

MMRC



**ANNUAL REPORT OF MEDICAL MYCOLOGY
RESEARCH CENTER, CHIBA UNIVERSITY 2014**

千葉大学 真菌医学研究センター 報告

18



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はじめに

千葉大学真菌医学研究センターは、真菌感染症の基盤・臨床研究、共同利用・共同研究拠点事業、真菌バイオリソース事業を活動の3本柱とする、我が国で唯一の真菌感染症の研究・教育に特化した公立の大学附属研究センターです。

平成26年7月、千葉大学亥鼻キャンパスでは、次世代対応型医療人の育成と「治療学」拠点形成を目的として、徳久剛史学長の主導のもとに、医学研究院の中山俊憲教授を機構長とする「未来医療教育研究機構」が設立されました。真菌医学研究センターは、医学部、薬学部、看護学部とともに機構のメンバーとして研究・教育活動に参加しています。これに伴い、本センターには、准教授、助教ポジションと事業費が配分されました。また学長裁量による特別経費で、新たな研究チームを立ち上げるために、センターA棟の諸設備の更新、実験室新設、客員教授居室新設等、一連の工事を行っています。また平成27年3月までには、A棟の耐震補強工事が完了するとともに、B棟1階にはBSL-3レベル実験施設が設置され、より安全性の高い病原真菌の研究環境が整備される予定です。

本センターでは、超高齢社会における真菌感染症の増加、及び経済のグローバル化を通じてもたせられる輸入真菌症など、新たな課題の解決に貢献するため、真菌感染症の診断・治療への取り組みを強化すると共に、深在性真菌症を中心とする難治性感染症研究拠点の形成を目指して、医学部附属病院との連携を強化しました。具体的なアクションとして、平成26年11月に附属病院において、亀井克彦教授、渡邊哲准教授が「真菌症専門外来」を開設しました。また11月には、医学部附属病院の感染症管理治療部講師石和田稔彦先生を准教授として迎えました。その結果、本センターと附属病院との連携が従来にもまして深まり、本センターにおける感染症臨床研究のさらなる発展が期待されます。一方で、病原真菌の基礎研究の強化及び感染免疫研究の裾野を広げる目的で、共同利用・共同研究拠点「真菌感染症研究拠点」事業を活用して、異分野との連携を積極的に行い、平成26年度は全国の研究機関と24件の共同研究課題を実施しました。また、平成25年度の研究成果は本年報に掲載してありますが、一部の採択課題の成果について、平成26年3月7日に東大医科学研究所と合同で開催した成果報告会にて報告を行いました。

本センターでは7名のPrincipal investigator (PI) による研究グループが研究・教育活動を行い、世界レベルの研究成果が発信できる研究センターを目指しています。本年度は、西城忍特任准教授が九大の山崎晶教授（本センター客員教授）との共同研究を行い、その成果がImmunity誌に発表され、新聞等でも紹介され注目を集めました。また米山光俊教授が、トムソンロイターが本年発表した、「Highly Cited Researchers」に、免疫学の分野で世界87名の最も影響力のある研究者の一人に選ばれました（センターホームページ参照）。一方、病原真菌の基礎研究では、11月に川本進教授が、「Cryptococcus neoformansの細胞周期制御と低酸素ストレス応答の分子細胞シグナリング解析」で、日本医真菌学会より学会賞を受賞されました。これらは、いずれも日頃の研究活動の成果が評価されたものでありセンターとしても大変喜ばしいことであります。

センターでは、平成26年6月より国内外から第一線で活躍する研究者をセンターへ招き、6回の「Monthly seminar」を開催しました。また11月15日には共同利用・共同研究拠点事業の一環として、医学研究院の横須賀収先生及び教室の方々の御支援をいただき、「感染症研究グローバルネットワークフォーラム2014」を開催しました。今回で3回目となり、学内外から著名な先生をお招きして講演会を行い、成功裏に終了することができました。本年度は、これら一連の活動を通じて、本センターに対する、社会、大学、研究コミュニティからの期待が極めて大きいことを改めて認識させられました。

我が国はすでに超高齢社会に突入し、本センターには真菌をはじめとする難治性感染症の基盤研究を推進し、これらの研究成果を臨床や創薬開発へ繋げてゆくことが強く求められています。一方、世界規模では、高度病原性病原体による感染症の脅威に加えて、多剤耐性病原体の拡散、あるいは高度医療の普及に伴う難治性感染症の増加が大きな問題となっています。本センターでは、これらの現状と今後の社会情勢を踏まえ、学内外からの御支援を賜り、次世代の研究者の育成と同時に、医療へ貢献できる研究センターを目指す所存でおります。

平成27年1月

千葉大学真菌医学研究センター長

笹川千尋

Preface

It is my great pleasure to present our Annual Report 2014 for the Medical Mycology Research Center (MMRC), Chiba University. MMRC was originally founded in 1946 as the Institute for Food-Microbiology Chiba Medical College. The Institute was renovated to the Research Institute for Chemobiodynamics in 1973, and after 4 years the Institute was rebuilt to focus largely on pathogenic fungi and its infectious diseases, for which the Institute was renamed as MMRC in 1997. In 2004, MMRC was certified to act as National University Cooperation. In 2010, MMRC was reorganized into 1 department composing of 4 research divisions to further strengthen the research activity, those include divisions of molecular biology of pathogenic fungi, infection and immunology, clinical research, and bioresources. In 2010, MMRC was certified for Joint/Research Center for promoting the central role in leading medical mycology research in Japan. We are aiming at achievement of high level of research and clinical activities, for which we employ multi-disciplinary approaches to infection biology, comprising concepts and methodologies of molecular genetics, bioinformatics, immunology, cell biology, protein chemistry, and clinical research. MMRC also encourages to promote the applications of their researches toward prevention, diagnosis, and control of fungal and bacterial infectious diseases by cooperating with the Faculty of Medicine, Medical Hospital, and Faculty of Pharmacy at Chiba University.

This annual report summarizes our scientific achievements in 2014, which I hope will be useful to promote domestic as well as worldwide collaboration with our scientists, and ultimately contribute to medical fungal research and medicine.

