and is involved in a number of activities including the collection, preservation, and distribution of pathogenic fungi and Actinomycetales, as well as fungal genome analysis aimed at investigating drug-resistance and virulence genes. In parallel, MMRC continues to support basic, clinical, and drug-development research through a principal investigator system. A specialty outpatient facility for fungal infections was opened at the Chiba University Hospital in October 2014. Additionally, in 2016, MMRC began exploratory research aimed at identifying seed compounds and novel antifungal agents, as well as interactions between microbial flora and host immunity, with research groups selected by the Institute for Global Prominent Research and the Leading Research Promotion Program. We envision MMRC as the leading institution for scientific research excellence in microbiology, immunology, and clinical and drug-development research, as well as a joint-use, collaborative, bioresource center, with the ultimate goal of advancing the field of medical mycology.

January, 2017

Chihiro Sasakawa

Director of MMRC

米山教授　感染応答プロジェクト

Project for Immune Response in Infections Diseases

研究概要（Summary）

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に注目し，感染センサーRIG-I-like受容体（RLR）によるウイルス由来の非自己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の開発を目指した解析を行っている．

Innate immune system plays an essential role for self-defense against infection of a variety of pathogens. In this project, we focus on antiviral innate immunity, especially molecular machinery for detection of viral RNA by retinoic acid-inducible gene I（RIG-I）-like receptors（RLRs）and the 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.

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助　　教	尾野本浩司	Assistant Professor	Koji Onomoto
非常勤技術職員	滝澤香代子	Adjunct Research Technician	Kayoko Takizawa
技術補佐員	常喜　儒彦	Research Promotion Technician	Michihiko Jogi
技術補佐員	滝沢みゆき	Research Promotion Technician	Miyuki Takizawa

1. Leader-containing uncapped viral transcript activates RIG-I in antiviral stress granules

Seong-Wook Oh[1], Koji Onomoto[2], Mai Wakimoto[2,3], Kazuhide Onoguchi[4], Fumiyoshi Ishidate[5,6], Takahiro Fujiwara[6], Mitsutoshi Yoneyama[2], Hiroki Kato[1,3] and Takashi Fujita[1,3]

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RIG-I triggers antiviral responses by recognizing viral RNA（vRNA）in the cytoplasm. However, the spatio-temporal dynamics of vRNA sensing and signal transduction remain elusive. We investigated the time course of events in cells infected with Newcastle disease virus（NDV）, a non-segmented negative-strand RNA virus. RIG-I was recruited to viral replication complexes（vRC）and triggered minimal primary type I interferon（IFN）production. RIG-I subsequently localized to antiviral stress granules（avSG）induced after vRC formation. The inhibition of avSG attenuated secondary IFN production, suggesting avSG as a platform for efficient vRNA detection. avSG selectively captured positive-strand vRNA, and pol derived from read-through transcription was sensed by RIG-I in avSG. These results highlight how viral infections stimulate host stress

responses, thereby selectively recruiting uncapped vRNA to avSG, in which RIG-I and other components cooperate in an efficient antiviral program.

2. Functional analysis of RNA binding proteins (RBPs), which are responsible for induction of anti-viral innate immunity via RNA-granule formation

Koji Onomoto, Takaaki Ito, Marie Ban, Eri Miyamoto and Mitsutoshi Yoneyama

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

We demonstrated that viral infection induces RIG-I to accumulate in cytoplasmic granular-like structure, avSG. We further revealed that avSG plays a critical role as platform for initiation of RIG-I-mediated antiviral signaling. To understand the molecular machinery for avSG formation, we identified several RBPs that are associated with RIG-I in virus-infected cells, and examined their function in antiviral immune responses. As a result, we found out that several RBPs play an important role for regulation of both RIG-I-mediated signal activation and avSG formation. This work was supported by MEXT KAKENHI, Grant-in-Aid for Scientific Research on Innovative Areas (15H01252) and JSPS KAKENHI, Grant-in-Aid for Young Scientist (B) (26293101).

3. Recognition of viral ribonucleoprotein complex (RNP) by RIG-I

Michihiko Jogi, Chiaki Sakuma and Mitsutoshi Yoneyama

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

It has remained unclear how RIG-I detects viral ribonucleoprotein complex (RNP), which consists of viral genomic RNA and viral proteins, in the virus-infected cells. Recently, we established *in vitro* reconstitution system for RIG-I activation and examined whether viral RNP can activate RIG-I *in vitro*. As a model RNP, we prepared artificial influenza A virus (IAV) RNP generated in 293T cells and confirmed that the artificial RNP forms a horseshoe-like structure using the atomic force microscope, as reported in the previous report. Our data indicated that IAV RNP can activate recombinant RIG-I (rRIG-I)-mediated antiviral signaling in our assay system. However, we failed to observe direct interaction between viral RNP and rRIG-I by density gradient centrifugation analysis, suggesting possible requirement of additional molecule(s) for the interaction between them. This work was supported by JSPS KAKENHI, Grant-in-Aid for Scientific Research (B) (26293101) and Grant-in-Aid for Challenging Exploratory Research (16K15279).

4. Functional analysis of RLR-mediated signal transduction

Koji Onomoto, Koutaro Okita and Mitsutoshi Yoneyama

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

RLRs are viral RNA sensors to initiate antiviral innate immunity, including gene activation of type I and III IFNs. To understand RLR-mediated signaling, we established the artificial RLR activation system that is based on chemically-induced oligomerization of caspase recruitment domain of RIG-I (ARIAD Pharmaceuticals). This system can deliver RLR-mediated signaling into the cells without viral infection. The recent data indicated that the RLR-mediated signaling induces both cell death and growth inhibition to the cells. We try to reveal the molecular machinery underlying these RLR-induced cellular responses.

Publications

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2) Yoneyama M, Jogi M, Onomoto K: Regulation of antiviral innate immune signaling by stress-induced RNA granules. J Biochem, 159: 279-286, 2016.

西城准教授　サイトカインプロジェクト

Project for Cytokine Research

研究概要（Summary）

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．

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.

准　教　授	西城　忍	Associate Professor	Shinobu Saijo
助　　教	矢部　力朗	Assistant Professor	Rikio Yabe
技術補佐員	森本　雅子	Research Promotion Technician	Masako Morimoto
技術補佐員	鈴木　智明	Research Promotion Technician	Tomoaki Suzuki

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

Shinobu Saijo and Rikio Yabe

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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. Dectin-1 and Dectin-2 promote control of the fungal pathogen Trichophyton rubrum independently of IL-17 and adaptive immunity in experimental deep dermatophytosis

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Dermatophytoses are chronic fungal infections, the main causative agent of which is *Trichophyton rubrum*（*T. rubrum*）. Despite their high occurrence worldwide, the immunological mechanisms underlying these diseases remain largely unknown. Here, we uncovered the C-type lectin receptors, Dectin-1 and Dectin-2, as key elements in the immune response to *T. rubrum* infection in a model of deep dermatophytosis. In vitro, we observed that deficiency in Dectin-1 and Dectin-2 severely compromised cytokine production by dendritic cells. In vivo, mice lacking Dectin-1 and/or Dectin-2 showed an inadequate pro-inflammatory cytokine production in response to *T. rubrum* infection, impairing its resolution. Strikingly, neither adaptive immunity nor IL-17 response were required for fungal clearance, highlighting innate immunity as the main checkpoint in the pathogenesis of *T. rubrum* infection.

Publications

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後藤准教授　微生物・免疫制御プロジェクト

Project for Host-Microbial Interactions in Symbiosis and Pathogenesis

研究概要（Summary）

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-フコースを介した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．

Gastrointestinal tract is a unique organ which is constitutively exposed by various antigens including dietary materials, commensal bacteria, and fungi. In order to exclude pathogens and create symbiotic environment to non-pahogenic microorganisms, intestinal epithelial cells（ECs）and immune cells contribute to establish homeostasis of intestinal microenvironment. Disruption of symbiotic relationship between host and commensals predispose to the development of inflammatory bowel diseases and systemic disorders such as obesity and diabetes. Therefore, it is important to understand the mechanism of symbiotic and homeostatic condition regulated by intestinal ECs and immune cells. In this project, we aim to uncover the symbiotic system with commensal micro- and mycobiota mediated by epithelial α1, 2-fucose. 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 infection and metabolic syndrome caused by the disruption of intestinal homeostasis.

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研究生・技術補佐員	松尾　謙蔵	Research student/Research Promotion Technician	Kenzo Matsuo
技 術 補 佐 員	高木　弘子	Research Promotion Technician	Hiroko Takagi

1. The role of commensal bacteria and fungi against infection of pathogenic microorganisms

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In our gastrointestinal tract, numerous numbers of bacteria and fungi peacefully colonize and create mutual relationship with their host. These commensals contribute to prevent infection of pathogenic microorganisms. However, it is still unclear what kind of and how commensal bacteria and fungi interrupt the pathogenic infections. In this study, we

investigate the mechanism of symbiotic colonization by bacteria and fungi in the intestine. We also aim to isolate beneficial commensal bacteria and fungi for the development of novel therapeutic targets for human infectious diseases. After the establishment of this system, we further target other human diseases such as inflammatory bowel diseases, allergy, cancer, and metabolic syndrome.

2. Epithelial α1, 2-fucose induced by commensal bacteria and immune cells regulate gut and systemic homeostasis

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Intestinal epithelial cells apically express glycans, especially α1, 2-fucosyl linkages, which work as a biological interface for the host–microbe interaction. Emerging studies have shown that epithelial α1, 2-fucosylation is regulated by commensal microbes and by group 3 innate lymphoid cells (ILC3). Dysregulation of the gene (*FUT2*) encoding fucosyltransferase 2, an enzyme governing epithelial α1, 2-fucosylation, is associated with various human disorders, including pathogenic infection, Crohn's diseases, type I diabetes, primary sclerosing cholangitis, psoriasis, and Behcet's disease. This suggests a critical role for an interaction between microbes, epithelial cells and ILC3 mediated via glycan residues. We aim to identify detail molecular mechanism how epithelial glycosylation is controlled by immune cells and luminal microbes. We also address the pathophysiological contribution of epithelial α1, 2-fucosylation to pathogenic and commensal microbes as well as the potential of α1, 2-fucose and its regulatory pathway as previously unexploited targets in the development of new therapeutic approaches for human diseases.

Publications

1) Goto Y, Uematsu S, Kiyono H: Epithelial glycosylation in gut homeostasis and inflammation. Nat Immunol, 17: 1244-1251, 2016.

芦田特任准教授　細菌感染免疫プロジェクト

Project for Bacterial Infection Immunology

研究概要（Summary）

病原細菌と疾患との関係は比較的明瞭だが，感染の成立と発症の分子メカニズムはまだよく理解されていない．本プロジェクトでは腸管病原細菌をモデルとして，菌の病原因子と宿主因子の感染における相互作用を細胞，組織で時空列的に解明することにより，病原細菌の感染メカニズムと新規な感染現象を明らかにし，その知見を細菌感染症の予防・治療へ積極的に応用ことを目的としている．

Research in this project is directed toward understanding the complex interactions that occur between pathogenic bacteria and the gastrointestinal epithelium and the process of infectious diseases. Our special interest is focused upon the molecular pathogenicity of enteropathogenic bacteria, such as *Shigella*, enteropathogenic *E. coli* and enterohemorrhagic *E. coli*, and aim to dissect the mechanisms of bacterial infectious systems and discover new infectious aspects. We are also searching for effective methods to protect or regulate bacterial infection by using knowledge accumulated, and interested in developing vaccine and antibacterial drug.

特任准教授　芦田　浩　Associate Professor　Hiroshi Ashida

1. Bacterial pathogens manipulate host immune responses by delivering effector proteins

Hiroshi Ashida

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

The intestinal epithelium deploys multiple defense systems against microbial infection to sense bacterial components and danger alarms, as well as to induce intracellular signal transduction cascades that trigger both the innate and adaptive immune system, which are pivotal for bacterial elimination. However, many enteric bacterial pathogens, including *Shigella*, deliver a subset of virulence proteins (effectors) via the type III secretion system (T3SS) that enable bacterial evasion from host immune systems; consequently, these pathogens are able to efficiently colonize the intestinal epithelium. We are searching interactions between *Shigella*, enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC) and host immune responses, with particular emphasis on strategies that bacteria use to manipulate inflammatory outputs of host cell responses such as membrane trafficking and innate immune responses.

2. Manipulation of the host cell death pathway by bacterial pathogens

Hiroshi Ashida

Host cells deploy multiple defenses against microbial infection. One prominent host defense mechanism, the death of infected cells, plays a pivotal role in clearing damaged cells, eliminating pathogens, removing replicative niches, exposing intracellular bacterial pathogens to extracellular immune surveillance, and presenting bacteria-derived antigens to the adaptive immune system. Although cell death can occur under either physiological or pathophysiological conditions, it acts as an innate defense mechanism against bacterial pathogens by limiting their persistent colonization. However, many bacterial pathogens, including *Shigella*, EPEC, and EHEC, have evolved mechanisms that manipulate host cell

death for their own benefit.

3. Developing animal model for studying the bacterial pathogens

Hiroshi Ashida

Because many enteric bacterial pathogens are highly human adapted and unable to colonize mice intestine, lack of appropriate animal infection models are becoming bottleneck for developing vaccine and novel drugs. Therefore, we are trying to develop mice intestinal infection model that would be suitable to *in vivo* bacteria-host interplay, vaccine development, and screening for novel antibacterial drug.

Publications

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知花准教授　カンジダ・グラブラータフェノームプロジェクト

Candida glabrata phenome project

研究概要（Summary）

病原性酵母カンジダ・グラブラータの全遺伝子改変株を作製し，病原性に関する遺伝子の特定と機能解析ならびに抗真菌薬の開発を行う．

Using the pathogenic yeast *Candida glabrata*, we are systematically constructing mutants for gene identification and functional analyses working on the pathogenicity and for developing of anti-fungal drug targets.

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1. Membrane Proteome-Wide Response to the Antifungal Drug Clotrimazole in *Candida glabrata*: Role of the Transcription Factor *CgPdr1* and the Drug:H+ Antiporters *CgTpo1_1* and *CgTpo1_2*

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Azoles are widely used antifungal drugs. This family of compounds includes triazoles, mostly used in the treatment of systemic infections, and imidazoles, such as clotrimazole, often used in the case of superficial infections. *Candida glabrata* is the second most common cause of candidemia worldwide and presents higher levels of intrinsic azole resistance when compared with *Candida albicans*, thus being an interesting subject for the study of azole resistance mechanisms in fungal pathogens. Since resistance often relies on the action of membrane transporters, including drug efflux pumps from the ATP-binding cassette family or from the Drug:H(+) antiporter (DHA) (1) family, an iTRAQ-based membrane proteomics analysis was performed to identify all the membrane-associated proteins whose abundance changes in *C. glabrata* cells exposed to the azole drug clotrimazole. Proteins found to have significant expression changes in this context were clustered into functional groups, namely: glucose metabolism, oxidative phosphorylation, mitochondrial import, ribosome components and translation machinery, lipid metabolism, multidrug resistance transporters, cell wall assembly, and stress response, comprising a total of 37 proteins. Among these, the DHA transporter *CgTpo1_2* (ORF CAGL0E03674g) was identified as overexpressed in the *C. glabrata* membrane in response to clotrimazole. Functional characterization of this putative drug:H(+) antiporter, and of its homolog *CgTpo1_1* (ORF CAGL0G03927g), allowed the identification of these

proteins as localized to the plasma membrane and conferring azole drug resistance in this fungal pathogen by actively extruding the drug to the external medium. The cell wall protein *CgGas1* was also shown to confer azole drug resistance through cell wall remodeling. Finally, the transcription factor *CgPdr1* in the clotrimazole response was observed to control the expression of 20 of the identified proteins, thus highlighting the existence of additional unforeseen targets of this transcription factor, recognized as a major regulator of azole drug resistance in clinical isolates.

2. Microbially cleaved immunoglobulins are sensed by the innate immune receptor LILRA2

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Despite the crucial role played by the glyoxylate cycle in the virulence of pathogens, seed germination in plants, and sexual development in fungi, we still have much to learn about its regulation. Here, we show that a previously uncharacterized SCF(Ucc1) ubiquitin ligase mediates proteasomal degradation of citrate synthase in the glyoxylate cycle to maintain metabolic homeostasis in glucose-grown cells. Conversely, transcription of the F box subunit Ucc1 is downregulated in C2-compound-grown cells, which require increased metabolic flux for gluconeogenesis. Moreover, in vitro analysis demonstrates that oxaloacetate regenerated through the glyoxylate cycle induces a conformational change in citrate synthase and inhibits its recognition and ubiquitination by SCF(Ucc1), suggesting the existence of an oxaloacetate-dependent positive feedback loop that stabilizes citrate synthase. We propose that SCF(Ucc1)-mediated regulation of citrate synthase acts as a metabolic switch for the glyoxylate cycle in response to changes in carbon source, thereby ensuring metabolic versatility and flexibility.

3. Quantitative measurement of hydrophilicity/hydrophobicity of the plasma-polymerized naphthalene film (Super Support Film) and other support films and grids in electron microscopy

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Hydrophilicity/hydrophobicity of the surfaces of plasma-polymerized naphthalene film (Super Support Film, Nisshin EM Co. Ltd., Tokyo), carbon and formvar support films, and copper and nickel grids were quantitatively estimated by contact angles measured from diameters and heights of water droplets placed on various specimens. With treatment of glow discharge, the surfaces of plasma-polymerized naphthalene and carbon support films became fully hydrophilic in 20 s.

They remained hydrophilic for 6 h. The surfaces of copper and nickel grids became fully hydrophilic with 60 s of glow discharge treatment. They remained hydrophilic for only 1 h. This information is useful for nega-tive staining, ultrathin sectioning and rapid freezing of biological specimens using the sandwich method.

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亀井教授　臨床感染症プロジェクト

Project to Link Basic Sciences and Clinical Medicine

研究概要（Summary）

附属病院の真菌症専門外来における診療と並行して，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している．また，一般施設では実施困難な菌種同定，遺伝子検査などの特殊検査を年400件あまり受け入れており，名実ともに我が国における「真菌症リファレンスセンター」（輸入真菌症を含む）として活動している．研究面では臨床研究に加えて，特にアスペルギルス症に代表される難治性真菌症の感染機構の研究とこれに基づく診断・治療法の開発を中心に進めているが，近年はアスペルギルス耐性株の疫学と耐性機構の研究なども積極的に進め，本年からSATREPSによるブラジル・カンピーナス大学感染症内科との共同研究も開始している．

We have been doing basic and clinical research primarily on fungal infections along with seeing patients in the Specialty Clinic for Fungal Infections at the University Hospital. Working as the Reference Center for fungal infections, we also take ca. 400 consulting services on fungal diseases from all over the country every year. From the aspect of research, we are investigating the mechanisms of infection of intractable fungal diseases, particularly aspergillosis, while developing their diagnostic and therapeutic methods in collaboration with various universities/pharmaceutical companies. The epidemiology and mechanisms of antifungal resistance also hold a prominent part of our research, and a collaborative study with Sao Paulo State University of Campinas (Brazil) was started this year (SATREPS).

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1. Epidemiological study of *Fusarium* species causing invasive and superficial fusariosis in Japan

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In Japan, *Fusarium* species are known etiological agents of human fungal infection; however, a large-scale epidemiological study on the etiological agents of fusariosis has not been

reported. A total of 73 *Fusarium* isolates from patients with invasive fusariosis (IF, n = 36) or superficial fusariosis (SF, n = 37), which were obtained at hospitals located in 28 prefectures in Japan between 1998 and 2015, were used for this study. *Fusarium* isolates were identified using *Fusarium*- and *Fusarium solani* species complex (FSSC)-specific real-time PCR and partial DNA sequences of the elongation factor-1 alpha gene and the nuclear ribosomal internal transcribed spacer region. FSSC was predominately isolated from both patients with IF and SF (IF, 77.8% and SF, 67.6%). Distribution of the phylogenetic species of FSSC isolates from patients with IF and SF exhibited different spectra; specifically, *F. keratoplasticum* (FSSC 2) (25.0%) was the most frequent isolate from patients with IF, whereas *F. falciforme* (FSSC 3 + 4) (32.4%) was the most frequent isolate from patients with SF. *Fusarium* sp. (FSSC 5) was the second most frequent isolate from both patients with IF and SF (IF, 22.2% and SF, 24.3%). Notably, *F. petroliphilum* (FSSC 1) was isolated only from patients with IF. Each species was isolated from a broad geographic area, and an epidemic was not observed. This is the first epidemiological study of *Fusarium* species causing IF and SF in Japan.

2. Sensitisation of an azole-resistant *Aspergillus fumigatus* strain containing the Cyp51A-related mutation by deleting the SrbA gene

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Azoles are widely used for controlling fungal growth in both agricultural and medical settings. The target protein of azoles is CYP51, a lanosterol 14-α-demethylase involved in the biosynthesis of ergosterol. Recently, a novel azole resistance mechanism has arisen in pathogenic fungal species *Aspergillus fumigatus*. Resistant strains contain a 34-bp or 46-bp tandem repeat (TR) in the promoter of *cyp51A*, and have disseminated globally in a short period of time. In this study, we investigated whether an azole-resistant strain with a 46-bp TR (TR46/Y121F/T289A) could be sensitised to azoles by deletion of *srbA*, encoding a direct regulator of *cyp51A*. The loss of SrbA did not affect colony growth or conidia production, but decreased expression of *cyp51A*. The *srbA* deletion strain showed hyper-susceptibility to medical azoles as well as azole fungicides, while its sensitivity to non-azole fungicides was unchanged. This is the first demonstration that deletion of a regulator of *cyp51A* can sensitise an azole-resistant *A. fumigatus* strain. This finding may assist in the development of new drugs to help combat life-threatening azole-resistant fungal pathogens.

3. Multi-azole resistant *Aspergillus fumigatus* harboring Cyp51A TR46/Y121F/T289A isolated in Japan

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Multi-azole resistant *Aspergillus fumigatus* carrying TR46/Y121F/T289A was isolated from a patient inJapan in Dec 2013. This strain grouped into the same clade of the ones which were clinically isolated in France and Germany. *A. fumigatus* harboring this mutation could be rapidly diffused outside the Eurasian continent.

4. Comparative transcriptome analysis revealing dormant conidia and germination associated genes in *Aspergillus* species: an essential role for AtfA in conidial dormancy.

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BACKGROUND: Fungal conidia are usually dormant unless the extracellular conditions are right for germination. Despite the importance of dormancy, little is known about the molecular mechanism underlying entry to, maintenance of, and exit from dormancy. To gain comprehensive and inter-species insights, transcriptome analyses were conducted across *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus oryzae*.
RESULTS: We found transcripts of 687, 694, and 812 genes were enriched in the resting conidia compared with hyphae in *A. fumigatus*, *A. niger*, and *A. oryzae*, respectively (conidia-associated genes). Similarly, transcripts of 766, 1,241, and 749 genes were increased in the 1 h-cultured conidia compared with the resting conidia (germination-associated genes). Among the three *Aspergillus* species, we identified orthologous 6,172 genes, 91 and 391 of which are common conidia-and germination-associated genes, respectively. A variety of stress-related genes, including the catalase genes, were found in the common conidia-associated gene set, and ribosome-related genes were significantly enriched among the germination-associated genes. Among the germination-associated genes, we found that calA-family genes encoding a thaumatin-like protein were extraordinary expressed in early germination stage in all *Aspergillus* species tested here. In *A. fumigatus* 63% of the common conidia-associated genes were expressed in a bZIP-type transcriptional regulator AtfA-dependent manner, indicating that AtfA plays a pivotal role in the maintenance of resting conidial physiology. Unexpectedly, the precocious expression of the germination-associated calA and an abnormal metabolic activity were detected in the resting conidia of the atfA mutant, suggesting that AtfA was involved in the retention of conidial dormancy.
CONCLUSIONS: A comparison among transcriptomes of hyphae, resting conidia, and 1 h-grown conidia in the three *Aspergillus* species revealed likely common factors involved in conidial dormancy. AtfA positively regulates conidial stress-related genes and negatively mediates the gene expressions related to germination, suggesting a major role for AtfA in *Aspergillus* conidial dormancy.

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山本特任教授　感染宿主応答ネットワークプロジェクト

Project for Host Response Network of Bacterial Infection

研究概要（Summary）

本プロジェクトでは，細菌感染と発症のメカニズムを分子レベルで解明し，研究成果を感染症の予防と治療へ役立てることを目指している．感染現象は，２つの異なる生物（病原体と宿主）の間に形成される新たな生命現象である．細菌感染のメカニズムを分子レベルで解き明かすことにより，細菌と生体の間に展開される複雑系の生命現象を解き明かすことを合わせて目指している．

「主要な研究テーマ」

（Ⅰ）サルモネラ属細菌をモデルとした，食細胞内寄生性を有する病原細菌の全身感染症発症機序並びに持続感染機構の解明

（Ⅱ）RNAエピジェネティクスとリボソームターゲッティング薬の抗菌活性

（Ⅲ）AAA^+プロテアーゼClpXPの研究成果に基づいた慢性感染症治療薬となるanti-persisterの探索研究

Research Focus

(I) Dissecting the molecular mechanisms of systemic infection and persistent infection by facultative intracellular bacteria through the study of *Salmonella*-host interplay: We focus on the *Salmonella* effectors, which we have identified through a meta-analytic approach to the accurate prediction of effectors, to elucidate the dynamic interplay with their host targets and bacterial strategies for withstanding the host innate- and acquired-immune systems.

(II) RNA epigenetics and bacterial susceptibility to ribosome-targeting antibiotics: This project is based on our recent findings that post-transcriptional modifications (epigenetic alterations) of 23S rRNA by intrinsic enzymes are essentially responsible for susceptibility of pathogenic bacteria to several ribosome-targeting antibiotics.

(III) Identification and development of anti-persister compounds as a new class of antibiotics to treat chronic infection: Our previous studies on the AAA^+ protease, ClpXP allowed us to hypothesize that the dysregulation of proteolysis by activation of ClpP core in the absence of the regulatory ClpX ATPase may lead to corruption of bacterial physiology and eventually death of dormant cells. The compounds leading such uncontrolled proteolysis could be potential as a new class of antibiotics to treat chronic infection.

特任教授　山本　友子　Professor　Tomoko Yamamoto

技術補佐員　野村祐理子　Research Promotion Technician　Yuriko Nomura

1. The RNA chaperons, CspC and CspE, mediate the upregulation of mRNAs in *Salmonella* surviving in macrophages after phagocytosis

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CspC and CspE, members of bacterial cold shock protein family that includes well-conserved small acidic proteins, function as RNA chaperones through direct binding to RNA, thereby modulating the target RNAs at the post-transcriptional level. We here demonstrate that CspC and CspE are involved in the systemic infection of *Salmonella enterica* serovar Typhimurium. Double disruption of *cspC* and *cspE* impairs the ability to survive intracellularly at early stages after phagocytosis within murine macrophage-like RAW264.7 cells. By analysis of RNA-seq and qRT-PCR using RNAs prepared from the intracellularly surviving bacteria, 4 transcripts, *STM1630*, *STM1867*, *STM3132* and *STM3133*, were found to be regulated post-transcriptionally by both CspC and CspE after phagocytosis by macrophages. *STM1867* encodes PagK1, which is a virulence protein. *STM3132* and *STM3133* are located in *Salmonella* pathogenicity island 13. Furthermore, CspC and CspE stabilized *STM1630* transcript, newly identified among mRNAs upregulated in response to the intracellular environment after phagocytosis. Analysis of a target region of CspC and CspE with transcriptional *STM1630-gfp*-fusions suggested that CspE, but not CspC, could bind to multiple stem-loops within the coding region of *STM1630*. These findings suggest that CspC and CspE are critical in the early intracellular survival of *Salmonella* induced by post-transcriptional regulation of several RNA molecules in response to the phagosomal environment.

2. Collapse of nuclear RNA decay machines by infection invokes immune response

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Eukaryotic genomes are pervasively transcribed, resulting in the production of many unstable nuclear long noncoding RNAs such as nuclear short-lived noncoding transcripts (nSLiTs). However, the biological significance and turnover mechanism of nSLiTs are largely unknown. Here, we identified 145 nSLiTs that accumulated in HeLa cells upon *Salmonella* infection through RNA stabilisation, of which 26 mapped to enhancer regions (enSLiTs) and 119 mapped to non-enhancer regions (linSLiTs). Knockout studies showed that linSLiT08211 and enSLiT07573 regulated the expression of distinct classes of immune genes. enSLiT degradation was aided by the nuclear exosome targeting complex containing SKIV2L2/MTR4. In contrast, degradation of linSLiTs was executed by a novel HNRNPH1-dependent RNA decay pathway that also involves MTR4. Interestingly, MTR4 and the exosome component RRP6 disappeared upon infection, resulting in collapsing nuclear RNA decay complexes. Our findings highlight the importance of regulated nuclear RNA degradation in host immune responses.

3. Reduction of bone marrow immunoglobulin G-secreting plasma cells by *Salmonella* infection

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Host innate and adaptive immune responses confer crucial protection against *Salmonella* infection. On the other hand, *Salmonella* can escape from the host immune system by producing virulence factors, leading to establishment of systemic infection. To understand the mechanism by which *Salmonella* escapes the adaptive immune response, we focused on the influence of *Salmonella* infection on the production of immunoglobulin (Ig)-secreting plasma cells. We showed that the great majority of bone marrow IgG-secreting plasma cells

was diminished within 4 days after intraperitoneal infection of live Lon-deficient attenuated *Salmonella*, whereas bone marrow IgM-secreting and splenic IgG-secreting plasma cells were unaffected. The infection also reduced total IgG titers in serum. This selective diminishment was also induced by culture supernatants from *Salmonella* but not by those from *Escherichia coli* within 24 hours after intraperitoneal injection. The culture supernatant depleted of ligands for Toll-like receptors, lipopolysaccharide and flagellin, also reduced the plasma cells. This study revealed that *Salmonella* reduces bone marrow IgG-secreting plasma cells which are the main source of serum IgG, leading to escape from the host humoral immunity. It is suggested that *Salmonella*-specific component is involved in the reduction.

4. Invasive non-typhoidal *Salmonella* (iNTS) disease: Host pathogen interaction in iNTS infection and appropriate vaccine candidate

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Non-typhoidal *Salmonella* (NTS) serovars such as serovar Typhimurium generally cause in humans, self-limited gastroenteritis, associated with intestinal inflammation and diarrhea. Nevertheless, in developed countries up to 5% of NTS cases may be invasive, extra-intestinal disease leading to bacteremia and focal systemic infections. Moreover, in Sub-Saharan Africa invasive non-typhoidal *Salmonella* (iNTS) have emerged as a major cause of bloodstream infection in immunocompromised and debilitated host. Transmission of iNTS has been demonstrated to be primarily human to human, rather than zoonotic, but little is known about the relationship of invasive disease to diarrheal *Salmonella* disease. To understand the iNTS diseases, we focus on the host-pathogen interaction, i.e. bacterial pathogenesis and host immune responses, in iNTS infection. We analyzed the whole genomes of various iNTS strains isolated in Sub-Saharan Africa during the period 2011-2014 using next generation sequencing.

5. Identification and development of anti-persister compounds as a new class of antibiotics to treat chronic infection

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Chronic infection is often difficult to treat, even when caused by a pathogen that is not resistant to antibiotics. This is the essential paradox of chronic infection. In most cases, chronic infections are accompanied by dormant persisters. Regular antibiotics kill bacteria by corrupting their targets in active cells but targets are inactive in persisters, leading to tolerance to antibiotics. The tolerance is phenotypic variants of normal bacteria and pathways to dormancy are redundant, making it challenging to develop anti-persister compounds which could prevent persister formation or awake the dormant cells and thereby turn those susceptible to conventional antibiotics. We reasoned that a compound leading to corrupting physiology of dormant, energy-deprived cells will be an anti-persister. Our previous studies on the energy-dependent ClpXP-protease allowed us to hypothesize that the dysregulation of proteolysis by activation of ClpP

core in the absence of the regulatory ClpX ATPase may corrupt physiology of dormant cells. In the present project, we aim to identify the compounds to activate ClpP core, leading to the dysregulation of proteolysis. This study will provide innovative perspectives on developing a new class of antibiotics to treat chronic infection.

（i）We have completed the system evaluating ClpP proteolysis in vitro. We have already succeeded in purification of the components necessary for the system through the studies on ClpXP-protease.

（ii）We have started the high-through-put screening of compounds with activity provoking ClpP-protease by exploiting the chemical libraries mainly established in Chiba University. We will evaluate the bactericidal activity of candidates using a different species of bacteria（*Staphylococcus aureus*, *Pseudomonas aureginosa*, *Escherichia coli*, etc.）at several states, dormancy in stationary phase or in biofilm, and chronical infection in mouse at the next stage.

Publications

1）Ishiwada N, Takaya A, Kimura A, Watanabe M, Hino M, Ochiai H, Matsui M, Shibayama K, Yamamoto T: Linezolid-resistant *Staphylococcus epidermidis* associated with long-term, repeated linezolid use in a pediatric patient. J Infect Chemother, 22: 187-190, 2016.

石和田准教授　感染症制御プロジェクト

Project for Infection Control and Prevention

研究概要（Summary）

インフルエンザ菌の病原性解析ならびにインフルエンザ菌感染症，肺炎球菌感染症，B群溶血性レンサ球菌感染症の疫学調査を継続的に行っている．結合型ワクチン導入後，新しく問題となっているワクチン非含有型株による病原因子の解析を行い，新たな予防法の開発を目指す．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，附属病院における小児科・感染症内科での診療活動及び学内外でのコンサルテーションを行っている．

Our research focuses on sero-epidemiology and pathogenesis of *Haemophilus influenza*, *Streptococcus pneumoniae* and *Streptococcus agalactiae*. We organize several clinical researches for the development of diagnostic and therapeutic methods for respiratory infectious diseases collaborating with clinicians and also care for patients in the clinic of the University Hospital.

准　教　授	石和田稔彦	Associate Professor	Naruhiko Ishiwada
特任助教	竹内　典子	Assistant Professor	Noriko Takeuchi
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1. Inhaled Laninamivir Octanoate as Prophylaxis for Influenza in Children

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Background: A single 20-mg dose of inhaled laninamivir octanoate is an effective treatment of influenza. However, the efficacy of laninamivir octanoate for the prevention of influenza in children <10 years of age has not yet been established.

Methods: We conducted a double-blind, multicenter, randomized, placebo-controlled study to determine whether the efficacy of a single 20-mg dose of inhaled laninamivir octanoate to prevent the development of influenza was superior to that of placebo as prophylaxis for influenza in pediatric（<10 years）household members of index cases. Eligible subjects without influenza, in contact with an influenza-infected index case living in the same household, were blindly randomly assigned in a 1:1 ratio to receive 20 mg of laninamivir octanoate or placebo. The primary end point was the proportion of subjects who developed clinical influenza during a 10-day period.

Results: A total of 343 subjects were randomly assigned, with 341 subjects included in the full analysis set for the primary analysis. The proportions of subjects who developed clinical influenza were 11%（18/171）in the laninamivir octanoate group and 19%（33/170）in the placebo group（P = .02）. The relative risk reduction was 45.8%（95% confidence interval, 7.5% to 68.2%）. The incidence of adverse events was similar in both groups.

Conclusions: A single 20-mg dose of inhaled laninamivir

octanoate was effective and well tolerated as prophylaxis for influenza.

2. Putative orotate transporter of *Cryptococcus neoformans*, Oat1, is a member of the NCS1/PRT transporter super family and its loss causes attenuation of virulence

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It is well known that 5-fluoroorotic acid (5-FOA)-resistant mutants isolated from wild-type *Cryptococcus neoformans* are exclusively either ura3 or ura5 mutants. Unexpectedly, many of the 5-FOA-resistant mutants isolated in our selective regime were Ura+. We identified CNM00460 as the gene responsible for these mutations. Cnm00460 belongs to the nucleobase cation symporter 1/purine-related transporter (NCS1/PRT) super family of fungal transporters, representative members of which are uracil transporter, uridine transporter and allantoin transporter of *Saccharomyces cerevisiae*. Since the CNM00460 gene turned out to be involved in utilization of orotic acid, most probably as transporter, we designated this gene Orotic Acid Transporter 1 (OAT1). This is the first report of orotic acid transporter in this family. *C. neoformans* has four members of the NCS1/PRT family, including Cnm00460, Cnm02550, Cnj00690, and Cnn02280. Since the cnm02550Δ strain showed resistance to 5-fluorouridine, we concluded that CNM02550 encodes uridine permease and designated it URidine Permease 1 (URP1). We found that oat1 mutants were sensitive to 5-FOA in the medium containing proline as nitrogen source. A mutation in the GAT1 gene, a positive transcriptional regulator of genes under the control of nitrogen metabolite repression, in the genetic background of oat1 conferred the phenotype of weak resistance to 5-FOA even in the medium using proline as nitrogen source. Thus, we proposed the existence of another orotic acid utilization system (tentatively designated OAT2) whose expression is under the control of nitrogen metabolite repression at least in part. We found that the OAT1 gene is necessary for full pathogenic activity of *C. neoformans* var. *neoformans*.