January 26, 2015

Chihiro Sasakawa

Director of MMRC

川本 PI (分子細胞シグナリング解析)プロジェクト

Project for Molecular Signaling Analysis

研究概要 (Summary)

生化学・分子生物学・細胞生物学等の手法を用い、病原酵母・糸状菌の分子細胞研究を行い、病原機能などに関連するシグナリング解析を進め、抗真菌薬シーズ創出など真菌症の分子制御に向けた分子細胞医真菌学への貢献を目指す。

We are conducting basic research on the molecular and cellular biology of pathogenic fungi using biochemistry and molecular biology methods based on gene and protein science as well as ultrastructural morphology and cell biology methods such as electron microscopy.

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|----------|-------|-------------------------------|-------------------|
| 教授 | 川本 進 | Professor | Susumu Kawamoto |
| 准教授 | 横山 耕治 | Associate Professor | Koji Yokoyama |
| 助教 | 清水 公德 | Assistant Professor | Kiminori Shimizu |
| 技術職員 | 大楠美佐子 | Research Technician | Misako Ohkusu |
| グランドフェロー | 山口 正視 | Grandfellow | Masashi Yamaguchi |
| 特任助教 | 萩原 大祐 | Research Assistant Professor | Daisuke Hagiwara |
| 技術補佐員 | 中野百実子 | Research Promotion Technician | Yumiko Nakano |

1. Structure based Functional Distinction between Cln1 and Cln2 Depends on the Ubiquitin-Proteasome Pathway.

Akiko Suganami¹, Norio Takase¹, Hajime Sugiyama², Eric V Virtudazo³, Susumu Kawamoto³, Yutaka Tamura¹

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Cln1 and Cln2, G1/S cyclins of the ascomycetous budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), oscillate during the cell cycle, rising in late G1 and falling in early S phase. We have been tried to elucidate the structure basis of the functional distinction between Cln1 and Cln2. Here we performed *in silico* simulations: construction and evaluation of three dimensional structures of Cln1-Cdc28 and Cln2-

Cdc28 complexes. Our *in silico* simulations suggested that the interaction of Cln1 and Cln2 with Cdc28 were in the two distinct situations, designated as flip and flop conformation, at the extra amino acid region in the cyclin box of Cln1 and Cln2. We speculated the trigger of this flip-flop conversion of the extra amino acid region in the cyclin box of Cln1-Cdc28 and Cln2-Cdc28 might be regulated by the ubiquitination of the sequences rich in Pro (P), Glu (E), Ser (S) and Thr (T), so-called PEST motifs, in Cln1 and Cln2. Furthermore, we presumed that the functional superiority between Cln1 and Cln2 in the G1/S phase of *S. cerevisiae* might be controlled by flip-flop conversion and ubiquitin-proteasome pathway.

2. Functional characterization of *PMT2*, encoding a protein-O-mannosyltransferase, in the human pathogen *Cryptococcus neoformans*.

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Diazobenzoic acid B (DBB), also known as diazonium blue B or fast blue B, can be used to distinguish basidiomycetous yeasts from ascomycetes. This chemical has long been used for the taxonomic study of yeast species at the phylum level, but the mechanism underlying the DBB staining remains unknown. To identify molecular targets of DBB staining, we isolated *Agrobacterium tumefaciens*-mediated insertional mutants of *Cryptococcus neoformans*, a basidiomycetous pathogenic yeast, which were negative to DBB staining. In one of these mutants, we found that the *PMT2* gene, encoding a protein-O-mannosyltransferase, was interrupted by a T-DNA insertion. A complete gene knockout of the *PMT2* gene revealed that the gene was responsible for DBB staining in *C. neoformans*, suggesting that one of the targets of Pmt2-mediated glycosylation is responsible for interacting with DBB. We also determined that *Cryptococcus gattii*, a close relative of *C. neoformans*, was not stained by DBB when the *PMT2* gene was deleted. Our finding suggests that the protein-O-mannosylation by the *PMT2* gene product is required for DBB staining in *Cryptococcus* species in general. We also showed that glycosylation in *Cryptococcus* by Pmt2 plays important roles in controlling cell size, resistance to high temperature and osmolarity, capsule formation, sexual reproduction, and virulence.

3. The *a*-oxoamine synthase gene *fum8* is involved in fumonisin B2 biosynthesis in *Aspergillus niger*.

Kiminori Shimizu¹, Hiroyuki Nakagawa², Ruiko Hashimoto³, Daisuke Hagiwara¹, Yoshiki Onji⁴, Katsuyoshi Asano⁴, Susumu Kawamoto¹, Haruo Takahashi^{1,5}, Koji Yokoyama¹

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One of the secondary metabolite gene clusters of *Aspergillus niger* possesses highly homologous genes with those within the fumonisin gene cluster of *Fusarium* spp. At least 14 genes are considered to be present within approximately 50 kb genomic region. A number of strains of *A. niger* have been shown to produce fumonisin B2 (FB2), however, there has been no direct evidence that the gene cluster is responsible for FB2 production in this fungus. We investigated the involvement of the cluster in FB2 biosynthesis. The disruption of *fum8*, coding for an *a*-oxoamine synthase, resulted in loss of FB2 biosynthesis in *A. niger*, but did not influence the vegetative growth and sensitivity to high temperature or UV irradiation. The gene expression patterns of the transcription factor gene *fum21* and *fum8* were determined, but were different from those in *F. verticillioides*.

4. The role of AtfA and HOG MAPK pathway in stress tolerance in conidia of *Aspergillus fumigatus*.

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Aspergillus fumigatus is a life-threatening pathogenic fungus, whose conidium is the infectious agent of aspergillosis. To better understand the mechanism underlying the long-term viability of conidia, we characterized a bZip transcription factor, AtfA, with special reference to stress-tolerance in conidia. The *atfA* deletion mutant conidia showed significant sensitivity to high temperature and oxidative stress. The trehalose content that accumulated in conidia was reduced in the mutant conidia. Transcriptome analysis revealed that AtfA regulated several stress-protection-related genes such as *catA*, *dprA*, *scf1*, and *conJ* at the conidiation stage. The upstream high-osmolarity glycerol pathway was also involved in conferring stress tolerance in conidia because Δ *pbsB* showed stress sensitivity and reduced trehalose in conidia. However, a mutant lacking the SakA mitogen-activated protein kinase (MAPK) produced normal conidia. We investigated another MAPK, MpkC, in relation with SakA, and the double deletion mutant, Δ *sakA*, *mpkC*, was defective in conidia stress tolerance. We concluded that MpkC is able to bypass SakA, and the two MAPKs redundantly regulate the conidia-related function of AtfA in *A. fumigatus*.

5. Whole-Genome Comparison of *Aspergillus fumigatus* Strains Serially Isolated from Patients with Aspergillosis.

Daisuke Hagiwara, Hiroki Takahashi, Akira Watanabe, Azusa Takahashi-Nakaguchi, Susumu Kawamoto, Katsuhiko Kamei, Tohru Gono

Medical Mycology Research Center (MMRC), Chiba University, Chiba, Japan

The emergence of azole-resistant strains of *Aspergillus fumigatus* during treatment for aspergillosis occurs by a mutation selection process. Understanding how antifungal resistance mechanisms evolve in the host environment during infection is of great clinical importance and biological interest. Here, we used next-generation sequencing (NGS) to identify mutations that arose during infection by *A. fumigatus* strains sequentially isolated from two patients, one with invasive pulmonary aspergillosis (IPA) (five isolations) and the other with aspergilloma (three isolations). The serial isolates had identical microsatellite types, but their growth rates and conidia production levels were dissimilar. A whole-genome comparison showed that three of the five isolates from the IPA patient carried a mutation, while 22 mutations, including six nonsynonymous ones, were found among three isolates from the aspergilloma patient. One aspergilloma isolate carried the *cyp51A* mutation P216L, which is reported to confer azole resistance, and it displayed an MIC indicating resistance to itraconazole. This isolate harbored five other nonsynonymous mutations, some of which were found in the *afyap1* and *aldA* genes. We further identified a large deletion in the aspergilloma isolate in a region containing 11 genes. This finding suggested the possibility that genomic deletions can occur during chronic infection with *A. fumigatus*. Overall, our results revealed dynamic alterations that occur in the *A. fumigatus* genome within its host during infection and treatment.

6. Dynamics of cell components during budding of *Cryptococcus albidus* yeast cells.

Masashi Yamaguchi¹, Kiminori Shimizu¹, Susumu Kawamoto¹, Amaliya A Stepanova², Natalya V Vasilyeva²

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² Kashkin Research Institute of Medical Mycology of North-West State Medical University, St. Petersburg, Russia

Phase-contrast and freeze-substitution microscopy were used to study cellular division in the pathogenic fungus *Cryptococcus albidus* *in vitro*. It was shown that the mother and daughter cells (during exponential growth) varied in vacuolar contents and there was an absence of storage compounds in the cytosol. Ultrastructural signs of mother cells for transitioning to bud formation were a migration of the nucleus from distal to lateral, increasing the level of chromatization and nucleolus size, proliferation of mitochondria and changes in topography including formation of a “cover” around the nuclear envelope. Specific feature of cellular division in cultures was permanent nucleolus presence. For the first time, we described the nucleolus material being uniformly distributed between mother and daughter cells during cellular division. The formation of the septum separating the daughter cells was investigated in detail.

7. Genome sequence comparison of *Aspergillus fumigatus* strains isolated from patients with pulmonary aspergilloma and chronic necrotizing pulmonary aspergillosis.

Azusa Takahashi-Nakaguchi¹, Yasunori Muraosa¹, Daisuke Hagiwara¹, Kanae Sakai¹, Takahito Toyotome^{1,3}, Akira Watanabe^{1,2}, Susumu Kawamoto¹, Katsuhiko Kamei¹, Tohru Gono¹, Hiroki Takahashi¹

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Aspergillus fumigatus is the most important pathogenic fungus among *Aspergillus* species associated with aspergillosis. Of the various presentations of aspergillosis, one of the most common forms of pulmonary involvement by *A. fumigatus* is pulmonary aspergilloma (PA), which is a fungus ball composed of fungal hyphae, inflammatory cells, fibrin, mucus, and tissue debris. Chronic necrotizing pulmonary aspergillosis (CNPA), also known as semi-invasive or invasive aspergillosis, is locally invasive and predominantly seen in patients with mild immunodeficiency or with a chronic lung disease. In the present study, with the aid of a next-generation sequencer, we performed whole genome sequence (WGS) analysis of 17 strains isolated from patients with PA and CNPA in Japan. A total of 99,088 SNPs were identified by mapping the reads to *A. fumigatus* genome reference strain Af293, and according to genome-wide phylogenetic analysis, there were no correlations between the whole genome sequence typing, and pathological conditions. Here, we conducted the first multi-genome WGS study to focus on the *A. fumigatus* strains isolated from patients with PA and CNPA, and comprehensively characterized genetic variations of strains. WGS approach will help in better understanding of molecular mechanisms in aspergillosis caused by *A. fumigatus*.

8. Dendritic cell-based immunization ameliorates pulmonary infection with highly virulent *Cryptococcus gattii*.

Keigo Ueno¹, Yuki Kinjo¹, Yoichiro Okubo², Kyoko Aki², Makoto Urai¹, Yukihiko Kaneko^{1,3}, Kiminori Shimizu⁴, Dan-Ni Wang⁵, Akiko Okawara¹, Takuya Nara¹, Kayo Ohkouchi¹, Yuki Mizuguchi¹, Susumu Kawamoto⁴, Katsuhiko Kamei^{5,6}, Hideaki Ohno^{1,7}, Yoshihito Niki⁸, Kazutoshi Shibuya², Yoshitsugu Miyazaki¹

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Cryptococcosis due to a highly virulent fungus, *Cryptococcus gattii*, emerged as an infectious disease on Vancouver Island and surrounding areas in 1999, for which deaths were reported among immunocompetent individuals. Previous studies indicated that *C. gattii* strain R265 isolated from a Canadian outbreak had immune-avoidance or immune-suppression capabilities. However, any protective immunity against *C. gattii* has not been identified. In this study, we used a gain-of-function approach to investigate protective immunity against *C. gattii* infection using a dendritic cell-based (DC) vaccine. Bone marrow derived dendritic cells (BMDCs) efficiently engulfed an acapsular *C. gattii* mutant,

CAP60Δ, which resulted in their expression of co-stimulatory molecules and inflammatory cytokines. This was not observed for BMDCs that were cultured with encapsulated strains. When CAP60Δ-pulsed BMDCs were transferred to mice prior to intratracheal R265 infection, significant amelioration of pathology, fungal burden, and the survival rate resulted as compared to controls. Multi-nucleated giant cells (MGCs) that engulfed fungal cells were significantly increased in the lungs of immunized mice. IL-17A, IFN γ , and TNF α -producing lymphocytes were significantly increased in the spleens and lungs of immunized mice. Although only partially, the protective effect of this DC vaccine was significantly reduced in IFN γ knockout mice. These results demonstrated that an increase in cytokine-producing lymphocytes and the development of MGCs that engulfed fungal cells were associated with protection against pulmonary infection with highly virulent *C. gattii* and ¹suggested that IFN γ may have been an important mediator for this vaccine-induced protection.