3. Analysis of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolated from middle ear fluid before and after the introduction of government subsidies for pneumococcal and *H. influenzae* type b vaccines in Japan

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This study aimed to identify trends in frequency, serotype, and antimicrobial susceptibility of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolated from middle ear fluid specimens of children aged ≤15 years (mean, 2 years), before and after the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) and the *H. influenzae* type b vaccine, at a pediatric facility in Japan. Sixty-six *S. pneumoniae* and 88 *H. influenzae* strains were isolated from 820 middle ear fluid samples. Serotyping and antimicrobial susceptibility testing were performed. The study time-frame was divided into period 1 (2007-2010) and period 2 (2011-2014), according to the availability of vaccine public funding. The *S. pneumoniae* detection rate decreased from 9.6% in period 1-6.1% in period 2 ($p = 0.042$). PCV7 serotypes decreased from 56.8% to 9.1% ($p = 0.0002$). No significant change was observed for the 13-valent pneumococcal conjugate vaccine (PCV13) serotypes: 72.7% in period 1 and 59.1% in period 2. Penicillin-resistant strains (penicillin G-MIC ≥2μg/mL)

decreased from 25% to 4.5% (p = 0.038). Detection rates for *H. influenzae* did not change significantly: 10.3% in period 1 and 11.3% in period 2. Serotypes were mostly non-typeable: 97.9% in period 1 and 90.2% in period 2, and only one serotype b strain was isolated in each period. The frequency of ampicillin-resistant strains (MIC ≥4μg/mL) did not change. These results show a preventative effect of PCV7 on otitis media due to *S. pneumoniae*. PCV7 was replaced with PCV13 in 2013 in Japan; therefore, a further decrease in pneumococcal otitis media is anticipated in the future.

4. Clinical and bacteriological analyses of bacteremia due to *Corynebacterium striatum*

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Background: *Corynebacterium striatum* was recently recognized as a potential pathogen of various infectious diseases. However, the clinical entity of this microorganism has not been clearly identified. Therefore, we analyzed *C. striatum* isolates from blood culture and explored their clinical determinants.
Methods: We reviewed the medical records of all patients from whom *C. striatum* isolates were recovered from blood culture for analysis of the patients' backgrounds and clinical course including response to antimicrobial therapy and prognosis.
Results: During the 5-year study period (January 2010 to December 2014), 24 *C. striatum* strains were isolated from blood samples, and the frequency of *C. striatum* bacteremia increased. The majority of the strains were multidrug resistant. All of the tested strains were susceptible to only vancomycin. The age at onset of *C. striatum* bacteremia encompassed all adult age groups, and at least one underlying condition was documented in all patients. Thirteen of the 24 patients were cured using appropriate antibiotics (true infection group); however, 11 of the 24 patients were cured using inappropriate antibiotic therapy or no antibiotics (contamination group). Malignancy and neutropenia significantly increased the odds of true *C. striatum* bloodstream infection.
Conclusions: The Corynebacterium species is often considered a contaminant when isolated in culture. Instead, particularly when the strain is isolated from blood, the species should be considered clinically relevant and identified to the species level; in addition, antimicrobial susceptibility testing is recommended.

5. Characterization and Evaluation of Newly Developed Immune- Chromatographic Method Targeting *Mycoplasma pneumoniae* Ribosomal Protein L7/L12

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Point-of-care testing (POCT) for Mycoplasma pneumoniae infection may be ideal and useful, because significant numbers of the cases will be seen as an outpatient. Recently, a new immune-chromatographic method (ICM)

targeting *M. pneumoniae* ribosomal protein L7/L12 (RP-L7/L12) became available in Japan, although clinical data and basic information regarding efficacy and characterization of this ICM are limited. The present study examined the fate of *M. pneumoniae* ribosomal protein L7/L12 (RP-L7/L12) during the growth in-vitro and correlation between *M. pneumoniae* number in clinical samples and positivity in RP-L7/L12 ICM. Also, usefulness of RP-L7/L12 ICM was investigated in 176 pharyngeal swabs from *M. pneumoniae* infection-suspected patients (children 137 cases; adults 39 cases). In-vitro analysis, the detection limit was shown to be 1.1 × 104 CFU/mL for *M. pneumoniae*. Production of RP-L7/L12 was paralleled with viable *M. pneumoniae* number, and a half-life was calculated to be approximately 2 days in-vitro. Five other Mycoplasma species and 14 representative respiratory pathogens exhibited negative even in a concentration of 106 CFU/mL. The sensitivity and specificity of RP-L7/L12 were respectively 57.1% (20/35) and 92.2% (130/141), comparing to culture method. Clinical samples with *M. pneumoniae* burden of 106 CFU/mL or more exhibited ICM positive in 13 of 18 cases (72.2%). These data demonstrated characteristics of ICM targeting *M. pneumoniae* RP-L7/L12 in-vitro, in addition to sensitivity, specificity and positivity in clinical samples with different bacterial number of *M. pneumoniae*.

6. Congenital Rubella Syndrome: A Case Report on Changes in Viral Load and Rubella Antibody Titers.

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To our knowledge, this is the first report of the use of real-time reverse transcription-polymerase chain reaction to assess changes in viral load in a patient with congenital rubella syndrome (CRS). Rubella-specific antibody titers were also determined. The patient was a male neonate born to a primipara with rubella infection at 10 weeks of gestation. He had no symptoms at birth, but rubella virus was detected in his pharynx, blood, and urine. His mental and physical development was normal for 1 year; however, he was diagnosed with deafness at 13 months of age. Thus, the patient was diagnosed with CRS. Rubella infection in the pharynx was almost constant until 5 months of age; however, it increased dramatically at 6 months of age. No infection was detected at 13 months. Rubella-specific immunoglobulin M titer was consistently low until 9 months of age and then decreased gradually until it became negative at 20 months of age. Rubella-specific immunoglobulin G titer was high at birth. However, it decreased at 3 months and increased again at 4 months. This titer peaked at ～9 months and then decreased again at 13 months. This case shows that the period after the decline in maternal antibody titers, not the neonatal period, may be the most contagious period in patients with CRS.

7. The impact of heptavalent pneumococcal conjugate vaccine on the incidence of childhood community-acquired pneumonia and bacteriologically confirmed pneumococcal pneumonia in Japan

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Heptavalent pneumococcal conjugate vaccine (PCV7) was introduced to Japan in 2010. We investigated the impact of PCV7 on childhood community-acquired pneumonia (CAP) and pneumococcal pneumonia (PP). Children aged <5 years living in Chiba city, Japan, who were admitted to hospitals were enrolled to estimate the incidence of CAP based on the mid-year population. PP was determined by the presence of *Streptococcus pneumoniae* in cultured blood and/or sputum samples of CAP patients. The incidence of CAP and *S. pneumoniae* isolated from PP patients was compared before (April 2008-March 2009) and after (April 2012-March 2013) the introduction of PCV7 immunization. The annual incidence of CAP was reduced [incidence rate ratio 0·81, 95% confidence interval (CI) 0·73-0·90]. When comparing post-vaccine with pre-vaccine periods, the odds ratio for PP incidence was 0·60 (95% CI 0·39-0·93, P = 0·024). PCV7-covered serotypes markedly decreased (66·6% in pre-vaccine vs. 15·6% in post-vaccine, P < 0·01), and serotypes 6C, 15A, 15C and 19A increased. Multidrug-resistant international clones in the pre-vaccine period (Spain6B-2/ST90, Taiwan19F-14/ST236) decreased, while Sweden15A-25/ST63 was the dominant clone in the post-vaccine period. A significant reduction in the incidence of both CAP hospitalizations and culture-confirmed PP of vaccine serotypes was observed at 2 years after PCV7 vaccination.

8. The First Case of Invasive Mixed-Mold Infections Due to *Emericella nidulans* var. *echinulata* and *Rasamsonia piperina* in a Patient with Chronic Granulomatous Disease

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A 16-year-old boy with chronic granulomatous disease presented with pneumonia and rib osteomyelitis. *Emericella nidulans* var. *echinulata* was isolated from his sputum. After starting voriconazole, *Rasamsonia piperina* was isolated from the rib swelling. A combination therapy of voriconazole and micafungin effectively eradicated this invasive mixed-mold infection. In immunocompromised patients, a precise pathogenic diagnosis is clinically useful for administration of an appropriate treatment regimen.

9. Linezolid-resistant *Staphylococcus epidermidis* associated with long-term, repeated linezolid use in a pediatric patient

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We report an 8-year-old patient with catheter-related bacteremia caused by linezolid-resistant *Staphylococcus epidermidis* that was isolated after the long-term, repeated use of linezolid. Three *S. epidermidis* strains isolated from this patient were bacteriologically analyzed. While the strain isolated prior to linezolid initiation was susceptible to linezolid, two strains after linezolid therapy displayed low-level linezolid susceptibility (MIC, 4 mg/L) and linezolid resistance (MIC, 16 mg/L). T2500A mutation in two copies

and G2575T mutations in three copies of 23S rRNA were detected in the low-susceptible strain and the resistant strain, respectively. Linezolid-resistant *S. epidermidis* infection is rare, but may occur with the long-term administration of linezolid.

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伊庭特任教授　RNA制御プロジェクト

Project for RNA Regulation

研究概要（Summary）

細胞内でみられる遺伝子発現の制御ネットワークは，その細胞のもつ発生，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．平成28年４月に開始された本プロジェクトでは発現の様式を制御する因子のなかでも特に，１）各遺伝子のプロモーター上で作用する転写制御因子群，２）クロマチンの活性化状態を規定するクロマチン構造変換因子，３）多数の遺伝子群の発現をpost-transcriptionalレベルで一括して負に制御するmiRNAの３者に注目して，これらが形成する遺伝子制御ネットワークの解明を進め感染症をはじめとしたヒト疾患の制圧をめざす．

Gene regulatory networks in a cell determine not only cellular specificity of development, differentiation, and growth activity but also cellular response or competency to virus, bacterium, and mycete. Whereas these expression patterns are regulated by many factors, this project, which has started in April 2016, concentrate on the following three factors; 1) transcriptional factors, which operate on the promoter region of their target gene, 2) chromatin remodeling factors that modulate the state of chromatin activity, 3) miRNA, which suppresses expression of many genes at the post-transcriptional level to establish new therapeutic methods for human infectious diseases.

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1. MiR-199a Inhibits Secondary Envelopment of Herpes Simplex Virus-1 through downregulation of Cdc42-specific GTPase activating protein

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Several different research groups including us reported that miR-199a inhibits replication of herpesviruses（HSV-1, HCMV, MCMV, and MHV-68）, Semliki Forest Virus, hepatitis B virus, hepatitis C virus, retrovirus, and lentivirus. We here examined how miR-199a exerts its antiviral effects using epithelial tumour cell lines infected with herpes simplex virus-1（HSV-1）. Whereas exogenous expression

of either miR-199a-5p or -3p didn't affected HSV-1 virus gene expression, we found that both miR-199a-5p and -3p inhibit secondary envelopment of HSV-1 by suppressing their common target, ARHGAP21, a Golgi-localized GTPase-activating protein for Cdc42. We have also shown that the trans-cisterna of the Golgi apparatus is a potential membrane compartment for secondary envelopment of this virus. Exogenous expression of either pre-miR-199a or sh-ARHGAP21 exhibited shared phenotypes: alteration of Golgi function in uninfected cells, inhibition of HSV-1 secondary envelopment, and reduction of trans-Golgi proteins upon HSV-1 infection. Importantly, a constitutively active form of Cdc42 also inhibited HSV-1 secondary envelopment. Endogenous levels of miR-199a in a panel of epithelial tumour cell lines were negatively correlated with the efficiency of HSV-1 secondary envelopment in them. These results indicate that miR-199a is a crucial regulator of Cdc42 activity on Golgi membranes, which is important for the maintenance of Golgi function and for the secondary envelopment of HSV-1 upon its infection.

2. Tumor suppression via inhibition of SWI/SNF complex-dependent NF-κB activation

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NF-kappaB (NF-κB) is one of the most important transcriptional factor that are involved in infection, immunological response, inflammation and cancer. Indeed, in numerous epithelial tumor cell lines, an elevated basal expression level of NF-kappaB (NF-κB) strongly contributes to tumor formation activity. Although it has been considered a promising cancer therapy target, few inhibitors of NF-κB can effectively suppress cancer. This may be partly because NF-κB has a wide variety of target genes that are involved in development, differentiation, growth, and inflammation, as well as those showing widespread expression (housekeeping genes). For this reason, complete suppression of NF-κB target genes could affect normal cells, including immune cells that contribute to tumor-eliminating activity. Therefore, there is a need for new NF-κB inhibitors that can suppress only a specific subset of NF-κB target genes and have strong tumor suppressor activity without effects on housekeeping genes.

In this report, we show that the d4 protein family, which we have previously reported to function as adaptor proteins between the SWI/SNF complex and NF-κB dimers, are excellent targets for cancer therapy. We first show that several epithelial tumor cell lines express the genes of this family —DPF1, DPF2, DPF3a, and DPF3b—at various levels and that knockdown of any of them reduces anchorage-independent growth (AIG). We found that a peptide (which we have named "CT1") composed of the 84 highly homologous N-terminal amino acids of the d4 protein family can function as a strong dominant negative mutant that reduces the NF-κB–activating activity of the entire d4 protein family. Proximity ligation assays clearly showed that CT1 retains full adaptor function linking SWI/SNF complex with NF-κB dimers, indicating that the C-terminal domain of d4 protein family contains essential function for NF-κB activation. CT1 suppressed AIG much more effectively than knockdown of any d4 protein alone without affecting growth in monolayer cultures. The peptide also suppressed tumorigenicity in a mouse xenograft model.

Using microarray analysis, we were able to separate NF-κB target genes into two subtypes, SWI/SNF complex-dependent (CT1-sensitive) genes and SWI/SNF complex-independent (CT1-insensitive) genes. The CT1-insensitive genes comprised about three-quarters of all NF-κB genes and, unlike the CT1-sensitive genes, included many housekeeping genes and key transcription factors that are important to amplify the immunoresponse. We further indentified IL-6 as one of the CT-sensitive genes that are required for AIG activity.

3. Inhibition of miR-200-family suppresses tumor formation by triple-negative breast cancer

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Mammary tumors have been shown to contain multiple subpopulations with distinct phenotypic characteristics. In heterogeneous populations, there are cancer stem cells with malignant characteristics that are high tumorigenicity, self-renewal, differentiation, and drug-resistance. Therefore, it is a promising therapeutic target to differentiate cancer stem cells to non-cancer stem cells.

SUM149PT, a cell line originated from triple-negative breast cancer, consists of ESA (epithelial specific antigen) (+) and ESA(-) cells. Each subpopulation can convert to the other subpopulation. Cells in ESA(+) fraction has strong tumorigenicity indicating it contains breast cancer initiating cells, whereas cells in ESA(-) fraction has low tumorigenicity. We found that the expression of miR-200 family members in the ESA(+) fraction was enriched and miR-200 family was a key regulator of transition between two subpopulations. We suppressed the miR-200 family in either the ESA(+) or ESA(-) fraction by transducing lentivirus vectors expressing TuD RNA against miR-200 family members. We found that the strong tumorigenicity of ESA(+) fraction was drastically reduced, and that the marginal tumorigenicity of the ESA(-) fraction, which likely arose from cells that had stochastically converted to ESA(+) cells in vivo, was completely suppressed. This result showed that the strong suppression of miR-200 family activity could have potential therapeutic benefit in triple-negative breast cancer.

Publications

1) Haraguchi T, Kondo M, Uchikawa R, Kobayashi K, Hiramatsu H, Kobayashi K, Chit UW, Shimizu T, Iba H: Dynamics and plasticity of the epitherial to mesenchymal transition induced by miR-200 family inhibition. Sci Rep, 6: 21117, 2016.

五ノ井教授　真菌・放線菌と宿主の分子相互作用研究プロジェクト

Project for Host Pathogen（fungi/actinomycetes）Molecular Interaction

研究概要（Summary）

微生物資源分野では，バイオリソース管理室と協力し，日本国内および海外のヒトや動物に由来する病原真菌・病原放線菌を収集，管理，分譲している．これらの菌株数は，現在約２万に達するが，菌のマーカー遺伝子やゲノムを解析し，また薬剤感受性や電子顕微鏡による形態観察，２次代謝産物の解析などを行い菌株資源，遺伝子資源としての付加価値の向上に努めている．さらなる独自の研究テーマ（PIプロジェクト）については下記『主なテーマ』を参照してください．

主なテーマ（Research Focus）

1）ヒト・動物の病原真菌・病原放線菌の収集，分類，系統解析，２次代謝産物の解析，病原因子解析，２次代謝産物生合成遺伝子，ゲノムの解析を行っている．

2）真菌・放線菌のヒトへの感染機構の解明を分子生物学的手法，動物モデル，ゲノム解析などを用いて行っている．特に，近年は，（Ⅰ）糖鎖と糖鎖受容体を介した菌と宿主の相互作用解明，（II）ヒト病原糸状菌の寄生ウイルスが病原性に及ぼす影響を調べることに力を入れている．

3）真菌感染発症と宿主の栄養状態やストレス状態との関連を，動物モデルなどを用いて研究している．特に代謝関連分子と免疫関連分子の機能的リンクに焦点を当てている．

In cooperation with Bio-Resource management office, we collect pathogenic fungi and actinomycetes in both inside and outside of Japan. We identify pathogenic fungi and actinomycetes as a public service, and analyze their phylogenetic relations. We store fungi and actinomycetes with the support of the National BioResource Projects in Japan, and distribute them upon request. Currently we stock approximately 20,000 strains. We analyze sequences of marker genes and genomes, drug-sensitivities, and observe fine structures using electron-microscopy, to enhance biodiversity values. Other projects are listed below.

1）We collect, identify and phylogenetically analyze of human and animal pathogenic fungi and actinomycetes. We also analyze 2nd metabolites and their synthetic enzymes, pathogenic factors, and genomes.

2）We analyze infection mechanisms of human pathogenic fungi and actinomycetes using molecular methods, animal models, and genome analysis. In particular, we are trying to understand（I）roles of cell surface glycans and their receptors（lectins）of human and fungi in infection, and also（II）studying influence of mycovirus on pathogenicity of filamentous fungi.

3）We study effects of diets and mental stresses on fungal infections mainly using animal models and molecular methods. We are trying to clarify yet unknown links between metabolism and immune-related molecules.

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1. *Nocardia shinanonensis* sp nov., isolated from a patient with endophthalmitis

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A nocardioform strain IFM 11456(T) was isolated from the aqueous humor from a patient with endophthalmitis and was characterized to its taxonomic position. IFM 11456(T) contained arabinose, galactose and meso-diaminopimelic acid in whole-cell hydrolysates and mycolic acids that co-migrated with those from the type strain of *Nocardia asteroides*. The acyl type of muramic acid was N-glycolyl. The diagnostic polar lipids were phosphatidylethanolamine, diphosphatidylglycerol and two unidentified glycolipids and the predominant menaquinone was MK-8 (H-4, omega-cycl.). These characteristics are typical of members of the genus *Nocardia*. Results of phylogenetic analyses based on 16S rRNA gene sequences indicated that the isolate represented a novel species of the genus *Nocardia* and was most closely related to the type strains of *Nocardia mikamii* JCM 15508(T) (98.1%) and *Nocardia aobensis* IFM 0372(T) (98.1%). However, analysis of partial *gyrB* sequences showed that strain IFM 11456 (T) had 90.2% similarity to *Nocardia concava* IFM 0354(T) and 90% to *Nocardia niigatensis* IFM 0330(T). The DNA-DNA relatedness values for strain IFM 11456(T) compared with *N. mikamii* JCM 15508(T), *N. aobensis* IFM 0372(T) and *N. concava* IFM 0354(T) ranged from 24.4 to 39.9%. Phenotypic characteristics that differentiated IFM 11456 (T) from phylogenetically related species were growth at 45 degrees C, utilization of citrate and growth with inositol as a sole carbon source. On the basis of this polyphasic study, the isolate represents a novel species within the genus Nocardia, for which the name *Nocardia shinanonensis* sp. nov. is proposed. The type strain is IFM 11456(T) (= NBRC 109590(T) = TBRC 5149(T)).

2. Successful treatment of primary cutaneous *Nocardia brasiliensis* infection with oral potassium iodide

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Nocardia species are soil-borne aerobic actinomycetes responsible for systemic or cutaneous infections. *Nocardia brasiliens* is the predominate cutaneous nocardiosis. Although combined antibiotics therapies are commonly effective in nocardiosis, few reports show efficacy of the treatment with potassium iodide (KI). We report a case of primary cutaneous nocardiosis due to *N. brasiliensis* who was successfully treated with oral KI with a review of the published work.

3. Multi-azole resistant *Aspergillus fumigatus* harboring Cyp51A TR46/Y121F/T289A isolated in Japan

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Multi-azole resistant *Aspergillus fumigatus* carrying TR46/Y121F/T289A was isolated from a patient in Japan in Dec

2013. This strain grouped into the same clade of the ones which were clinically isolated in France and Germany. *A. fumigatus* harboring this mutation could be rapidly diffused outside the Eurasian continent.

4. Hyrtinadines C and D, New Azepinoindole-Type Alkaloids from a Marine Sponge *Hyrtios* sp.

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New bisindole alkaloids, hyrtinadines C (1) and D (2), have been isolated from an Okinawan marine sponge *Hyrtios* sp. The structures of hyrtinadines C (1) and D (2) were elucidated based on analyses of the spectral data. Hyrtinadines C (1) and D (2) were the relatively rare alkaloids possessing a 3, 4-fused azepinoindole skeleton. Hyrtinadines C (1) and D (2) showed antimicrobial activity.

5. Comparative transcriptome analysis revealing dormant conidia and germination associated genes in *Aspergillus* species: an essential role for AtfA in conidial dormancy

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Background: Fungal conidia are usually dormant unless the extracellular conditions are right for germination. Despite the importance of dormancy, little is known about the molecular mechanism underlying entry to, maintenance of, and exit from dormancy. To gain comprehensive and inter-species insights, transcriptome analyses were conducted across *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus oryzae*.
Results: We found transcripts of 687, 694, and 812 genes were enriched in the resting conidia compared with hyphae in *A. fumigatus*, *A. niger*, and *A. oryzae*, respectively (conidia-associated genes). Similarly, transcripts of 766, 1,241, and 749 genes were increased in the 1 h-cultured conidia compared with the resting conidia (germination-associated genes). Among the three *Aspergillus* species, we identified orthologous 6,172 genes, 91 and 391 of which are common conidia-and germination-associated genes, respectively. A variety of stress-related genes, including the catalase genes, were found in the common conidia-associated gene set, and ribosome-related genes were significantly enriched among the germination-associated genes. Among the germination-associated genes, we found that calA-family genes encoding a thaumatin-like protein were extraordinary expressed in early germination stage in all *Aspergillus* species tested here. In *A. fumigatus* 63% of the common conidia-associated genes were expressed in a bZIP-type transcriptional regulator AtfA-dependent manner, indicating that AtfA plays a pivotal role in the maintenance of resting conidial physiology. Unexpectedly, the precocious expression of the germination-associated calA and an abnormal metabolic activity were detected in the resting conidia of the atfA mutant, suggesting that AtfA was involved in the retention of conidial dormancy.
Conclusions: A comparison among transcriptomes of hyphae, resting conidia, and 1 h-grown conidia in the three *Aspergillus* species revealed likely common factors involved in conidial dormancy. AtfA positively regulates conidial stress-related genes and negatively mediates the gene expressions related to germination, suggesting a major role for AtfA in *Aspergillus* conidial dormancy.

6. Aflatoxin M1 Contamination of Milk and Its Products in Bomet County, Kenya

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Aflatoxin M1 (AFM1) is a major carcinogenic compound that may be found in milk and dairy products resulting from ingestion of aflatoxin B1 by dairy animals. The study aimed at determining the level of aflatoxin M1 in milk and milk products from Bomet County. A total of 185 samples (150 raw milk and 35 processed milk and milk products) were randomly collected from milk collection sites and randomly selected milk kiosks respectively. The AFM1 was analyzed using a commercial ELISA kit (Ridascreen, aflatoxin M1 R-Biopharm, Product code, R5812, Darmstadt, Germany). Out of 185 samples investigated, 156 samples were positive for AFM1, an overall contamination rate of 84.32%. The samples with levels higher than the tolerance limit of 0.05μg/l recommended by Food and Agriculture Organization (FAO) and World Health Organization (WHO) limits were 43.8% mainly contributed by the raw milk compared to processed milk (52.0% versus 8.6%). Processed milk had insignificant level of contamination with aflatoxin M1 (Median 0.00 (IQR: 0.00, 0.00μg/l) with a minimum of 0.00μg/l and a maximum of 0.69μg/l. Raw milk showed significant contamination, median 0.09 (IQR: 0.00, 0.50) μg/l with a minimum of 0.00 μg/l and a maximum of 2.93μg/l. Although there was no significant differences in AFM1 levels with study sites (P = 0.217); the median levels of aflatoxin M1 was high in sites 1, 3, and 7. The sites that had median aflatoxin M1 levels below the WHO/FAO acceptable limits of 0.05μg/l were sites 2, 4 and 6. Due to high incidence of AFM1 contamination of milk and milk samples in Bomet County, there is need for regular monitoring and regulation of AFM1 contamination in milk and its products in the County.

7. *Nocardia elegans* infection: a case report and literature review

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A case of disseminated nocardiosis caused by Nocardia elegans in a 72-year-old man with rheumatoid arthritis, treated with tacrolimus and prednisolone, is reported herein. The patient had impaired vision and was diagnosed with endophthalmitis and an abdominal skin abscess. He was started on trimethoprim–sulfamethoxazole treatment, followed by cefepime. The patient was then switched to a combination of imipenem–cilastatin and minocycline. Although the patient survived as a result of surgery and prolonged antibiotic treatment, he eventually lost vision after the infection became resistant to antibiotic treatment. Molecular analysis of samples from the abscess and vitreous fluid confirmed the extremely rare pathogen N. elegans, which accounts for only 0.3–0.6% of infections caused by Nocardia species. This organism is almost always associated with pulmonary infection, and disseminated infections are rare. As with previously reported norcardial infections, the current case was treated successfully with trimethoprim–sulfamethoxazole, carbapenems, and aminoglycosides. However, the clinical characteristics of this organism remain unclear. Further studies are therefore required to develop more effective treatment protocols for disseminated nocardiosis caused by this problematic pathogen.

8. First case report of pulmonary nocardiosis caused by *Nocardia mexicana*

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Introduction: *Nocardia* species usually cause opportunistic infections, and the frequency of these infections is increasing owing to the growing population of immunocompromised hosts. However, *Nocardia* species may sometimes cause an infection disease in immunocompetent hosts. *Nocardia mexicana* infections are the least common and are very rare.
Case presentation: Herein, we report the first case of a pulmonary infection with *N. mexicana* in a 61-year-old Japanese woman with a history of hyperlipidaemia and bronchiectasis and a 6-month history of non-productive hacking cough. A sample of bronchial lavage fluid obtained by bronchofiberscopy showed filamentous branching gram-positive rods and acid-fast filamentous branching rods, and a colony of suspected *Nocardia* was cultured. Based on 16S rRNA, *gyrB*, *rpoB*, *secA1* and *hsp65*gene sequence analyses and biochemical and physiological properties, the strain was identified as *N. mexicana*. The strain was resistant to the antimicrobial agents amoxicillin-clavulanic acid, clarithromycin, minocycline, gentamycin, tobramycin, ciprofloxacin and trimethoprim-sulfamethoxazole. The patient was treated with biapenem followed by intravenous amikacin and oral linezolid.
Conclusion: Despite its rarity, the species require attention owing to the existence of multidrug-resistant strains.

Publications

1）Hagiwara D, Takahashi H, Fujimoto M, Sugahara M, Misawa Y, Gonoi T, Itoyama S, Watanabe A, Kamei K: Multi-azole resistant *Aspergillus fumigatus* harboring Cyp51A TR46/Y121F/T289A isolated in Japan. J Infect Chemothera, 22: 7-8, 2016.
2）Hagiwara D, Takahashi H, Kusuya Y, Kawamoto S, Kamei K, Gonoi T: Comparative transcriptome analysis revealing dormant conidia and germination associated genes in *Aspergillus* species: an essential role for AtfA in conidial dormancy. BMC Genomics, 17: 358, 2016.
3）Kubota T, Nakamura K, Sakai K, Fromont J, Gonoi T, Kobayashi J: Hyrtinadines C and D, New Azepinoindole-Type Alkaloids from a Marine Sponge *Hyrtios* sp. Chem Pharm Bull, 64: 975-978, 2016.
4）Langat G, Matsusawa T, Gonoi T, Matiru V, Bii C: Aflatoxin M1 Contamination of Milk and Its Products in Bomet County, Kenya. Adv Microbiol, 6: 528-536, 2016.
5）Matsumoto T, Negishi T, Hamada M, Komaki H, Gonoi T, Yaguchi T: *Nocardia shinanonensis* sp nov., isolated from a patient with endophthalmitis. Int J Syst Evol Microbiol, 66: 3324-3328, 2016.
6）Nakamura I, Nagakura T, Fujita H, Fukusima S, Gonoi T: *Nocardia elegans* infection: a case report and literature review. Int J Infect Dis, 54: 15–17, 2016.
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高橋准教授　微生物創生プロジェクト

Project for Systems Biology of Microorganisms

研究概要（Summary）

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．

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.

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1. Comparative transcriptome analysis revealing dormant conidia and germination associated genes in Aspergillus species: an essential role for AtfA in conidial dormancy

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BACKGROUND: Fungal conidia are usually dormant unless the extracellular conditions are right for germination. Despite the importance of dormancy, little is known about the molecular mechanism underlying entry to, maintenance of, and exit from dormancy. To gain comprehensive and inter-species insights, transcriptome analyses were conducted across Aspergillus fumigatus, Aspergillus niger, and Aspergillus oryzae.

RESULTS: We found transcripts of 687, 694, and 812 genes were enriched in the resting conidia compared with hyphae in A. fumigatus, A. niger, and A. oryzae, respectively (conidia-associated genes). Similarly, transcripts of 766, 1,241, and 749 genes were increased in the 1 h-cultured conidia compared with the resting conidia (germination-associated genes). Among the three Aspergillus species, we identified orthologous 6,172 genes, 91 and 391 of which are common conidia-and germination-associated genes, respectively. A variety of stress-related genes, including the catalase genes, were found in the common conidia-associated gene set, and ribosome-related genes were significantly enriched among the germination-associated genes. Among the germination-associated genes, we found that calA-family genes encoding a thaumatin-like protein were extraordinary expressed in early germination stage in all Aspergillus species tested here. In A. fumigatus 63% of the common conidia-associated genes were expressed in a bZIP-type transcriptional regulator AtfA-dependent manner, indicating that AtfA plays a pivotal role in the maintenance of resting conidial physiology. Unexpectedly, the precocious expression of the germination-associated calA and an abnormal metabolic activity were detected in the resting conidia of the atfA mutant, suggesting

that AtfA was involved in the retention of conidial dormancy. CONCLUSIONS: A comparison among transcriptomes of hyphae, resting conidia, and 1 h-grown conidia in the three Aspergillus species revealed likely common factors involved in conidial dormancy. AtfA positively regulates conidial stress-related genes and negatively mediates the gene expressions related to germination, suggesting a major role for AtfA in Aspergillus conidial dormancy.

2. Control of transcriptional pausing by biased thermal fluctuations on repetitive genomic sequences

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In the process of transcription elongation, RNA polymerase (RNAP) pauses at highly nonrandom positions across genomic DNA, broadly regulating transcription; however, molecular mechanisms responsible for the recognition of such pausing positions remain poorly understood. Here, using a combination of statistical mechanical modeling and high-throughput sequencing and biochemical data, we evaluate the effect of thermal fluctuations on the regulation of RNAP pausing. We demonstrate that diffusive backtracking of RNAP, which is biased by repetitive DNA sequence elements, causes transcriptional pausing. This effect stems from the increased microscopic heterogeneity of an elongation complex, and thus is entropy-dominated. This report shows a linkage between repetitive sequence elements encoded in the genome and regulation of RNAP pausing driven by thermal fluctuations.

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研究概要（Summary）

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．

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.

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1. Studies in phylogeny, development of rapid identification, antifungal susceptibility and growth rates on clinical strains of *Sporothrix schenckii* complex in Japan

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Sporotrichosis is a fungal infection caused by the *Sporothrix* species, which have distinct virulence profiles and geographic distributions. We performed a phylogenetic study in strains morphologically identified as *S. schenckii* from clinical specimens in Japan, which were preserved at the Medical Mycology Research Center, Chiba University. In addition, we examined the in vitro antifungal susceptibility and growth rate to evaluate their physiological features. 300 strains were examined by sequence analysis of the partial calmodulin gene, or polymerase chain reaction (PCR) method using newly designed species-specific primers; 291 strains were *S. globosa* and 9 strains were *S. schenckii* sensu stricto (in narrow sense, s. s.). *S. globosa* strains were further clustered into two subclades and *S. schenckii* s. s. strains were divided into three subclades. In 38 strains of *S. globosa* which were determined antifungal profiles, four strains (11%) showed high minimal inhibitory concentrations (MIC) value for itraconazole. All tested strains of *S. schenckii* s. s. and *S. globosa* showed low sensitivity for amphotericin B. These antifungals are used for treatment of sporotrichosis when infection is severe. *S. schenckii* s. s. grew better than *S. globosa*, especially *S. globosa* showed restricted growth at 35℃ and didn't grow at 37℃. Our molecular data showed that *S. globosa* is the main causal agent of sporotrichosis in Japan. It is important to determine the antifungal profiles of each case, in addition to species-level accurate identification, to strategize the therapy for sporotrichosis.

2. Development of rapid identification and risk analysis of *Moniliella* spp. in acidic processed foods

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The number of spoilage incidents in the food industry attributable to a species of the genus *Moniliella* has recently increased, but the risk of food spoilage has not yet been evaluated. The purpose of this study was to develop a method to rapidly identify high-risk species and to conduct a risk analysis study of *Moniliella* spp. Acetic acid resistance of *M. acetoabutens* and ethanol resistance of *M. suaveolens* were higher than for other *Moniliella* species. All examined strains of *M. acetoabutens* developed a high tolerance to acetic acid by culturing twice in liquid media containing low concentrations of acetic acid. These finding indicate that *M. acetoabutens* and *M. suaveolens* are high-risk species for food spoilage and must be discriminated from other fungi. We developed species-specific primers to identify *M. acetoabutens* and *M. suaveolens* using polymerase chain reaction to amplify the D1/D2 domain of 28S rDNA. Polymerase chain reaction using the primer sets designed for *M. acetoabutens* (Mac_F1/R1) and *M. suaveolens* (Msu_F1/R1) were specific to the target species and did not detect other fungi involved in food spoilage or environmental contamination. This method is expected to be effective for the surveillance of raw materials and components of the food production proess.

3. Verification of ribosomal proteins of *Aspergillus fumigatus* for the use of biomarkers in MALDI–TOF MS identification

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We have previously proposed a rapid identification method for bacterial strains based on the profiles of their ribosomal subunit proteins (RSPs), observed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This method can perform phylogenetic characterization based on the mass of housekeeping RSP biomarkers, ideally calculated from amino acid sequence information registered in public protein databases. With the aim of extending its field of application to medical mycology, this study investigates the actual state of information of RSPs of eukaryotic fungi registered in public protein databases through the characterization of ribosomal protein fractions extracted from genome-sequenced *Aspergillus fumigatus* strains Af293 and A1163 as a model. In this process, we have found that the public protein databases harbor problems. The RSP names are in confusion, so we have provisionally unified them using the yeast naming system. The most serious problem is that many incorrect sequences are registered in the public protein databases. Surprisingly, more than half of the sequences are incorrect, due chiefly to mis-annotation of exon/intron structures. These errors could be corrected by a combination of in silico inspection by sequence homology analysis and MALDI-TOF MS measurements. We were also able to confirm conserved post-translational modifications in eleven RSPs. After these verifications, the masses of 31 expressed RSPs under 20,000 Da could be accurately confirmed. These RSPs have a potential to be useful biomarkers for identifying clinical isolates of *A. fumigatus*.

4. Draft genome sequence of the pathogenic filamentous fungus *Aspergillus lentulus* IFM 54703^T

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Aspergillus lentulus, a sibling species of *Aspergillus fumigatus*, has been reported as a causative agent of aspergillosis, and exhibited low susceptibility to azole. Here, we present the draft genome sequence of *A. lentulus* strain IFM 54703^T for the first time.

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文部科学省　ナショナルバイオリソースプロジェクト「病原微生物」

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

文部科学省では2002年度からナショナルバイオリソースプロジェクト（NBRP）を開始し，国が戦略的に整備することが重要なものについて体系的に収集，保存，提供などを行うための体制を整備してきた．その後５年ごとの見直しを行い，現在，第４期の公募が開始された．

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），大阪大学微生物病研究所および岐阜大学大学院医学研究科（病原細菌）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．

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, now the selection of the forth phase has been 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), Osaka University's Research Institute for Microbial Diseases (pathogenic bacteria), Gifu University's Graduate School of Medicine (pathogenic bacteria), 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.

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

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所ケニア拠点の助力を得て，ケニアを中心に上記表題のプロジェクトを展開しています．現在までにケニア全土の主要穀物（トウモロコシ，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ました．結果は，順次現地のマスコミにも取り上げられ，大きな反響を呼び起こしました．さらに，フザリウム属菌，アスペルギルス属菌など，食糧植物を汚染し人体に健康被害を及ぼす菌についても現地研究員を日本に招聘するなどして共同研究を続けています．さらに現地に蔓延するエイズの患者さんの命を奪う原因菌のひとつであるクリプトコッカス属菌を中心に疫学的調査を行なっています．

海外での研究は，現地の研究者や監督官庁と信頼関係を築き，適切な許可を得るなど多くの問題を解決しなければ前進できません．しかし，現地の医療に貢献し，人々の生活の質（QOL）の向上を図り，さらに日本との友好を深めるために努力を重ねています．一方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，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す．

2012 年 2 月　ケニア国スキム市の病院・研究施設訪問

Under assistance of Kenya Research Station, Inst. NEKKEN, Nagasaki univ., we are analyzing toxins contaminating major local grains（maze, wheat, rice etc. ）milks, local beers and so on. We also analyze fungal producers of toxins. We found the local foods are contaminated by several toxins at concentrations far above the international standards. The result has been published in a scientific papers and announced in newspapers, receiving large public attention. A new project for epidemiological study of Cryptococcal fungi in HIV-infected patients has been launched in collaboration with Kenya Medical Res. Insti.（KEMRI）and doctors from UCSF, USA.