9. Ultrastructural observation of cell components during budding in yeast *Malassezia pachydermatis*.

Masashi Yamaguchi¹, Kiminori Shimizu¹, Susumu Kawamoto¹, Amaliya A Stepanova², Natalya V Vasilyeva²

¹ Medical Mycology Research Center, Chiba University, Chiba, Japan

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The phase-contrast, scanning and transmission electron microscopy were used for observation of the cell components in exponential phase of growth in *M. pachydermatis*. Lower level of vacuolization, small number of mitochondria, presence of single cisterns of endoplasmic reticulum (ER), absence of secretory vesicles and presence of a large lipid inclusion opposite budding scar was typical for mother cells. It was revealed the uniformity in bud formation, the number of mitochondria, storage inclusion, and cisterns of ER were not increased compared with the cytosol volume and

number of free ribosomes. An increase in nucleus sizes and level of chomatisation were observed in mother cell before budding. The mother cell, after septum formation, differs from daughter cell in larger volume of cytosol and presence of thicker cell wall. In contrast, daughter cell was typical in smaller volume of cytosol and presence of large lipid inclusion. A diagram of organelles transition in *M. pachydermatis* budding was presented.

10. The Effects of F-Actin Inhibitor Latrunculin A on Pathogenic Yeast *Cryptococcus neoformans*.

Marie Kopecká¹, Masashi Yamaguchi², Susumu Kawamoto²

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Background: This basic research aimed to investigate the effects of actin inhibitor latrunculin A on human pathogen *Cryptococcus neoformans* by freeze-substitution and electron microscopy to find out whether the actin cytoskeleton can become a new antifungal target for inhibition of cell division. Methods: Cells treated by latrunculin A for 20 h in yeast extract peptone dextrose medium were investigated by phase contrast and fluorescent microscopy, freeze-substitution and transmission electron microscopy, counted in a Bürker chamber and absorbance was measured. Results: The cells of *Cryptococcus neoformans* responded to the presence of latrunculin A, an actin inhibitor, by disappearance of actin patches, actin cables and actin rings. Removal of actin cables and patches arrested proliferation and led to the production of cells that had ultrastructural disorder and irregular morphology of mitochondria and thick aberrant cell walls. Budding cells lysed in buds and in septa. Conclusion: Latrunculin A has fungistatic, fungicidal and fungilytic effects on human pathogenic yeast *Cryptococcus neoformans*.

11. Positional cloning in *Cryptococcus neoformans* and its application for identification and cloning of the gene encoding methylenetetrahydrofolate reductase.

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Cryptococcus neoformans, a basidiomycetous human pathogenic yeast, has been widely used in research fields in medical mycology as well as basic biology. Gene cloning or identification of the gene responsible for a mutation of interest is a key step for functional analysis of a particular gene. The availability therefore, of the multiple methods for cloning is desirable. In this study, we proposed a method for a mapping-based gene identification/cloning (positional cloning) method in *C. neoformans*. To this end, we constructed a series of tester strains, one of whose chromosomes was labeled with the *URA5* gene. A heterozygous diploid constructed by crossing one of the tester strains to a mutant strain of interest loses a chromosome (s) spontaneously, which is the basis for assigning a recessive mutant gene to a particular chromosome in the mitotic mapping method. Once the gene of interest is mapped to one of the 14 chromosomes, classical genetic crosses can then be performed to determine its more precise location. The positional information thus obtained can then be used to significantly narrow down candidate genes by referring to the *Cryptococcus* genome database. Each candidate gene is then examined whether it would complement the mutation. We successfully applied this method to identify *CNA07390* encoding methylenetetrahydrofolate reductase as the gene responsible for a methionie-requiring mutant in our mutant collection.

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知花PI (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標的探索を行う。

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

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1. Aoyama T, Nakayama H, Ueno K, Inukai T, Tanabe K, Nagi M, Bard M, Chibana H: **Genome-wide survey of transcriptional initiation in the pathogenic fungus, *Candida glabrata*. *Genes Cells*. 19 (6): 478-503, 2014.**

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DNA sequencing of the 5'-flanking region of the transcriptome effectively identifies transcription initiation sites and also aids in identifying unknown genes. This study describes a comprehensive polling of transcription start sites and an analysis of full-length complementary DNAs derived from the genome of the pathogenic fungus *Candida glabrata*. A comparison of the sequence reads derived from a cDNA library prepared from cells grown under different culture conditions against the reference genomic sequence of the *Candida* Genome Database (CGD:<http://www.candidagenome.org/>) revealed the expression of 4,316 genes and their acknowledged transcription start sites (TSSs). In addition this analysis also predicted 59 new genes including 22 that showed no homology to the genome of *Saccharomyces cerevisiae*, a genetically close relative of *C. glabrata*. Furthermore, comparison of the 5'-untranslated regions (5'-UTRs) and core promoters of *C. glabrata* to those of *S. cerevisiae* showed various global similarities and differences among orthologous genes. Thus, the *C. glabrata* transcriptome can complement the annotation of the genome database and should provide new insights into the organization, regulation, and function of genes of this important human pathogen.

2. Costa C, Nunes J, Henriques A, Mira NP, Nakayama H, Chibana H, Teixeira MC: The *Candida glabrata* drug:H⁺ antiporter *CgTpo3* (ORF CAGL0I10384g): role in azole drug resistance and polyamine homeostasis. *Journal of Antimicrobial Chemotherapy*. 57(7): 3159-67, 2014.

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Objectives The ability of opportunistic pathogenic *Candida* species to persist and invade specific niches in the human host depends on their resistance to natural growth inhibitors and antifungal therapy. This work describes the role of the *Candida glabrata* drug:H⁺ antiporter *CgTpo3* (ORF CAGL0I10384g) in this context.