In 2016 we imported approximately 300 hundreds of fungal strains from Kenya with collaboration of Kenyan researchers and under appropriate permission of Kenyan and Japanese regulatory agencies, started analyzing them by morphological, molecular and physiological methods. We are preparing new papers describing new fungal species from Kenya.

In 2014 we published on the high risk mycotoxin contamination of the domestic beer, busaa, which is very popular in Kenyan local areas and served in several types of celebrations. The results were published in an academic journal and also announced in nationwide papers. In October 2014, we invited Mr. Olga, a research officer of Kenya Medical Research Institute to Medical Mycology Research Center, Chiba University, and started collaborative works on food contaminating fungi and mycotoxins, human pathogenic *Cryptococcus* in HIV patients, and dermatophytes in Kenya.

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

The Project for Prophylaxis, Diagnosis, and Treatment for Aspergillosis and the Other Mycoses in Aged and Neonate Patients

真菌が内臓に感染する深在性真菌症は難治性で死亡率が高く重要な疾患である．我が国で最も死亡者が多い真菌症はアスペルギルス症であるが．我が国で懸念されている高齢化やCOPDの増加はいずれも慢性肺アスペルギルス症の発症要因であり，本疾患の増加に対する対策が急務と考えられる．さらに近年は原因菌であるアスペルギルスにおいて，その主力薬剤のアゾール薬に対する耐性化が欧米を中心に急速に進んでいることから，５年生存率50-60％とされている本疾患の予後が更に悪化することが予測されている．一方，新生児領域におけるアスペルギルス症を始めとした深在性真菌感染症はしばしば致死的とされるが，その症例数や疾患の種類，詳細な予後などの実態は全く不明である．本プロジェクトは，このようなCOPD等の慢性呼吸器疾患に併発するアスペルギルス症，さらには新生児における真菌症を含めて疫学調査を行いその実態を把握するとともに，新規診断法，治療法，予防法の開発を行って本疾患の制圧を目指すものである．

本年，慢性呼吸器疾患を対象とした慢性肺アスペルギルス症の研究では，COPDなどの慢性肺疾患に合併する慢性肺アスペルギルス症の疫学調査において，慶応大学呼吸器内科と共同研究を開始した．その検討の中で，対象をCOPD患者から更に拡大して，気管支拡張症，非結核性抗酸菌症，ALI（acute lung injury, 急性肺傷害）を基礎疾患にもつ患者も対象とすることとし，同大の担当教官と症例登録を進めている．更に症例を増やして検討するため，新たに同大感染制御センターとも協力関係を確立した．

一方，新生児領域における深在性真菌感染症の研究では，日本新生児成育医学会・感染対策予防接種委員会の協力を得て，新生児を取扱う全国の医療機関を対象に，国内ではじめてとなる深在性真菌感染の発症状況調査を行った．その結果，新生児に対する抗真菌薬の予防投与は55施設で，母体への予防投与は31施設で行われていた．深在性真菌症は34症例の報告があったが，原因菌はカンジダ属が27例と最も多く，また６例が死亡していた．これは日本における新生児深在性真菌症の実態を明らかにしたはじめての研究であり，新生児深在性真菌症の発症率は低いものの致死率が約20％の予後不良な感染症であることが明らかとなったことから，その診断・治療・予防法に関する指針を策定する必要があると考えられた（第61回日本新生児成育医学会において発表）．この結果を基に，日本新生児成育医学会・感染対策予防接種委員会において，新生児深在性真菌感染症の前向き調査を実施すること，症例から分離された菌株の保存の徹底と真菌センターでの解析を行うことを提言した．現在，全国調査結果に関して論文を作成中である．

Aspergillosis is the most serious deep-seated mycosis in Japan with high mortality rate. The recent changes in our society, such as aging of the population and the increase of COPD patients, are both predisposing factors for chronic pulmonary aspergillosis. Rapidly prevailing azole drug resistance among aspergilli is expected to make the disease more intractable. Deep-seated mycosis among neonates is another threat to our society. It is often fatal, but the epidemiology of the disease is not yet known. The aim of this project is to make the epidemiological analyses of these diseases, i. e. aspergillosis among chronic pulmonary disease patients and deep-seated mycoses among neonates, and to find seeds for the new diagnostic/therapeutic/preventive measures to counteract the plagues.

In the study for the aspergillosis among chronic pulmonary diseases patients, we started out a collaborative study with the Department of Pulmonary Medicine, Keio University for the surveillance of the disease, and registered patients are now being accumulated. To enhance the network for the study, collaboration with the Department of Infectious Diseases is

also discussed.

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) from January in 2014 to October in 2015. This is the first study on nationwide surveillance of neonatal IFI in Japan. The primary questionnaire survey was performed for 309 medical facilities that regularly take care of the high-risk neonate. The 128 centers replied the primary questionnaire and the rate of reply was 41.4%. In 24 of 128 centers experienced 34 IFI. The estimated annual IFI incidence was 0.57 per 1,000 beds. The patient's information at the onset of IFI was available all 34 patients. The causative microorganisms was identified in 29 patients. *Candida* species (n= 27) was the mist common pathogen. The mortality rate of IFI cases was 17.6%. Neonatal fugal prophylaxis was done in, 55 of 128 medical facilities (43.0%). Fungal prophylaxis for mothers who colonized fungus was done in 31 of 128 facilities (24.2%). In Japan, diagnosis, treatment and prevention of neonatal IFI were varied. Continuous surveillance and established the treatment regimen against neonatal IFI were required for the improvement of outcome of high risk neonates. We are planning prospective surveillance on neonatal IFI collaboration with the members of Japanese Society for Neonatal Health and Development.

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

「薬剤耐性真菌検出のための新規検査法の開発とブラジルにおける疫学調査等への応用」

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

“Development of innovative diagnostic tools to detect drug-resistant fungi and their application to the epidemiological surveillance in Brazil”

SATREPS（感染症分野）とは，国立研究開発法人日本医療研究開発機構（AMED）と独立行政法人国際協力機構（JICA）が共同で実施している，地球規模課題解決と将来的な社会実装に向けて日本と開発途上国の研究者が共同で研究を行う研究プログラムである．本課題は平成28年に採択された．

真菌感染症患者は世界的に急増している．なかでも薬剤耐性真菌による感染症は致死率が極めて高い．真菌が耐性を獲得するメカニズムについては十分に解明されていないが，農地に散布される農薬（抗真菌薬類似の成分を含む）の影響によるもの，慢性真菌感染症患者に対するアゾール系抗真菌薬の長期使用などが原因として考えられている．その一方，ブラジルでは薬剤耐性真菌の実態は十分解明されていない状況にある．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．平成29年からのプロジェクト開始に向け，平成29年１月に現地に詳細計画策定調査団（渡辺哲准教授が参加）が赴き，カンピーナス大学のスタッフとプロジェクトについて詳細な意見交換を行う予定である．

SATREPS is a Japanese government program that promotes international joint research. The program is structured as a collaboration between the Japan Agency for Medical Research and Development（AMED）which provides competitive research funds for science and technology projects, and the Japan International Cooperation Agency（JICA）which provides development assistance（ODA）. Based on the needs of developing countries, the program aims to address global issues and lead to research outcomes of practical benefit to both local and global society. Our proposal was selected in 2016.

The incidence of fungal infections is increasing worldwide. The fungi’s drug resistance has strengthened along with the increased frequency and the mortality rate of the patients having contracted drug-resistant fungal infections is high. The mechanism how fungi gains drug resistance has not been clarified. For example, it could be through the exposure to pesticides containing ingredients similar to medical antifungal drugs in the fields（fungicides）, or in the body of a patient with chronic fungal infection who has undergone a treatment using azole-based drugs for a long time. Moreover, there are few public data within Brazil that shows the frequency of identification regarding fungal strains that cause drug resistance. In these situation, we planned our poroject in collabration with the State University of Campinas in Brazil（UNICAMP）. Aims of our project are to clarify the epidemiology of drug-resistant fungi’s causing drug resistance in Campinas Metropolitan area, develop a new detection method for the drug resistant fungi and establish an optimum treatment system and research network regarding the drug-fungi centered in UNICAMP.

The project will be started in 2017, and detailed planning survey team（including Associate Professor A. Watanabe）

will visit Brazil, and framework of the project will be thoroughly discussed with the staff of UNICAMP.

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」

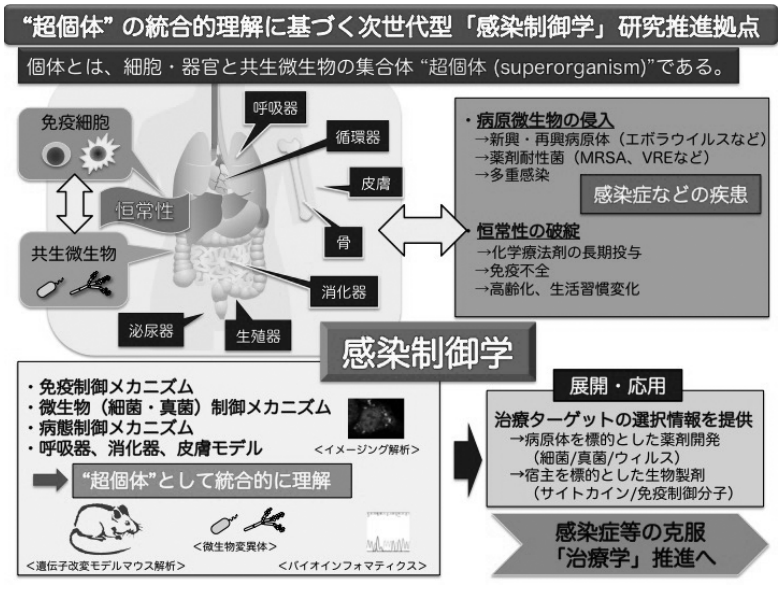
Leading Research Promotion Program, Institute for Global Prominet Research

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

千葉大学で2016年度から開始された『グローバルプロミネント研究基幹』において，当センターの教員が中心となって申請した研究プロジェクトが，リーディング研究育成プログラムの一つとして採択された．

本プログラムでは，当センターの感染免疫分野及び微生物資源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て，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．

The research proposal submitted by MMRC, School of Medicine, Faculty of Pharmaceutical Sciences and University Hospital has been selected as Leading Research Promotion Program by Chiba University. In this program, we focus on understanding of molecular interactions between hosts and microbes, especially commensal fungi and bacteria, using the model assay system in skin, respiratory and digestive organs of mouse and human. We also aim to reveal the molecular machinery underlying disruption of the homeostatic balance by invasive pathogens and the pathogenesis of infectious diseases. The results obtained from our projects will help to create innovative achievements in therapeutics of the infectious diseases and lead to improvement of human health.



平成 27 年度 共同利用・共同研究報告

2015 Fiscal Year Cooperative Research Program Report

研究課題　’15－１

糸状菌の感染免疫応答と形態形成を支配する細胞表層α-1，3-グルカンの生合成制御

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Biogenesis of cell wall α-1，3-glucan controlling fungi-host immune-responses and fungal morphogenesis

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研究成果

（１）「*A. nidulans* のCWI経路，フェロモン経路，AG生合成酵素遺伝子の各欠損株の有性生殖能の解析」および「AG合成酵素遺伝子の転写制御のCWI経路とフェロモン経路への依存度の評価」

糸状菌の細胞壁α-1，3-グルカン（AG）は，細胞壁の最外層に位置し，内層のβ-1，3-グルカン（BG）やキチンを被覆することで感染宿主の免疫認識を回避するステルス因子として機能することが知られている．しかし，麹菌などの非感染性の糸状菌にもAGが存在することから，宿主に対するステルス性以外にもAGには未知の生物学的機能があると思われる．我々は*A. nidulans* のAG欠損株を用いて，AGが菌糸同士の接着因子であることを明らかにしたが，その他の生物学的機能はいまだ不明である．*A. nidulans* には無性世代と有性世代の生活環が存在することが知られている．有性生殖器官（クライストセシア）の形成には，菌糸先端にある細胞の菌糸融合が必要である．そこで，２種あるAG合成酵素遺伝子*agsA* および*agsB* の遺伝子破壊株を用いて，有性生殖能を検討した結果，*agsA*Δ株および*agsB*Δ株は有性世代不全であることを見出した．有性世代不全は*agsA*Δ株でより顕著であった．以上より，AGの接着因子としての機能が有性生殖にも寄与することが予想された．*A. nidulans* の栄養生長時には，*agsB* が主として機能しており，CWI経路による制御を受ける（総説１，関連原著論文１，　関連総説１）．以上の知見を総合すると，*A. nidulans* に存在する２種のα-1,3-グルカン合成酵素は，栄養生長時と有性生殖器官形成時にAG合成の役割分担をしており，*agsA* がフェロモン経路の制御により有性生殖へ寄与するという全く新しい知見を与える可能性が考えられた．現在，フェロモン経路の人為的活性化株を作出中であり，引き続き，フェロモン経路の人為的活性化時の*agsA* および*agsB* 遺伝子の転写レベルを確認する予定である．

（２）「Aspergillus属糸状菌におけるα-1,3-グルカンの細胞接着性への寄与の検証」

糸状菌は液体培養において菌糸が絡まり菌糸塊となってしまうことが多いことから，AGがAspergillus属糸状菌一般の菌糸接着因子であることが予想された．モデル糸状菌*A. nidulans* においても，産業糸状菌*Aspergillus oryzae* でも液体培養時に菌糸塊が形成される．麹菌*Aspergillus oryzae* は *agsA*, *agsB*, *agsC* の３種のAG合成酵素遺伝子を有していることから，これら３種の遺伝子を破壊した*agsA*Δ*agsB*Δ*agsC*Δ株（AG欠損株）を造成した．その結果，液体培養において，麹菌のAG欠損株の菌糸は，*A. nidulans* のような菌糸の完全分散性状にはならなかったが，野生株に比べて小さな菌糸塊を形成し（野生株比直径が約60%に減少），培養総菌体量は増加した．

さらに，組換えタンパク質の生産量も野生株比1.7倍に増加した．これらの結果は，麹菌においてもAGは菌糸接着因子であるが，AGの他にも菌糸接着因子が存在することを示唆している．AG欠損株における酵素生産性の向上は，AG欠損株を物質生産用の宿主株として用いることにより，従来よりも一培養当たりの生産性の大幅な改善が可能であることを示唆した（論文投稿中）．

発表論文

1）Yoshimi A, Fujioka T, Mizutani O, Marui J, Hagiwara D, Abe K: Mitogen-activated protein kinases MpkA and MpkB independently affect micafungin sensitivity in *Aspergillus nidulans*. Biosci Biotech Biochem, 79: 836-844, 2015

研究課題　'15－2

Aspergillus fumigatus の転写因子AtrRによるアゾール系薬剤耐性関連遺伝子の発現制御の分子メカニズム

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Molecular mechanisms for the regulation of genes involved in azole drug resistance by the transcription factor, AtrR, in *Aspergillus fumigatus*

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研究成果

麹菌*Aspergillus oryzae*において見出されたアゾール系薬剤耐性に関する新規Zn_2Cys_6型転写因子AtrRは，モデル糸状菌の*Aspergillus nidulans*やアスペルギルス症起因菌である*Aspergillus fumigatus*にも共通のオーソログとして見出され，それぞれの*atrR*遺伝子の破壊株では，野生株より100倍も低い薬剤濃度でも感受性を示すことが見出された．*A. fumigatus*においてAtrRの機能解析を行った結果，AtrRがアゾール耐性に関わるABCトランスポーターCdr1Bの発現を制御していることを世界で初めて明らかにするとともに，アゾール薬標的分子であるCyp51Aをはじめとしてエルゴステロール生合成に関与する酵素遺伝子の発現にも関わっていることを見出した．AtrRが本菌のアゾール薬耐性メカニズムにおいて中心的な役割を果たしており，AtrRの機能阻害剤の開発はアゾール薬の治療効果を格段に高め，耐性株に対しても卓効を示すことが期待される．本研究ではAtrRによる*cdr1B*および*cyp51A*遺伝子の発現調節機構の解明を目指すとともに，Cyp51Aに耐性変異を有するアゾール耐性*A. fumigatus*株におけるAtrRの機能阻害の重要性を検証することを目的とした．

Cyp51Aを含むエルゴステロール生合成酵素遺伝子の発現制御を担う転写因子としてすでにbHLH型転写因子SrbAが報告されていたことから，*A. fumigatus*の*atrR*破壊株に加えて*srbA*破壊株も作製して，アゾール耐性や低酸素条件下における生育，マウスに対する病原性などの違いを比較した．*atrR*破壊株と*srbA*破壊株ともにアゾール薬剤に感受性が高くなっていたが，*atrR*破壊株の方が感受性が高くなっていた．一方，低酸素条件下における生育はともに悪くなったが，株間での差異はほとんど認められず，マウスに対する病原性も同等に低下していた．両破壊株ともに*cyp51A*, *erg3B*, *erg24A*, *erg25A*のエルゴステロール生合成遺伝子の顕著な発現量低下を示したが，ABCトランスポーター遺伝子（*cdr1B*）の発現は*atrR*破壊株のみで著しく低下していた．また，マウスを用いた感染性試験により，いずれの破壊株も病原性の著しい低下が認められた．さらに，HAタグを付加したAtrRを用いたクロマチン免疫沈降（ChiP）解析を行ったところ，コントロールのアクチン遺伝子（*act1*）プロモーターは濃縮されなかったのに対して，*cyp51A*および*cdr1B*のプロモーターはそれぞれ20倍，33倍に濃縮されたことから，AtrRがこれらのプロモーター領域に結合することが示された．また，AtrRは*cyp51A*の上流－69～－415の領域に，*cdr1B*の上流－675～－982の領域に強く結合することが示唆された．Cyp51AのG54E置換変異による*A. fumigatus*のアゾール耐性株（IFM 61567）につ

いて*atrR*の破壊を行ったところ，イトラコナゾールやポサコナゾールに対して高い感受性を示しただけでなく，野生株でも耐性を示すフルコナゾールに対しても感受性を示すことが分かった．これはアゾール耐性株の*atrR*破壊によって*cyp51A*および*cdr1B*のいずれも発現量が著しく低下したことによるものと考えられた．

なお，以上の得られた成果を取りまとめた論文を５月中旬に*PLoS Pathogen*誌に投稿した．

研究課題　’15－３

新興強毒性真菌*Cryptococcus gattii*の高病原性機序の免疫学的解析 ― その２

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Immunological analysis of a mechanism for high pathogenicity of *Cryptococcus gattii*