Methods Deletion and cloning of *CgTPO3* was achieved using molecular biology tools. *C. glabrata* strain susceptibility was assayed based on growth in liquid and solid media and through MIC determination. Radiolabelled compound accumulation or HPLC were used for the assessment of the role of *CgTpo3* as a drug or polyamine transporter. Quantitative RT–PCR was used for expression analysis.

Results *CgTpo3* was found to confer resistance to azole drugs in *C. glabrata*. This protein was found to be localized to the plasma membrane and to decrease the intracellular accumulation of [³H] clotrimazole, playing a direct role in its extrusion from pre-loaded *C. glabrata* cells. *CgTPO3*

was further found to confer resistance to spermine, complementing the susceptibility phenotypes exhibited by the deletion of its *Saccharomyces cerevisiae* homologue, *TPO3*. In spermine-stressed *C. glabrata* cells, *CgTPO3* is transcriptionally activated in a CgPdr1-dependent manner, contributing to a decrease in the intracellular concentration of this polyamine. Clotrimazole exposure was found to lead to the intracellular accumulation of spermine, and pre-exposure to this polyamine was found consistently to lead to increased clotrimazole resistance.

Conclusions Altogether, these results point to a significant role for *CgTpo3* in azole drug resistance and in the tolerance to high polyamine concentrations, such as those found in the urogenital tract.

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米山 PI (感染応答) プロジェクト

Project for Immune Response in Infections Diseases

研究概要 (Summary)

感染に対する我々の生体防御は、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、特にウイルス感染に応答した自然免疫誘導に注目し、感染センサー分子によるウイルス由来の非自己核酸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 infection and subsequent immune responses. The observations obtained from this study will help us to establish a novel therapeutic or preventive strategy against infectious diseases by viruses.

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|---------|-------|-------------------------------|---------------------|
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1. Identification RNA binding proteins (RBPs), which are responsible for the formation of antiviral stress granules (avSGs).

Koji Onomoto, and Mitsutoshi Yoneyama

We demonstrated that viral infection including influenza A virus (IAV) induces RIG-I to accumulate in cytoplasmic granular-like structure, which we termed antiviral stress granule (avSG) (Onomoto *et al.*, *PLoS One*, 2012). Furthermore, we revealed that avSG plays a critical role as platform for initiation of RIG-I-mediated antiviral signaling. To understand how avSG is formed in response to viral infection and responsible for anti-viral signal activation, we are trying to identify regulatory molecule(s), which is responsible for avSG formation. We partially purified avSG and analyzed its components by mass spectrometry. As a result, we identified several RBPs in virus-induced samples. We are analyzing functional significance of the RBPs in antiviral innate immunity.

2. A novel function of human Pumilio proteins in cytoplasmic sensing of viral infection.

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RIG-I-like receptor (RLR) plays a pivotal role in the

detection of invading pathogens to initiate type I interferon (IFN) gene transcription. Since aberrant IFN production is harmful, RLR signaling is strictly regulated. However, the regulatory mechanisms are not fully understood. By expression cloning, we identified Pumilio proteins, PUM1 and PUM2, as candidate positive regulators of RIG-I signaling. Overexpression of Pumilio proteins and their knockdown augmented and diminished IFN- β promoter activity induced by Newcastle disease virus (NDV), respectively. Both proteins showed a specific association with LGP2, but not with RIG-I or MDA5. Furthermore, all of these components were recruited to NDV-induced antiviral stress granules. Interestingly, biochemical analyses revealed that Pumilio increased double-stranded (ds) RNA binding affinity of LGP2; however, Pumilio was absent in the dsRNA-LGP2 complex, suggesting that Pumilio facilitates viral RNA recognition by LGP2 through its chaperon-like function. Collectively, our results demonstrate an unknown function of Pumilio in viral recognition by LGP2.

3 . Molecular mechanism for detection of viral ribonucleoprotein complex by RLRs.

Reiko Hirai, Michihiko Jogi and Mitsutoshi Yoneyama

It has remained unclear how RIG-I detects viral ribonucleoprotein complex (RNP), which consists of viral genomic RNA and viral proteins such as nucleocapsid protein (NP). Recently, we established *in vitro* reconstitution assay system for RIG-I-mediated signaling and examined whether viral RNP could activate RIG-I *in vitro*. As a model RNP, we prepared artificial IAV RNP generated in 293T cells. As a result, we have successfully detected RIG-I activation by viral RNP *in vitro*. Now, we are trying to visualize interaction between RIG-I and viral RNP *in vitro* using Atomic force microscope (AFM).

4 . DHX36 enhances RIG-I signaling by facilitating PKR-mediated antiviral stress granule formation.

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RIG-I is a DExD/H-box RNA helicase and functions as a critical cytoplasmic sensor for RNA viruses to initiate antiviral interferon (IFN) responses. Here we demonstrate that another DExD/H-box RNA helicase DHX36 is a key molecule for RIG-I signaling by regulating double-stranded RNA (dsRNA)-dependent protein kinase (PKR) activation, which has been shown to be essential for the formation of antiviral stress granule (avSG). We found that DHX36 and PKR form a complex in a dsRNA-dependent manner. By forming this complex, DHX36 facilitates dsRNA binding and phosphorylation of PKR through its ATPase/helicase activity. Using DHX36 KO-inducible MEF cells, we demonstrated that DHX36 deficient cells showed defect in IFN production and higher susceptibility in RNA virus infection, indicating the physiological importance of this complex in host defense. In summary, we identify a novel function of DHX36 as a critical regulator of PKR-dependent avSG to facilitate viral RNA recognition by RIG-I-like receptor (RLR).

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西城PI (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.

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

Shinobu Saijo, Rikio Yabe and Akimasa Seno

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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- κ B 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 . C-Type Lectin Receptors in Host Defense Against Microbial Pathogens.