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研究成果

1999年にカナダのバンクーバー島で*Cryptococcus gattii*によるクリプトコックス症のアウトブレイクが発生し，その後アメリカ合衆国の北西沿岸地域を中心に拡大しつつある．2007年には，わが国でも国内感染と考えられる*C. gattii*によるクリプトコックス症例が報告され，その後も症例が増加している．通常の*C. neoformans*によるクリプトコックス症と異なり，健常者でも中枢神経感染症を発症し，その高い致死率から高病原性クリプトコックス症とも呼ばれており，今後新興感染症として重要な問題に発展することが懸念される．本研究では，*C. gattii*と*C. neoformans*に対する免疫応答性を比較することで，本感染症の病態解明の手掛かりを探ることを目的とした．

異なるクリプトコックス菌種での解析では抗原性の違いにより正確な解析が妨げられる可能性が懸念されるため，卵白アルブミン（OVA）遺伝子を導入することで両真菌種における共通抗原として発現させた*C. neoformans*株（YC-13, YC-11, H99）と*C. gattii*株（R265）を作製した．その中で，YC-13とH99にOVA遺伝子を導入した各１株（YC-13-OVA6, H99-OVA4）においてOVA mRNAの発現が検出され，YC-13-OVA6では感染マウスの所属リンパ節細胞がOVAの刺激によりIFN-γ産生を示した．また，OVA特異的なTCRを高発現したトランスジェニックマウス（OT-II）の脾細胞はYC-13-OVA6に反応してIFN-γを産生した．さらに，OT-IIマウスにYC-13またはYC-13-OVA6を感染させると，YC-13-OVA6感染マウスにおいて肺内のIFN-γ産生及び菌の排除が有意に亢進していた．一方，OT-IIマウス由来の脾細胞を抗原エピトープである$OVA_{323\text{-}339}$で刺激する際に，H99またはR265の破砕物を添加したところ，H99の破砕物でのみ有意なIFN-γ産生の増加が認められた．この違いは各菌種のDNAに起因しており，TLR9を介することが明らかとなった．さらに，H99由来のDNAはR265と比較して，骨髄由来樹状細胞からのIL-12産生をより強く誘導する結果が得られた．現在，両菌種でTLR9を刺激するCpG DNAのメチル化に相違がないか検討しているところである．

本研究を通して*C. gattii*感染症の高病原性における免疫機序が明らかになることにより，新たな治療・予防法の開発に繋がることが期待される．これらの成果は，第26回日本生体防御学会学術総会及び第44回日本免疫学会学術集会にて報告した．

研究課題　’15－４

*Cryptococcus neoformans*の特異な染色体末端維持機構を標的とした新規治療戦略の開発に向けて

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Towards development of novel therapeutic strategies targeting the specific regulation of telomere maintenance in *Cryptococcus neoformans*

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研究成果

*Cryptococcus neoformans*は環境に常在する担子菌酵母であり，主に免疫機能の低下した人に感染し重篤なクリプトコックス症を引き起こす日和見感染真菌として知られている．本菌は，環状プラスミドが維持できない，遺伝子ターゲティングの効率が悪く，導入された直鎖状DNA断片の末端に高頻度でテロメア反復配列が付加される，などDNA修復に関連するユニークな性質をもつことが明らかにされている（Edman, 1992）．本研究では，染色体末端の維持機構という観点から*C. neoformans*特有のゲノム維持機構を明らかにし，さらにこの特有なゲノム維持機構と*C. neoformans*の生活環との関連を明らかにすることを目的としている．

これまでに，*C. neoformans*よりテロメアDNA伸長酵素テロメラーゼ触媒サブユニットの相同遺伝子*CnEST2*を単離している．*C. neoformans*一倍体細胞で*CnEST2*の遺伝子破壊を行い，*CnEST2*遺伝子の細胞増殖における機能を解析した結果，本菌においても他生物種と同様，染色体末端の維持にはテロメラーゼの活性が必須であることが明らかになった．しかし，一倍体細胞での*CnEST2*破壊株の取得率は極めて低く，*CnEST2*遺伝子が増殖に極めて重要な役割を果たしていることが示唆された．*S. cerevisiae*など他の生物種ではテロメラーゼ遺伝子を欠損した後，数十回は正常に分裂を続けることが可能であることが知られている．我々の発見は，*C. neoformans*特有のゲノム維持機能にテロメラーゼが深く関与し，テロメラーゼの欠損が早期に増殖阻害をもたらす可能性を示唆している．

そこで本年度は，二倍体*C. neoformans*においてCnEST２欠損のヘテロ接合体を取得した．胞子形成により一倍体を取得し，テロメラーゼ欠損後どのタイミングで増殖が低下するかを確認したところ，テロメラーゼと相同組換え遺伝子との二重欠損株が取得できなかったのみならず，テロメラーゼ単独変異株の出現率も極めて低いことが示された．この結果により，テロメラーゼを抑制した条件で*C. neoformans*の増殖率が大幅に低下することが確認され，テロメラーゼを標的として*C. neoformans*特異的治療が可能であることが示された．

今後は，テロメラーゼ活性の制御に必要な分子の探索をさらに進めつつ，テロメラーゼ，組換え関連因子を含めたDNA末端修復機構の選択制の違いが生じる機構を分子の側から解析することで，この生物種の特異なDNA損傷修復のメカニズムと生理的意義を明らかにし，本菌に対する新規治療戦略の開発につなげたいと考えている．

研究課題　’15－５

病原真菌における一酸化窒素の合成機構と生理的役割の解析

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Analysis of synthetic mechanism and physiological role of nitric oxide in pathogenic fungus

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研究成果

一酸化窒素（NO）はシグナル分子として，哺乳類の幅広い生命現象に関与している．高木らは酵母*Saccharomyces cerevisiae*において，NOがアセチルトランスフェラーゼMpr 1およびフラボタンパク質Tah18依存的にアルギニンから合成され，酸化ストレス耐性に寄与することを見出した．また，Tah18と複合体を形成するDre2タンパク質が酸化ストレスセンサーとして働き，Tah18依存的なNO合成を制御する機構を提唱した．一方，病原真菌はヒトに感染する際，温度・低酸素などのストレスに応答して耐性を獲得し，病原性を示すことから，NOがストレス耐性や病原性に関与する可能性がある．

本研究では，*S. cerevisiae*と同様のNO合成経路の存在が示唆される病原真菌（*Candida glabrata, Cryptococcus neoformans, Aspergillus fumigatus*）について，NOと増殖・感染・病原性との関連性を解析する．平成27年度には，以下の研究成果が得られた．

１）*C. glabrata*：カイコ幼虫のテトラサイクリン転写抑制系を用いた*in vivo*必須遺伝子の判定法を開発し，Tah18のオルソログ遺伝子（*CgTAH18*）が*C. grabrata*の生育および病原性の発現に必須であることを示す結果を得た（知花，高木ら：論文投稿中）．

２）*C. neoformans*：プロモーター部位を改変してTah18のオルソログ遺伝子（*CnTAH18*）の発現抑制株を構築したところ，生育が抑制されたことから，*CnTAH18*は必須遺伝子であることが示唆された．

３）*A. fumigatus*：培養時に，過酸化水素添加や高温ストレス処理すると，NO特異的蛍光プローブによる染色が菌糸内に確認できたことから，これらのストレスに応答したNOの生成が示唆された．

今後，Mpr1，Tah18，Dre2のオルソログ遺伝子の破壊株，発現抑制株，高発現株を用いて，細胞内NOレベルを定量するとともに，カイコやマウスの感染実験によってNOがストレス耐性や病原性に及ぼす影響を多面的に評価する．

研究課題　’15－６

マイコウイルス由来タンパク質の異種発現システムを利用した病原性真菌の生育制御系の開発

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Development of novel regulation system for the human pathogenic fungi utilizing mycoviral proteins.

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研究成果

我々はイネいもち病菌に感染するマイコウイルス*Magnaporthe oryzae* chrysovirus, MoCV1-Aが宿主菌に対して，菌糸生育抑制，異常な色素沈着や分生子形成抑

制などの生育阻害現象をもたらすことを見出しており，MoCV1-Aウイルスの遺伝子がコードするタンパク質のうち，パン酵母*Saccharomyces cerevisiae*の遺伝子発現系の利用によりORF4が抗菌性タンパク質をコードすることを明らかにしてきた．また，メタノール資化酵母（*Pichia pastoris*）で産生させたORF4タンパク質をイネいもち病菌の分生子に添加すると菌糸の伸長抑制が見られることも示してきた．

本研究成果としてORF4タンパク質の部分領域をパン酵母に発現させることで，抗菌活性領域を調査したところ，MoCV1-Aの近縁ウイルス間で保存性の高い，中央部の領域（SUa領域）に活性があることが確認された．そこで，ピキア酵母の高密度培養によるSUaタンパク質の産生を試みている．さらに，パン酵母細胞内でのORF4タンパク質のC末端側領域（24領域）発現時に出現した，生育促進を示す変異株を利用した抗菌活性タンパク質産生法の検討中である．

病原真菌*Aspergillus fumigatus*はアスペルギルス症の主な原因菌である．五ノ井教授の研究グループにより，マウスに対する*A. fumigatus*の病原性を抑制する２種のマイコウイルスが見出されており，新規抗真菌薬としての応用を目指している．本共同研究プロジェクトにおいては，マイコウイルスの各ゲノムがコードするORFタンパク質（以下ORF）のうち，宿主菌の病原性抑制に関与するORFを特定するため，ウィルス未保有株*A. fumigatus*（KU株）にウイルス由来の各ORFをそれぞれ個別に強制発現させ，宿主の形態･生育速度･ストレス耐性などの表現型を比較した結果，生育不良が生じるORFが存在することが確認され，病原性の抑制に，マイコウイルス由来のタンパク質が関与する事が示唆された．

今後，*C. neoformans*に短縮など加工されたORF4タンパク質を発現させたり，異種発現させたORF4タンパク質を外部から作用させた時の生育抑制効果などを検討していきたい．またA. fumigatusに関しても，MoCV1-A由来のORF4タンパク質や，短縮配列であるSUa配列を発現させて，その生育阻害効果を評価する．

研究課題　’15－７

抗真菌薬標的タンパクのインシリコ予測と実験的検証

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An *in silico* prediction and experimental evaluation of antifungal drug targets

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研究成果

我々の研究分野は，ゲノム情報（*in silico*）の解析に焦点を絞り，実験研究者と連携することで生物学的な発見や医療などへの貢献を目的としている．近年，深在性真菌感染症は増加傾向にあるが，既存の抗真菌薬は４系統しか存在せず，副作用や耐性菌の問題が生じている．そこで，本研究課題では，新しい抗真菌薬の開発に貢献することを目的に，副作用が少なく，広域性の高い抗真菌薬の開発が期待できる分子標的の同定を目的としている．まず，パン酵母，分裂酵母を中心とする真菌の培地上での（*in vitro*）生育必須遺伝子情報を収集し，カンジダグラブラータのゲノムに含まれる相同遺伝子，1,160個を抽出した．それら1,160遺伝子について知花准教授が作製した遺伝子改変株を用いてカンジダグラブラータにおいて生育上必須か否かの評価を行ったところ，約900についての生育上必須であるとの判定が出された．これら900遺伝子についてヒト遺伝子との相同性解析を行った結果，192遺伝子が極めて低い相同性しか示さなかった．現在，これらの192遺伝子の改変株を用いてカイコあるいはマウスを用いた感染時（*in vivo*）必須遺伝子の評価実験を進めている．

研究課題　’15－８

Candida glabrata 細胞壁構築関連遺伝子欠損が菌体の性質に及ぼす影響

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Deletion effect of genes involved in cell wall integrity of *Candida glabrata*

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研究成果

真菌の細胞壁は環境に対する適応に必要なだけでなく病原性にも関与していることが指摘されている．そこで *C. glabrata* の細胞壁糖鎖生合成に関与する各種の遺伝子欠損株について細胞壁の構築，化学構造さらに病原性を含む性質を調べることにより，*ALG6*，*MNN2*，*MNN10*，*HOC1* の細胞壁構築への関与について解析した．マンナンの構造解析はアセトリシス，^{1}H NMR分析，メチル化分析により行った．細胞壁成分はアルカリ可溶性画分，酸可溶性画分，アルカリ・酸不溶性画分に分画し，キチンの定量はエルソン・モルガン法により行った．細胞壁グルカン層およびマンナン層の形態変化は透過型電子顕微鏡により解析した．また，病原性の変化はカイコへの感染実験により解析した．Δ*alg6* 株は細胞壁成分に親和性を示すCalcofluor White，Congo Red，SDSなどの薬剤に対する感受性，β-1,3-グルカナーゼ感受性が上昇していた．一方，micafunginに対する感受性は逆に低下し，キチン含量は野生株の２倍以上に増加していた．電子顕微鏡による細胞壁の解析でもマンナン層が薄く不鮮明になっていた．野生株と比較してマンナンの主鎖と側鎖から成るくし型構造に変化は見られなかったが，その分子量はかなり低下していた．一方，Δ*mnn2* 株では薬剤感受性に著しい変化は見られなかったが，マンナンの側鎖が失われα-1,6-結合マンノースからなる主鎖のみに変化していた．Alg6pは小胞体での糖タンパク質合成に関与しているが，Δ*alg6* 株は細胞壁へのストレスをシグナル伝達するMAPK（Slt2p）の活性化が起きていた．これはAlg6pの欠損で生じる小胞体ストレスが細胞壁の構築不全が起きており，その結果がSlt2の活性化に表れているものと考えられる．カイコを用いた感染実験でも野生型と比較してΔ*alg6* 株では病原性が低下していたが，復帰株では野生株と同等の病原性を示したことから，細胞壁の強度の維持は病原性に関与すると考えられる．この細胞壁構築不全に関連する遺伝子は，抗真菌薬の新たなターゲットとなる可能性を示唆している．

研究課題　’15－９

ゲノム情報を利用した*Aspergillus niger*及び醸造黒麹菌のアレルゲン遺伝子の同定

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Identification of allergen genes of *Aspergillus niger* and its related koji molds by using of their genomic information

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研究成果

これまでの千葉大学真菌医学研究所共同研究の成果として，カビアレルゲンに特化したデータベースを作製していた．麹菌，白麹菌，*A. niger*のゲノム情報を入手し，作製したカビアレルゲンデータベースとの相同性検索を行い，同データベースに追加した．相同性検索の結果，10種の既知カビアレルゲン遺伝子と類似のアレルゲン候補遺伝子が，上記麹菌等にあることを明らかにした．複数種の*Aspergillus* 属菌からゲノムDNAおよびRNAを抽出した．RNAはcDNA化し，DNAと共に次世代シークエンシンサーによるゲノムおよび発現遺伝子解析を行った．その成績をカビアレルゲンデータベースと照合することによって，実験を行わずに，アレルゲン遺伝子を選抜できるか検討した．*Aspergillus*属中に，未同定であるが，現在までに報告されているアレルゲン遺伝子と高度に相同性のある遺伝子が10種以上検出された．cDNAの次世代シークエンシングでは，その塩基配列の解析結果が直ちに遺伝子のクローニングに有効だった．合計６種のアレルゲン候補遺伝子の全長のクローニングと，発現ベクターへの導入，大腸菌における組換えアレルゲンタンパク質の発現が確認された．６種の内，可溶性タンパク質としてペルオキシゾーム局在性のタンパク質が精製された．同組換えアレルゲンタンパク質は，アレルギー性気管支肺アスペルギルス症患者血清中のIgE抗体と結合した．同組換えアレルゲンの接種は，マウスにIgE抗体産生を誘導した．以上の成績は，カビアレルゲンの同定に，従来の粗アレルゲンと患者血清との反応，粗アレルゲンの精製・アミノ酸配列解析・遺伝子クローニング，さらに組換えタンパク質の調製という過程を経ずにカビアレルゲンが同定できることを示し，カビアレルゲンの同定にゲノム情報を利用する有効性が確認された．

研究課題　’15－10

千葉大学が保有する化合物ライブラリーを用いた抗真菌薬シーズ開発

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Development of antifungal seeds from chemical compound library owned by Chiba University

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研究成果

千葉大学が保有する1,000種類のオリジナル合成化合物のうち450種類の化合物について，一次スクリーニングを終了した．その結果，６種類の化合物について*Candida albicans*, *C. glabrata*, *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，１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，特許出願を行った．また，日本化学療法学会のこれを元に製薬企業に対して抗真菌薬の共同研究を提案し，平成28年度に共同研究契約を締結することになった．平成28年度は550種類の化合物について，これまでと同様に，シーズ候補のスクリーニングを進めると共に，化合物の作用機序の解明に取組んで行くことにした．

研究課題　’15－11

新規ユビキチンリガーゼSCFUcc1によるカンジダ・グラブラータの感染制御機構の解明

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Functional analysis of a novel ubiquitin ligase complex, SCFUcc1, in the virulence of *Candida glabrata*

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研究成果

微生物や植物に特有の代謝経路であるグリオキシル酸回路は，病原性酵母の感染や植物の種子の発芽において必須の役割を果たすことが知られている．これらの生物はグルコースが不足すると酢酸や脂肪酸を材料にグリオキシル酸回路→クエン酸回路→糖新生という一連の代謝反応によりグルコースを合成する．これまで，代謝酵素の転写を介したグリオキシル酸回路の活性化，不活性化の機構は報告されていた．我々は本研究において，ユビキチン・プロテアソーム系を介した代謝酵素の分解によるグリオキシル酸回路の制御機構を見出した．グリオキシル酸回路において最初の反応を触媒するクエン酸合成酵素Cit2は，グルコースが豊富にあるとユビキチンリガーゼ複合体SCF^{Ucc1}によるユビキチン修飾を受け，プロテアソームによって分解された．また，グルコースが不足して酢酸や脂肪酸からグルコースを合成する必要が生じると，SCF^{Ucc1}はCit2をユビキチン化できなくなり安定化したCit2がグリオキシル酸回路を活性化した．この研究は，代謝経路の活性が代謝酵素の分解により制御されるというユニークなスイッチング機構を明らかにしたものと言える．現在，*Candida glabrata*の感染におけるSCF^{Ucc1}ホモログの役割について，in vivo解析を進めた．

まず，カイコ幼虫の体液中に菌体を感染させ生存率を比較した結果，Cit2の欠損株は野生株と比較して有意差が得られなかった．次にマウスのマクロファージを用いた貪食実験系を確立させ，現在本実験を用いたCit2の機能解析を進めている．

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研究課題　’15－12

真菌感染におけるペア型受容体を介した生体防御機構の解明

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Host defense mechanism by paired immune receptors in fungal infection

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研究成果

ペア型受容体は，細胞外ドメインが非常に相同性の高い活性化型と抑制化型のペアからなる受容体であり，主に自然免疫系の細胞に発現している．代表者は，ペア型レセプターの一つである活性化レセプターLILRA2が細菌によって壊された免疫グロブリンをリガンドとして認識することを発見した．しかしながら，真菌感染におけるLILRA2の役割は明らかではなかった．本研究課題において，病原真菌カンジダアルビカンスから分泌されたプロテアーゼによって分解された免疫グロブリンもLILRA2を介した生体防御機構を活性化することを明らかにし，これまでの成果と合わせて論文発表を行った．

研究課題　’15－13

ショウジョウバエ感染系を用いた真菌病原性発現機構のゲノムワイド解析

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Genome-wide analysis of fungal virulence mechanism using Drosophila infection system

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研究成果

日和見感染症に代表される感染症を引き起こす病原真菌の病原性発現機構は，依然として不明であり，大きな脅威となっている．真菌医学研究センター知花准教授は，*Candida glabrata*を用いて，5249遺伝子を組換えた変異体ライブラリーを作成している．このリソースを用いると，病原性の発現に関わる遺伝子をゲノムワイドに探索できる．しかしながら，マウスなどの哺乳動物を宿主として用いて網羅的に解析することは，現実的に不可能である．一方，自然免疫研究によく利用されているショウジョウバエは，生活環が短く網羅的解析に優れている．また，すでに確立されている自然免疫変異体を用いることができる．そこで，本研究では，センターの有する真菌のリソースを利用した，ショウジョウバエでの解析から病原真菌の病原性発現機構の解明に迫ることにした．

そのために，昨年度の本共同利用・共同研究（採択番号14-11）で確立した易感染ショウジョウバエ系統を用いた網羅的感染実験系を用いて，倍地上での生育が正常な2,026の遺伝子欠損株を解析した．合計７万匹以上のショウジョウバエを用いた四次スクリーニングまで行い，病原性が低下した57株同定した．さらに，このうち14株において，ショウジョウバエ体内での菌の増殖を調べ，宿主体内での菌の増殖と，病原性の低下との関係を調べた．その結果，14株中11株では，宿主体内での増殖能が低下していたが，３株では野生型と同等の増殖能を示した．本菌において，このような病原因子を示唆する報告は未だない．今後，これら３株において，宿主体内で増殖しているにもかかわらず，病原性が低下する理由を明らかにすることで，病原真菌の病原性発現機構の本質を明らかにすることが期待できる．

研究課題　’15－14

感染に応答した自然免疫誘導の分子機構の解析

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Innate immune responses against pathogen infection

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研究成果

高等脊椎動物における抗ウイルス自然免疫において，RIG-I-like受容体（RLR）は細胞内に侵入した非自己RNAを検知するセンサー分子として必須な役割を担っている．本研究は，最近明らかにした細胞内ストレス顆粒（SG）の形成を介したRLRシグナル誘導の分子機構について解析することを目的とした．本年度は，これまでのSGについての共同研究を進め，New castle disease virus（NDV）感染に応答したRIG-Iの挙動とSG形成について詳細に解析した．その結果，NDV感染細胞内では，まずRIG-IはNDVウイルスの増殖複合体に集積することで少量のI型インターフェロン産生を誘導すること，またそのあと遅れて形成されてくるSGへ移動することによりSGを足場としてさらに強力なインターフェロン産生を誘導すること，さらに，この時NDV由来の５’末端にキャップ構造を持たないリードスルーRNAがSGに共局在しており，このRNAが非自己としてRIG-Iにより検知されていることを明らかにした．

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研究課題　’15－15

真菌核酸に対する自然免疫応答の解析

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Recognition of fungal nucleic acids by pattern recognition receptors

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研究成果

本研究では，ウイルスや真菌由来のRNAに対する自然免疫応答惹起のメカニズムを明らかにすることを目指した．そこで，自然免疫によるRNA認識機構の詳細をさらに理解するため，I型インターフェロン遺伝子の発現誘導可能な分子の発現スクリーニングを行い，Hu Antigen R（HuR）を得た．HuRはRNA結合ドメインを

持つタンパク質であり，AUリッチ配列をもつmRNAの安定化に寄与することが知られている．そこで，ゲノム編集技術を用いてHuRを欠損するマウスマクロファージRAW264.7細胞を樹立した．合成二本鎖RNAアナログPoly（I:C）に対する応答を解析したところ，HuR欠損細胞ではインターフェロンβやケモカイン遺伝子の発現が野生型細胞と比べ顕著に限弱していた．また，HuRは未刺激状態では核に局在しており，Poly（I:C）刺激により細胞質内に移行し顆粒状の局在を示した．このドットはストレス顆粒であることが示されている．ストレス顆粒は，ストレス刺激やウイルス感染によって形成される細胞質内顆粒であり，mRNA, RNA結合蛋白質，リボゾーム等が含まれており，ストレス時における翻訳抑制に関与すると考えられている．ウイルスRNAもストレス顆粒に局在することが報告されており，病原体のRNAがここで認識されることが示唆される．今後，HuRの細胞内局在の詳細や結合するRNAの同定を通して，真菌やウイルスに対する防御機構におけるHuRの役割を明らかにしていく必要がある．

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研究課題　’15－16

遺伝子サイレンシングと自然免疫反応のスイッチング機構の解析

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Switching mechanism between gene silencing and innate immune response

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研究成果

近年，ゲノムからはタンパク質をコードしないノンコーディングRNAが多く転写されることが明らかになっている．ノンコーディングRNAの中でもsmall interfering RNA（siRNA），microRNA（miRNA），PIWI-interacting RNA（piRNA）といった内在性の小分子２本鎖RNAは，遺伝子サイレンシングという塩基配列特異的な遺伝子抑制機構によって，広く多様な生命現象を制御している．一方，ウイルスは，ほぼすべての生物に感染する病原体であり，生物はウイルス感染に対するさまざまな防御機構を発達させてきているが，哺乳類特異的な現象として，ウイルスなどの外来性２本鎖RNAにより惹起される抗ウイルス応答反応が存在する．これら２つの機構は，共通にRNAによって惹起される機構であるにも関わらず，相互の関連は不明である．申請者らは，両方の経路に共通に関わる因子としてTAR-RNA binding protein（TRBP）を同定している．本研究では，TRBPの機能を足がかりに，これら２つの経路のクロストークやスイッチングの分子機構を明らかにすることを目指している．

RNAサイレンシングでは，小分子RNAがArgonauteと呼ばれるタンパク質へ取り込まれて，初めてその遺伝子抑制機能を発揮する．TRBPは小分子RNAのArgonauteへの取り込みを促進してRNAサイレンシン

グ活性を促進する．米山らはウイルス感染を細胞質内で感知するウイルスセンサータンパク質としてRIG-I, MDA-5, LGP2を発見している．TRBPは，これらのうちLGP2と直接相互作用するが，RIG-IやMDA5とは相互作用しないことが明らかになった．そこで，これらのウイルスセンサータンパク質のRNAサイレンシング効果に対する影響を，それらのノックダウンによるレポーターアッセイや強制発現系をもちいて検討したところ，RIG-IとLGP2はRNAサイレンシング活性を抑制する作用があるが，MDA5には抑制作用はないことがわかった．さらに，小分子RNAとTRBPやウイルスセンサータンパク質との相互作用をゲルシフトアッセイで解析した結果，TRBPだけでなく，RIG-IとLGP2も小分子RNAと結合するが，MDA5はほとんど結合しないことが明らかになった．RNAサイレンシング活性とsiRNA結合活性の結果は，RIG-IとLGP2のsiRNAへの結合が遺伝子サイレンシング活性を何らかの形で制御している結果と推定された．今後は，このような遺伝子サイレンシング活性制御の機構とTRBPとの相互作用の関連を明らかにすることで，２つの経路のクロストークの機構を解明する予定である．

研究課題　’15－17

同種造血幹細胞移植後のCandida感染による移植免疫反応の修飾

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α-Mannan induces Th17-mediated pulmonary graft-versus-host disease in mice

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研究成果

同種造血幹細胞移植後の真菌感染症は，しばしば重症化し致命率も高い．また，移植患者で感染症とならんで危険な合併症である移植片対宿主病（GVHD）は，ドナーの移植片に混入したTリンパ球がレシピエントの細胞を攻撃する難治性の疾患である．GVHDは細菌感染によって増悪をきたすことが知られているが，真菌感染症がGVHDに及ぼす影響は不明であった．そこで，本研究ではマウスモデルを利用して真菌感染症がGVHDに及ぼす影響を解明し，移植後の真菌感染症予防の意義について再検討することを目的とした．

まず，マウスGVHDモデルに*C. albicans*死菌，あるいはその細胞壁構成糖鎖であるα-マンナンを投与したところ，病態は有意に悪化し，特に肺で顕著であった．そのメカニズムを解析するため，肺に浸潤している炎症性細胞を調べたところ，α-マンナンを投与したレシピエントマウスで，ドナー由来T細胞が顕著にTh17細胞への分化していることが明らかとなった．また，このTh17細胞分化の亢進は，レシピエントマウスのDectin-2を介したものであった．これらの結果から，同種造血幹細胞移植後の真菌感染症は，単に感染症として問題になるばかりではなく，肺におけるGVHDの悪化要因として重篤な病態を引き起こしている可能性が示唆された．

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研究課題　’15－18

カンジダ感染に対するIL-17A/Fを介した皮膚真菌症防御機構の解明

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IL-17 is pivotal for host defense against epicutaneous candidiasis

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研究成果

Candida albicans（*C. albicans*）は，健常人においては皮膚や粘膜に常在真菌として定着し，通常は症状を起こすことはない．一方，多湿や不衛生，抗生剤投与による菌交代現象などにより，主に間擦部に紅斑，鱗屑，小膿疱，びらんなどを生じる．ヒトの慢性皮膚粘膜カンジダ症においてはDectin-1, CARD9, STAT3, IL-17レセプター遺伝子など複数の遺伝子異常が報告され，これらの分子を介した生体防御機構が*C. albicans*排除に重要であることが示唆される．しかしながらマウスの経皮感染モデルは確立されておらず，その詳細な分子機構は不明である．そこで本研究では，１）経皮感染マウスモデルの作成，２）各種ノックアウトマウスを用いた感染防御機構の解明，を目的とし研究を開始した．まず，マウスの背部を剃毛し，一定量の*C. albicans*懸濁液に浸したガーゼを貼付・密封したところ，Day2をピークに感染局所で好中球の浸潤を伴う炎症が認められ，Day7で*C. albicans*の排除と炎症の収束が認められ，*C. albicans*経皮感染モデルの確立に成功した．そこで，IL-17のノックアウト（KO）マウスを用いて同様の実験を行ったところ，Day7でも*C. albicans*が排除できず，皮膚における感染防御にIL-17が必須であることが示された．さらに，IL-17の産生細胞を探索したところ，これまで考えられてきたGroup3 innate lymphoid cells（ILC3）とγδT細胞であることが明らかとなった．これらの結果から，皮膚ではTh17細胞ではなく，ILC3とγδT細胞から分泌されるIL-17が感染防御に必須であることが示された．

研究課題　’15－19

肺炎球菌認識と感染防御におけるDectin-２の役割に関する研究

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Role of Dectin-2 in the host defense to pneumococcal infection

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研究成果

肺炎球菌は市中肺炎の原因細菌として最も頻度が高い．特に，高齢者や慢性心肺疾患，糖尿病などの基礎疾患を有する患者では重症化することが多く，高齢化社会のわが国では重要な問題となっている．これらのハイリスク患者には莢膜多糖ワクチンが用いられ一定の予防効果を上げてきたが，胸腺非異存性抗原であるため臨床効果に十分でない点も存在し，より有効なワクチンの開発が望まれている．肺炎球菌は，真菌と同様に多糖に富む莢膜を有しており，免疫細胞による認識に何らかのCタイプレクチン受容体が関与している可能性が予想される．

本研究では，マウス肺炎球菌肺炎モデルを用いることで，本細菌に対する感染防御へのDectin-2の役割について，Dectin-2遺伝子欠損（KO）マウスと野生型コントロール（WT）マウスを比較検討することで解析した．WTマウス，Dectin-2KOマウスの肺内に血清型３型の肺炎球菌を感染させたところ，KOマウスではWTマウスに比べて生存期間が短縮し，３日後の肺内菌数が有意に増

加した．Dectin-2KOマウスでは，好中球の感染局所への集積の異常はみられなかったが，好中球による菌の貪食が有意に低下した．一方，肺炎球菌のオプソニン化に重要と考えられる血清や気管支肺胞洗浄液中の莢膜多糖特異的なIgG量がDectin-2KOマウスではWTマウスと比べて有意な低下，もしくは低下傾向を示した．また，肺炎球菌培養上清による骨髄由来樹状細胞（BM-DCs）からのIL-12p40産生が，Dectin-2KOマウスではWTマウスに比べ有意に低下していた．肺炎球菌由来成分のうち，ConA-sepharose 結合性物質がBM-DCsのDectin-2によって認識され，IL-12p40産生を誘導した．以上の結果から，Dectin-2による肺炎球菌多糖の認識が，感染局所への好中球動員よりも好中球による貪食機能に関与することで，感染防御において重要な役割を担うことが明らかになった．

本研究を通して免疫系による肺炎球菌の認識機構が明らかになることで，より有効なワクチンの開発に繋がることが期待される．

研究課題　’15－20

肺炎球菌混合感染における*Aspergillus fumigatus*バイオフィルム産生についての研究

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Studies on biofilm formation by *Aspergillus fumigatus* co-cultured with *Streptococcus pneumoniae*

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研究成果

ヒトの病原体となる細菌の多くが，ヒト体内において定着しバイオフィルムを形成すると，化学療法や宿主防御機構に対する抵抗性が増強することが知られている．近年，真菌においても*Aspergillus fumigatus*や*Candida albicans*がバイオフィルムを形成することが報告された．感染症は，単独の病原体だけでなく，複数の病原体によるもの（混合感染，重複感染）もあり，複数の病原体による場合は，異なる病原体の間で相互作用が起き，重症化の可能性もある．臨床例として，*Streptococcus pneumoniae*と*A. fumigatus*の重複感染が報告されている．本研究では，未だ知見の乏しい*S. pneumoniae*と*A. fumigatus*の混合バイオフィルムの構造・性状について解析した．

*S. pneumoniae*と*A. fumigatus*を24ウェルプレートにおいて同時にバイオフィルム形成開始させると，24時間後では既に*S. pneumoniae*のみからなるバイオフィルムが観察された．予め24時間形成させた*A. fumigatus*バイオフィルムに*S. pneumoniae*を加えると，バイオフィルムの減少および菌糸の減少・切断が観察された．この現象は，*A. fumigatus*のバイオフィルム産生を増強させる血清糖タンパク質fetuinを添加しても観察された．*S. pneumoniae*培養上清を*A. fumigatus*バイオフィルムに加えると，バイオフィルムの減少および菌糸の減少・切断が見られたが，培養上清を熱処理すると影響は見られず，*S. pneumoniae*培養上清中の熱感受性因子が*A. fumigatus*バイオフィルム破壊に関与することが示唆された．

今後は，*S. pneumoniae*培養上清中の*A. fumigatus*バイオフィルム破壊因子の同定を行う予定である．

研究課題　’15－21

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

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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（菌球）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．平成27年度では，分泌蛋白質と予測される*A. fumigatus*の５遺伝子について血清刺激によるバイオフィルム形成に及ぼす影響を検討した．また，Cas9/CRISPRゲノム編集技術を*A. fumigatus*に導入した．

ハイグロマイシン耐性マーカーを含むカセットDNAを*A. fumigatus* AfS35株に形質転換し，５つの分泌蛋白質をコードする遺伝子の破壊株をそれぞれ作製した．親株では血清添加によってバイオフィルム形成量が増加するが，遺伝子破壊株ではその増加は観察できなかったことから，５つの分泌蛋白質が血清刺激に応答したバイオフィルム形成に関与することが示唆された．今後，血清刺激に応答したバイオフィルム形成におけるシグナル伝達機構の役割を解明していく．

今後の新規遺伝子探索に応用するために，幅広い生物種で用いられているCas9/CRISPRゲノム編集技術を*A. fumigatus*で利用可能にした．ゲノム上の標的部位の20塩基を含むsgRNAとCas9蛋白質を一度の形質転換で導入するためのプラスミドを構築した．このシステムを用いることにより，ゲノムの特定の部位を切断し，変異導入などの編集を行うことが可能になる．今後，この技術を用いて，血清刺激に応答する新しい遺伝子群の探索に応用したい．

研究課題　’15－22

動物感染モデルを用いたボリコナゾール局所投与による真菌症治療に向けた基礎研究

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Effects of local administration of voriconazole onto mycosis in animal infection model

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研究成果

ボリコナゾール（VRCZ）はカンジダやアスペルギルス症などの真菌感染症に対して広く用いられる抗真菌薬である．有効性が高く，広く利用されることが期待される反面，治療域が狭く薬物動態の個体間あるいは個体内の変動が大きいことから治療濃度を管理するのが難しい薬物である．本研究ではアスペルギルス膿胸に対し，VRCZを胸腔内投与することにより，胸腔内でのVRCZ濃度並びに全身での血中濃度がどのように変動するか，

また胸腔内投与による治療効果を検討することを目的とした．

まず，千葉大学医学部附属病院における患者症例を検討した．71歳，アスペルギルス膿胸の患者に対し，VRCZ200mg/回，１日２回を静脈内で投与したが，血中濃度は1.45mgと目標濃度に到達しなかった．そこで，胸腔内にVRCZ200mgを投与し，血中濃度の推移を検討したところ，12時間後濃度は一過性に1.69mgまで上昇したが，24時間後では再び1.53mgと低下した．そこで，肺ドレーンを留置し，週２～３回繰り返し投与することとし投与量も300mg/回，400mg/回と増量したところ，血中濃度は5.63mgまで上昇した．血中濃度が中毒域となったため，300mg/回に戻して治療した．B-D-グルカン値は開窓術を行ったDay51には陰性化し，その後患者は回復し退院することができた．以上のことから，アスペルギルス膿胸に対するVRCZの胸腔内局所投与は臨床上有効な治療法であることが示唆された．

なお，マウスでのアスペルギルス膿胸のモデル作成を試みたが，安定した胸腔内注入モデルの作製に手間取り，データを得るに至らなかったため報告書には記載しなかった．今後，動物実験感染モデルを確立し，投与量や投与スケジュールについて詳細な検討がなされることが期待される．

研究課題　’15－23

侵襲性感染症由来インフルエンザ菌の病原因子に関する研究

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Pathogenesis of Haemophilus influenzae isolated from patients with invasive disease