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C-type lectin receptors (CLRs) are a group of pattern recognition receptors (PRRs) that recognize carbohydrate structures in microbes, including fungi and bacteria, as pathogen-associated molecular patterns (PAMPs). They are expressed mainly in dendritic cells (DCs) and macrophages, and among these CLRs, DC-associated C-type lectin-1 (Dectin-1), DC-associated C-type lectin-2 (Dectin-2), macrophage-inducible C-type lectin (Mincle), and macrophage C-type lectin (Mcl) transduce their signaling through phosphorylation of spleen tyrosine kinase (Syk). On the other hand, human DC-specific intracellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) and its mouse homologue SIGN-related gene 3 (SIGNR3), members of the DC-SIGN superfamily of CLRs, transduce their signaling through intracellular tyrosine-containing motif. In addition to pathogen recognition, Mincle, DC-SIGN, and SIGNR3 have been shown to recognize molecules from self, suggesting pleiotropic roles of CLRs in the homeostasis of the body. Here, we review each of these receptors in detail describing their expression, ligand recognition, signaling, and associated human diseases.

3 . Excess IL-1 signaling enhances the development of Th17 cells by downregulating TGF- β -induced Foxp3 expression.

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IL-1R antagonist-deficient (*Il1rn*^{-/-}) mice develop autoimmune arthritis in which IL-17A plays a crucial role. Although many studies have shown that Th17 cell differentiation is dependent on TGF- β and IL-6, we found that Th17 cells developed normally in *Il1rn*^{-/-}*Il6*^{-/-} mice in vivo. Then, we analyzed the mechanisms of Th17 cell differentiation in *Il1rn*^{-/-}*Il6*^{-/-} mice. We found that IL-21 production was increased in the lymph nodes of *Il1rn*^{-/-} mice, naive *Il6*^{-/-} CD4⁺ T cells differentiated into Th17 cells when cultured with TGF- β and IL-21, and the differentiation was greatly enhanced when IL-1 was added to the culture. Th17 cell differentiation was not induced by either TGF- β or IL-1 alone or in combination. IL-21 induced IL-1R expression in naive CD4⁺ T cells, and IL-1 inhibited TGF- β -induced Foxp3 expression, resulting in the promotion of Th17 cell differentiation. Furthermore, IL-1 augmented the expression of Th17 cell-specific transcription factors such as Nfkbiz and Batf. These results indicate that excess IL-1 signaling can overcome the requirement of IL-6 in the differentiation of Th17 cells by suppressing Foxp3 expression and inducing Th17 cell-specific transcription factors.

4 . Rag2-deficient IL-1 Receptor Antagonist-deficient Mice Are a Novel Colitis Model in Which Innate Lymphoid Cell-derived IL-17 Is Involved in the Pathogenesis.

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Il1rn^{-/-} mice spontaneously develop arthritis and aortitis by an autoimmune mechanism and also develop dermatitis by an autoinflammatory mechanism. Here, we show that *Rag2*^{-/-} *Il1rn*^{-/-} mice develop spontaneous colitis with high mortality, making a contrast to the suppression of arthritis in these mice. Enhanced IL-17A expression in group 3 innate lymphoid cells (ILC3s) was observed in the colon of *Rag2*^{-/-} *Il1rn*^{-/-} mice. IL-17A-deficiency prolonged the survival of *Rag2*^{-/-} *Il1rn*^{-/-} mice, suggesting a pathogenic role of this cytokine in the development of intestinal inflammation. Although IL-17A-producing T cells were increased in *Il1rn*^{-/-} mice, these mice did not develop colitis, because CD4⁺Foxp3⁺ regulatory T cell population was also expanded. Thus, excess IL-1 signaling and IL-1-induced IL-17A from ILC3s cause colitis in *Rag2*^{-/-} *Il1rn*^{-/-} mice in which Treg cells are absent. These observations suggest that the balance between IL-17A-producing cells and Treg cells is important to keep the immune homeostasis of the colon.

5 . CTRP3 plays an important role in the development of collagen-induced arthritis in mice.

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Rheumatoid arthritis (RA) is an autoimmune inflammatory disease exhibited most commonly in joints. We found that the expression of *C1qtnf3*, which encodes C1q/TNF-related protein 3 (CTRP3), was highly increased in two mouse RA models with different etiology. To elucidate the pathogenic roles of CTRP3 in the development of arthritis, we generated *C1qtnf3*^{-/-} mice and examined the development of collagen-induced arthritis in these mice. We found that the incidence and severity score was higher in *C1qtnf3*^{-/-} mice compared with wild-type (WT) mice. Histopathology of the joints was also more severe in *C1qtnf3*^{-/-} mice. The levels of antibodies against type II collagen and pro-inflammatory cytokine mRNAs in *C1qtnf3*^{-/-} mice were higher than WT mice. These observations indicate that CTRP3 plays an important role in the development of autoimmune arthritis, suggesting CTRP3 as a possible medicine to treat RA.

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亀井PI（臨床感染症）プロジェクト

Project to Link Basic Sciences and Clinical Medicine

研究概要（Summary）

アスペルギルス症を中心とする難治性糸状菌感染症，輸入真菌症を主要な研究対象とし，感染機構，新しい診断や治療法の開発を中心に研究を行っている．並行して行っている診療活動では，10月から正式に開始した附属病院での真菌症専門外来に加え，学内外でのコンサルテーション活動を行っており，学外からの依頼は検査を含めて年間300件以上に達している．スタッフ構成では4月からこれまでの助教1名（田口）の退官に伴い准教授1名（渡辺）が着任した．

実施体制：教員2名，技官1名（以上常勤），特任助教2名，補助員2名およびグランドフェロー1名で研究及び大学院生4名（博士課程）の教育指導を行なっている．

ACTIVITIES: Our research focuses on the development of diagnostic / therapeutic methods for intractable fungal diseases such as aspergillosis through an investigation into the mechanism of infection. We also take care of patients in the speciality clinic (Fungal Disease Clinic) at the University Hospital, while providing more than 300 consulting services on fungal diseases to physicians/clinical technologists all over the country.

STAFF: Professors (2), technician (1), research assistant professors (2) and research assistants (2), grand fellow (1) are working in our group with four graduate school students.