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研究成果

インフルエンザ菌ｂ型ワクチン導入後に分離された小児侵襲性感染症由来インフルエンザ菌株を収集しPCR法で血清型解析を実施した．血清型解析で無莢膜株と判定した28株について，臨床背景，薬剤感受性，生物型，MLST解析，バイオフィルム産生能，付着因子について検討を行った．臨床背景として発症年齢が新生児から学童まで幅広い年齢層に認められた．基礎疾患を有する症例からの分離例が多く，全体の42.9％を占めた．３例が死亡しており，予後不良例も認めた．薬剤感受性は良好な株が多かったが，２株がβラクタマーゼ産生クラブラン酸アモキシシリン耐性株であり，今後臨床的に問題となる可能性が示唆された．ST型は28株中26株が異なっており多様であった．生物型はⅡ型が最も多くついでⅢ型であり，インフルエンザ菌ｂ型株とは異なっていた．バイオフィルム産生能，付着因子保有状況は多様であり，呼吸器から分離される無莢膜株との大きな違いは認めなかった．

研究課題　’15－24

天然物を素材とした新規抗感染症薬リード化合物の獲得

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Development of lead compound for new anti-infective agent from natural resources

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研究成果

沖縄で採取したVerongida目の海綿から，単離した新規ブロモチロシンアルカロイドTyrokeradine GおよびHを単離，構造決定した．Tyrokeradine GおよびHは*Aspergillus niger*に対して穏やかな抗真菌活性を示した．

Tyrokeradine G は *Cryptococcus neoformans* に対しても穏やかな抗細菌活性を示した．

また，沖縄で採取した *Hyrtios* 属の海綿から，単離したアゼピノインドール骨格を持つ新規ビスインドールアルカロイド Hyrtinadine B および C を単離，構造決定した．Hyrtinadine B は，*Aspergillus niger* に対して穏やかな抗真菌活性を示した．Hyrtinadine C は *Escherichia coli* および *Bacillus subtilis* に対して穏やかな抗細菌活性を示した．

今後も，顕著な抗菌活性を示す新たな天然物の探索を継続して行う予定である．

発表論文

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研究課題　’15－25

Aspergillus fumigatus 関連種における臨床株と環境株の比較

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Studies on comparison between clinical and environmental isolates on relatives of *Aspergillus fumigatus*

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研究成果

アスペルギルス症主要原因菌である *Aspergillus fumigatus* のアゾール薬に対する耐性株の増加は世界的に問題視されているが，日本国内においてはこれまでの所，耐性株は殆ど発見されていないのが現状である．一方，*A. fumigatus* の関連種は国内の多くの臨床分離株において，アゾール薬に対して耐性があることが知られている．しかし，国内の環境中から関連種の正式な分離報告はこれまでになく，その分布の実態は解明されていない．そこで，国内各地の土壌から関連種の分離を試み，その遺伝子型および薬剤感受性を解析し，耐性株の分布の実態把握を行った．この結果，*A. fumigatus* に比べ低頻度ではあるが南北問わず幅広い地域の土壌から *A. fumigatus* 関連種である *A. lentulus*, *A. udagawae*, *A. viridinutans* を分離することができ，国内の自然環境中にこれら関連種が生息していることが確認された．また，薬剤感受性は，*A. lentulus*, *A. udagawae* においては臨床および環境分離株の多くにおいて ITCZ に耐性があるのに対して，*A. viridinutans* は臨床株，環境株とも多くの株において ITCZ, VRCZ に耐性みられた．これらの耐性は環境株，臨床株とも同程度であることから，自然耐性と推定される．

研究課題　’15－26

白癬菌が産生・分泌するプロテアーゼによる表皮角層ケラチン分解様式に関する形態学的解析

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矢口貴志・田中玲子
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Morphological analyses of keratin degradation mechanisms by dermatophytes

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Takashi Yaguchi, Reiko Tanaka
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研究成果

白癬の原因菌である白癬菌による表皮角化組織への侵入様式は今なお不明な点が多いが，タイプの異なるプロテアーゼ群を段階的に利用した酵素的分解が組織への菌の侵入初期の重要なプロセスであると長年考えられてきた．申請者らは，角化組織の酵素的分解への関

与が指摘されているメタロプロテアーゼ４（MEP ４），ロイシンアミノペプチダーゼ２（Lap ２）およびジペプチジルペプチダーゼ４（DPPIV）について，角化組織への菌の侵入様式との関連性を解析した．白癬菌*A. vanbreuseghemii*をホストに用いて，上記プロテアーゼ遺伝子の一重および多重破壊株を作出し，（ヒト由来の）爪を唯一のタンパク質源とする最小寒天培地（簡易的*in vitro*爪白癬モデル）を用いて感染実験を行った．しかし，すべての一重破壊株とホスト株との間で爪への感染（増殖）速度の差が認められなかった他，光学顕微鏡観察においても形態学的差異は認められなかった．また，ホスト株およびMEP4一重破壊株の菌糸で覆われた爪の走査型電子顕微鏡観察を行ったところ，爪表面に見られる隙間を利用して菌糸が組織内部に伸長していることが判明した．さらに，作出に成功したMEP ４/DPPIV二重破壊株，そして別途作出したゲノム上に５個あるMEP遺伝子を全て破壊した五重破壊株においても同様の解析結果が得られた．

以上のことから，酵素的分解に基づく表皮角化組織への白癬菌の侵入様式に対し，MEPファミリー，Lap2およびDPPIVの関与の可能性は低いと考えられる．また，ホスト株およびMEP4一重破壊株感染爪の走査型電子顕微鏡観察結果から，酵素的分解よりもむしろ組織表面の物理的なスペース（細胞間の隙間，傷など）が角化組織内への菌の侵入を促す重要な要素になっているのではないかと考えられる．

感染症研究グローバルネットワークフォーラム2015

The 4th Global Network Forum on Infection and Immunity

共催：千葉大学真菌医学研究センター 共同利用・共同研究拠点事業『真菌感染症研究拠点』，東京大学医科学研究所 細菌感染生物学社会連携研究部門・特別推進研究『病原細菌の自然免疫克服戦略の解明とその応用』

研究成果

第４回目となる感染症研究グローバルネットワークフォーラムを開催した．当初，千葉大学内の感染症研究のネットワーク化を目指して始まったこのフォーラムであるが，毎年その規模を拡大し，日本の代表的研究者のみならず，世界各地の研究者が集う極めて高レベルの会となっている．本年度は医学部附属病院感染症内科/感染制御部の猪狩部長を代表世話人として下記のようなプログラムに基づき，平成27年11月14日に医学部記念講堂で開催された．当日の参加者は学外を含め，87名であり，活発な討議が交わされた．

日時：平成27年11月14日（土） 9：30～16：40
場所：千葉大学医学部記念講堂

猪狩英俊（千葉大学医学部附属病院 感染制御部　部長）
亀井克彦（千葉大学真菌医学研究センター　教授）
笹川千尋（千葉大学真菌医学研究センター長，東京大学名誉教授）

【午前の部】

【開会の挨拶】

徳久剛史（千葉大学学長）

座長：野村文夫（千葉大学医学部附属病院　特任教授）
谷口俊文（千葉大学医学部附属病院 感染制御部　助教）
『米国におけるHIV診療最前線』

座長：羽田　明（千葉大学大学院医学研究院　教授）
加藤誠也（公益財団法人 結核予防会結核研究所　副所長）
『結核をめぐる国際的な課題と国内の課題について』

座長：山本友子（千葉大学真菌医学研究センター　特任教授）
松本智成（一般財団法人 大阪府結核予防会 大阪病院 診断検査部長）
『結核菌のゲノム解析』

座長：巽浩一郎（千葉大学大学院医学研究院　教授）
伊藤功朗（京都大学医学部附属病院 呼吸器内科学　助教）
『COPDと感染症』

座長：猪狩英俊（千葉大学医学部附属病院 感染制御部 部長）
加藤康幸（国立研究開発法人 国立国際医療研究センター 医長）
『エボラ出血熱など輸入感染症について』

【午後の部】 International Forum

Chair: Yasuyuki Kato（Medical director, National Center for Global Health and Medicine）
Cevayir Coban（Professor , IFReC, Osaka University）
“Tissue-specific immunopathology during malaria”

Chair: Mitsutoshi Yoneyama（Professor , Medical Mycology Research Center, Chiba University）
Gordon Brown（Professor , The Institute of Medical Sciences, University of Aberdeen, UK）
“C-type lectins: key players in antifungal immunity”

Chair: Naoki Shimojo（Professor , Graduate School of Medicine, Chiba University）
Yoshiyuki Goto（Associate Professor , Medical Mycology Research Center, Chiba University
Visiting Lecturer, The Institute of Medical Science, The University of Tokyo）
“Innate lymphoid cells regulate intestinal epithelial barrier

system against bacterial infection"

Chair: Kiyoshi Hirahara (Associate Professor, Graduate School of Medicine, Chiba University)
Yumiko Imai (Professor, Graduate School of Medicine, Akita University)
"Dynamic nuclear interactions between influenza virus and its host"

【Closing Remarks】

Chihiro Sasakawa (Director, Medical Mycology Research Center, Chiba University
Emeritus Professor, The University of Tokyo)

2016年講演会

2016 Scientific Meetings & Seminars

「感染症研究グローバルネットワークフォーラム 2016」
"The 5th Global Network Forum on Infection and Immunity"
（千葉大学真菌医学研究センター 共同利用・共同研究拠点「真菌感染症研究拠点」，千葉大学大学院医学研究院 小児病態学 共催）

日時：平成28年11月12日　9：30～17：00
場所：千葉大学附属病院３階　ガーネットホール

【午前の部】
【特別講演】
金井　隆典
（慶應義塾大学医学部 消化器内科　教授）
『腸内細菌が教える「医と食」の融合研究』

長澤　耕男
（国立感染症研究所 感染症疫学センター　任期付研究員）
『Human respiratory syncytial virus（HRSV）F遺伝子の分子進化』

大塚　岳人
（新潟大学医学部 小児科学　助教）
『アンチセンス療法：新しい抗微生物薬とその応用』

松岡　悠美
（千葉大学大学院医学研究院 皮膚科学　助教）
『アトピー性皮膚炎および健常者における黄色ブドウ球菌ゲノム進化と生着のメカニズム』

【午後の部】International Forum
Trinad Chakraborty
（Professor, Institute of Medical Microbiology, Biomedical Research Centre Seltersberg, Justus-Liebig-University, Giessen, Germany）
『Exploring the One Health paradigm with antimicrobial resistance: Insights from genome-based epidemiology』

Yasushi Kawaguchi
（Professor, The Institute of Medical Science, The University of Tokyo）
『Strategies of herpesviruses to hijack host cell machinery』

Tomoyoshi Nozaki
（Director, National Institute of Infectious Diseases）
『Unique evolution of mitochondria and metabolism in the human enteric parasite』

Eric G. Pamer
（Head, Memorial Sloan Kettering Cancer Center, New York, USA）
『Microbiota-mediated defense against intestinal infection』

「東京大学医科学研究所―千葉大学真菌医学研究センター　共同利用・共同研究拠点事業　平成27年度 合同成果報告会」

日時：平成28年３月15日（火）
　　13：00～16：50（午後の部）
場所：東京大学医科学研究所 ２号館２階 大講義室

【午後の部】
【特別講演】
荒瀬　尚
（大阪大学微生物病研究所　教授）
『ペア型レセプターを介した宿主病原体相互作用』

【合同成果報告会】
《千葉大学真菌医学研究センター成果報告》

中務　邦雄
（名古屋大学大学院　講師）
『新規ユビキチンリガーゼSCFUcc１ によるカンジダ・グラブラータの感染制御機構の解明』

倉田　祥一朗
（東北大学大学院　教授）
『ショウジョウバエ感染系を用いた真菌病原性発現機構のゲノムワイド解析』

程　久美子
（東京大学大学院　准教授）
『遺伝子サイレンシングと自然免疫応答のスイッチング機構の解析』

西城　忍
（千葉大学真菌医学研究センター　准教授）
『カンジダ感染に対するIL-17A/F を介した皮膚真菌症防御機構の解明』

《感染症・免疫共同研究領域》

朴　恩正
（三重大学大学院　准教授）
『免疫系と血栓・凝固系とのクロストークによる臓器特異的ホーミングの制御』

丸山　史人
（京都大学大学院　准教授）
『A群レンサ球菌における病原因子獲得機構の時空間的解析』

今村　健志
（愛媛大学大学院　教授）
『革新的in vivo 光イメージングを駆使した粘膜免疫機構における細胞動態とシグナル可視化』

西田　圭吾
（鈴鹿医療科学大学　准教授）
『細菌感染における亜鉛/ 亜鉛トランスポーターの役割』

中川　一路
（京都大学大学院　教授）
『細菌表層機能分子をターゲットとした分子標的薬開発』

福井　竜太郎
（東京大学医科学研究所　助教）
『自然炎症を基盤とするマウスの遺伝的表現型と関連遺伝子群の探索』

熊谷　雄太郎
（大阪大学免疫学フロンティア研究センター　特任助教）
『抗ウイルス免疫応答の網羅的データからのモデリングと制御』

「真菌医学研究センターMonthlyセミナー」
場所：真菌医学研究センター　大会議室

1）日時：平成28年２月16日　17：30～18：30
新　幸二
（慶應義塾大学 医学部 微生物学・免疫学教室　助教）
『腸内細菌による宿主免疫系の活性化機構』
（千葉大学リーディング研究育成プログラム推進候補課題・“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，東京大学医科学研究所 細菌感染生物学社会連携研究部門 共催）

2）日時：平成28年３月10日　17：30～18：30
今清水　正彦
（千葉大学真菌医学研究センター　特任助教）
『熱揺らぎの影響を踏まえた転写調節機構』
（千葉大学リーディング研究育成プログラム推進候補課題・“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，東京大学医科学研究所 細菌感染生物学社会連携研究部門 共催）

3）日時：平成28年３月23日　16：00～17：00
小野口　和英
（Department of Microbiology and Immunology, University of California, San Francisco, USA）
『ショウジョウバエをモデルにした小胞体ストレスと抗ウイルス免疫機構の解析』
（千葉大学リーディング研究育成プログラム推進候補課題・“超個体”の統合的理解に基づく次世代型「感染制御学」研究推進拠点共催）

4）日時：平成28年６月14日　11：00～12：00
原口　健
（千葉大学真菌医学研究センター　特任助教）
『高効率microRNA阻害法による乳癌幹細胞を標的とした治療法の開発』

5）日時：平成28年７月１日　16：00～17：30
佐野　晃之
（Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, USA）
『共生細菌により生み出されるTh17細胞の分化誘導とその活性化の分子機構』
（千葉大学リーディング研究育成プログラム推進候補課題・“超個体”の統合的理解に基づく次世代型「感染制御学」研究推進拠点共催）

6）日時：平成28年７月26日　16：00～17：00
芦田　浩
（千葉大学真菌医学研究センター　特任准教授）
『赤痢菌感染分子機構の解明とその応用』

7）日時：平成28年９月27日　16：00～17：00
西城　忍
（千葉大学真菌医学研究センター　准教授）
『C型レクチンによる生体防御機構の解明』

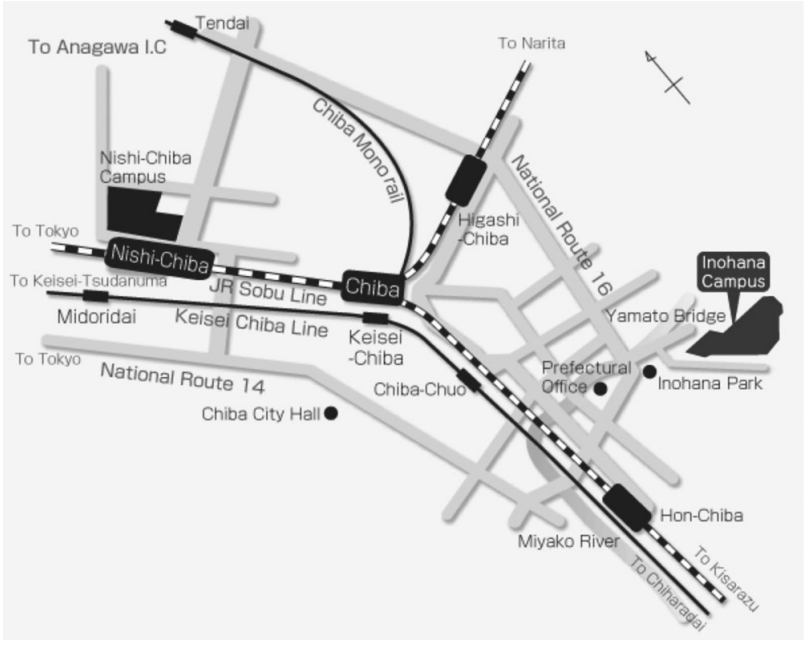
8）日時：平成28年10月25日　15：30～17：00
Jean-Marc Reichhart
（Professor, The University of Strasbourg, CNRS, France）
『The Drosophila Immune System, an evolutionary perspective』

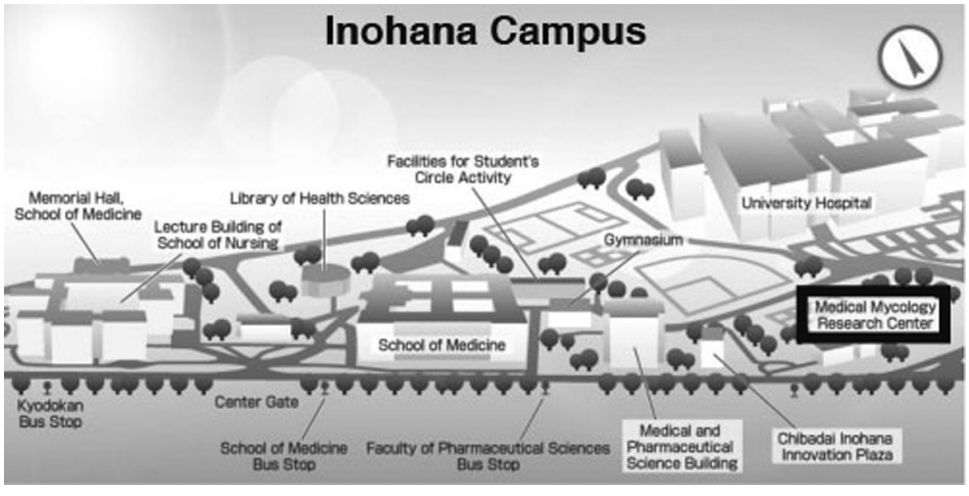
9）日時：平成28年11月15日　18：00～19：30
辻　典子
（国立研究開発法人 産業技術総合研究所 バイオメディカル研究部門 免疫恒常性研究特別チーム長）
『“管として生きる”個体の腸内環境と免疫機能』

10）日時：平成28年11月29日　16：00～17：00
知花　博治
（千葉大学真菌医学研究センター　准教授）
『カンジダ・グラブラータ全遺伝子組換え体を用いた病原性研究と抗真菌薬開発』

「第５回 細胞間クロノ・コミュニケーション研究会」
（千葉大学リーディング研究育成プログラム推進候補課題・細胞間クロノ・コミュニケーション主催）
日時：平成28年７月７日　17：00～18：00
場所：真菌医学研究センター　大会議室

大島　拓
（奈良先端科学技術大学院大学 バイオサイエンス研究科 助教）
『水平伝搬における細菌の転写サイレンサーの役割』





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