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|----------------------|-------|-------------------------------|------------------|
| 教授 | 亀井 克彦 | Professor | Katsuhiko Kamei |
| 准教授 (4月から着任) | 渡辺 哲 | Associate Professor | Akira Watanabe |
| 助教 (3月で退任) | 田口 英昭 | Assistant Professor | Hideaki Taguchi |
| 特任助教 | 村長 保憲 | Research Assistant Professor | Yasunori Muraosa |
| 特任助教 | 八尋 真希 | Research Assistant Professor | Maki Yahiro |
| グランドフェロー (4月から着任) | 田口 英昭 | Grand Fellow | Hideaki Taguchi |
| 技術職員 | 鎗田 響子 | Research Technician | Kyoko Yarita |
| 技術補佐員 | 関 里亜 | Research Promotion Technician | Rio Seki |
| 技術補佐員 | 井上 京子 | Research Promotion Technician | Kyoko Inoue |

1. Isolation and Drug Susceptibility of *Candida parapsilosis* Sensu Lato and other Species of *C. parapsilosis* Complex from Patients with Blood Stream Infections and Proposal of a Novel LAMP Identification Method for the Species.

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Candida parapsilosis complex (CPC) is the third *Candida* species isolated in blood cultures of patients from our Hospital, following *C. albicans* and *C. tropicalis*. From 2006 to 2010, the median annual distribution of CPC was 8 cases/year. Records of 36 patients were reviewed. CPC were 31 (86.1 %) *C. parapsilosis*; 4 (11.1 %) *C. orthopsilosis*; and 1 (2.8 %) *C. metapsilosis*. Clinical characteristics were central venous catheter, 34 (94.4 %); parental nutrition, 25 (70 %); surgery, 27 (57.9 %); prior bacteremia, 20 (51.3 %); malignancy, 18 (50 %). General mortality was 47.2 %. Death was higher in immunosuppressed patients (17 vs. 11; $p = 0.003$). Three out four (75 %) patients with *C. orthopsilosis* and 14 out 31 (45.2 %) with *C. parapsilosis* died ($p = 0.558$). Thirty-nine individual isolates were tested for susceptibility to seven antifungal drugs, with MICs values showing susceptibility to all of them. Two isolates, one *C. orthopsilosis* and one *C. parapsilosis*, had fluconazole MIC = 4 µg/mL. Differentiation among CPC has implication in caring for

patients with invasive candidiasis since there are differences in virulence, pathogenicity and drug susceptibility. A method targeting the topoisomerase II gene based on loop-mediated isothermal amplification (LAMP) was developed. LAMP emerges as a promising tool for the identification of fungal species due to the high sensitivity and specificity. LAMP can be performed at the point-of-care, being no necessary the use of expensive equipment. In our study, the method was successful comparing to the DNA sequencing and proved to be a reliable and fast assay to distinguish the three species of CPC.

2. Effect of Serum Components on Biofilm Formation by *Aspergillus fumigatus* and Other *Aspergillus* Species.

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Biofilm production by microorganisms is critical for their pathogenicity. Serum promotes biofilm production by *Aspergillus fumigatus*; however, its effects on other *Aspergillus* spp. have not been reported. We analyzed biofilm formation by five *Aspergillus* spp., i.e., *A. fumigatus*, *A. flavus*, *A. nidulans*, *A. niger*, and *A. terreus*, and examined the effects of serum/serum proteins such as fetal bovine serum (FBS), fetuin A, and bovine serum albumin (BSA) on hyphal growth, hyphal branching, and extracellular matrix (ECM) formation. The antifungal susceptibility of *A. fumigatus* isolates that formed biofilms was also examined. All serum/

serum proteins promoted the growth of all these fungal species; growth promotion was most evident with FBS, followed by fetuin A and BSA. This effect was most evident in case of *A. fumigatus* and least evident in case of *A. terreus*. Electron microscopy showed thick ECM layers surrounding fungal cell walls after culture with FBS, particularly in *A. fumigatus*. An increase in hyphal branching caused by fetuin A was the highest in case of *A. fumigatus* and *A. nidulans*. Biofilm-forming *A. fumigatus* showed resistance to most antifungal agents, although a synergism of micafungin and amphotericin B was suggested. Our results indicate that serum promotes biofilm formation, including thick ECM, by many *Aspergillus* spp., particularly *A. fumigatus*, and that this may be closely related to its virulence.

3. Glucoamylase is a major allergen of *Schizophyllum commune*.

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BACKGROUND:

Schizophyllum commune is one of the causative agents of basidiomycosis including disorders such as allergic bronchopulmonary mycosis, allergic fungal sinusitis, and mucoid impaction of bronchi, the incidence of those of which has been increasing. These mycoses are difficult to diagnose because only a limited number of diagnostic tools are currently available. The biggest problem is that no specific antigens of *S. commune* have been identified to enable serodiagnosis of the disease.

OBJECTIVE:

In this study, we attempted to identify a major antigen of *S. commune* to establish a reliable serodiagnostic method.

METHODS:

We used mass spectrometry to identify an antigen that reacted with the serum of a patient with allergic bronchopulmonary mycosis caused by *S. commune*. The protein was expressed in *Escherichia coli*, highly purified, and the patient sera IgG and IgE titres against the protein were determined by enzyme-linked immunosorbent assay.

RESULTS:

The protein identified as a major antigen of *S. commune* was named Sch c 1; it was a homolog of glucoamylase. The IgG and IgE titres against Sch c 1 in patient sera were significantly higher than those in healthy volunteer sera ($P < 0.01$).

CONCLUSIONS AND CLINICAL RELEVANCE:

Sch c 1 is recognized by the host immune system of patients as an antigen/allergen. The purified glucoamylase Sch c 1 is a promising candidate antigen for the serodiagnosis of *S. commune*-induced mycosis.

4. Antifungal susceptibility of *Aspergillus fumigatus* clinical isolates collected from various areas in Japan.

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Azole resistance among clinical isolates of *Aspergillus fumigatus* is becoming a serious problem in Europe, but the status in Japan is not yet known in detail. The aim of this study was to determine the present status of azole resistance in *A. fumigatus* in Japan. We employed 171 clinical isolates of *A. fumigatus sensu stricto* collected from 1987 to 2008 at the Medical Mycology Research Center, Chiba University, Japan for azole resistance determination. Identification of all

isolates were re-examined both from the aspect of morphology and molecular phylogeny. The antifungal susceptibility of these isolates was tested based on the CLSI M38-A2 broth microdilution method. In our collection, only 1 (0.6%) and 2 isolates (1.2%) showed elevated MIC to voriconazole and itraconazole, respectively. Our study disclosed that the frequency of azole resistance in *A. fumigatus* still remains low in this collection.

5. Development of cycling probe-based real-time PCR system to detect *Fusarium* species and species complex (FSSC).

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In the present study, we developed a new real-time PCR system based on the cycling probe technology (CPT), which is composed of two single tube real-time PCR assays: the *Fusarium* genus-specific assay and the *Fusarium solani* species complex (FSSC)-specific assay with primers targeting the 28s ribosomal RNA gene. The *Fusarium* genus-specific assay was shown to be highly specific, detecting all reference *Fusarium* strains with no cross-reaction with other reference fungal strains, such as *Aspergillus* spp. and human DNA. The FSSC-specific assay also reacted very specifically with FSSC, except for a cross-reaction with *Fusarium lunatum*. To validate the real-time PCR system, we tested 87 clinical isolates of *Fusarium* spp. Identification results from the real-time PCR system were found to be 100% concordant with those from DNA sequencing of EF-1 α gene. The sensitivity testing also

demonstrated high sensitivity, enabling detection of one copy of standard DNA with good reproducibility. Furthermore, both assays were shown to be extremely sensitive even when fungal cells were mixed with human cells, detecting 3 germinated conidia spiked in 3mL of human blood. To apply our new real-time PCR system to the molecular diagnosis of fusariosis, we evaluated its efficacy using a mouse model of invasive *F. solani* infection. Plasma and whole blood samples of infected mice were tested using the real-time PCR system. The sensitivity of the real-time PCR system was found to be 100% (n = 4) in plasma samples. In contrast, no amplification signal was detected in whole blood samples. This system could provide a rapid and precise diagnostic tool for early diagnosis, which is necessary for appropriate treatment and improvement of prognosis of disseminated fusariosis.

6. Opportunistic infection in patients with acute liver failure.

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Background

Treatment with systemic corticosteroids is often used for acute liver failure (ALF), but this has increased the number of profoundly immunocompromised patients and cases of opportunistic infection.

Methods

Between January 2007 and December 2012, all patients (n = 51) referred to the Chiba University Hospital for treatment of ALF were studied. Patients with prothrombin activity of 40 % or less of the standardized values were defined

as having ALF. Patient age, sex, cause of ALF, alanine aminotransferase and total bilirubin levels, prothrombin activity and total amount of corticosteroid were analyzed to determine the factors associated with the occurrence of opportunistic infection.

Results

Opportunistic infections occurred in 21.6 % (n = 11) of ALF patients. Thirty-five patients underwent systemic corticosteroid therapy, and 31.4 % of those patients showed opportunistic infections. Cytomegalovirus (n = 9, 81.8 %) and Pneumocystis jiroveci (n = 6, 54.5 %) were the microorganisms frequently suspected as the causes of opportunistic infection. In 7 (63.6 %) of the 11 cases of opportunistic infection, 2 or more species of microorganism were detected. Seven patients (63.6 %) with opportunistic infection were cured by treatment. Cox regression analysis for the patients who underwent systemic corticosteroid therapy revealed that age over 52 years (compared to younger patients: odds ratio = 9.62, 95 % confidence interval = 1.22–76.9) was only the predictive factor for the occurrence of opportunistic infection.

Conclusion

Opportunistic infections are not rare in ALF patients, and the appropriate diagnosis and treatment of these infections are critical during ALF treatment.

7. *Exophiala dermatitidis* pneumonia successfully treated with long-term itraconazole therapy.

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Exophiala dermatitidis pneumonia is extremely rare. Here we report a case of *E. dermatitidis* pneumonia successfully treated with long-term itraconazole therapy. A 63-year-old woman without a remarkable medical history developed a dry and chest pain. Chest radiographs revealed consolidation in the middle lobe of the lung. Cytologic examination by bronchoscopy showed filamentous fungi and *E. dermatitidis* was detected in the bronchoalveolar lavage fluid. After 5 months of itraconazole therapy, her symptoms improved and the area of consolidation diminished. Two weeks after discontinuing the itraconazole therapy, the area of consolidation reappeared. Itraconazole therapy was restarted and continued for 7 months. The abnormal shadow observed on the chest X-ray gradually diminished. Over a 27-month follow-up with periodic examination, there was no relapse and the patient had a favorable clinical course.

8. Pulmonary mucormycosis with embolism: two autopsied cases of acute myeloid leukemia.

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Mucormycosis is an increasingly important cause of morbidity and mortality for patients with hematological malignancies. The diagnosis of mucormycosis usually requires mycological evidence through tissue biopsy or autopsy because the signs and symptoms are nonspecific and there are currently no biomarkers to identify the disease. We herein present two autopsied cases of acute myeloid leukemia with prolonged neutropenia who developed invasive mucormycosis accompanied by pulmonary artery embolism. Our cases were featured by unexplained fever and rapidly progressive dyspnea. Computed tomography scan detected nodular lesions or nonspecific consolidations in the lungs. Cultures, cytological study, and serum fungal markers consistently gave negative results. Autopsy revealed embolism of the pulmonary artery which consisted of fibrin clots by filamentous fungi. Genomic DNA was extracted from the paraffin-embedded clots and was applied to polymerase chain reaction amplification, leading to the diagnosis of infection by *Rhizopus microsporus*. We should carefully search for life-threatening pulmonary embolism when patients with hematological malignancies develop pulmonary mucormycosis.

9. GliA in *Aspergillus fumigatus* is required for its tolerance to gliotoxin and affects the amount of extracellular and intracellular gliotoxin.

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Gliotoxin is an important virulence factor of *Aspergillus fumigatus*. Although GliA putatively belongs to the major facilitator superfamily in the gliotoxin biosynthesis cluster, its roles remain unclear. To determine the function of GliA, we disrupted gliA in *A. fumigatus*. gliA disruption increased the susceptibility of *A. fumigatus* to gliotoxin. The gliT and gliA double-disrupted mutant had even higher susceptibility to gliotoxin than each individual disruptant. The extracellular release of gliotoxin was greatly decreased in the gliA disruptant. Mice infected with the gliA disruptant of *A. fumigatus* showed higher survival rates than those

infected with the parent strain. These results strongly indicate that GliA, in addition to GliT, plays a significant role in the tolerance to gliotoxin and protection from extracellular gliotoxin in *A. fumigatus* by exporting the toxin. This also allows the fungus to evade the harmful effect of its own gliotoxin production. Moreover, GliA contributes to the virulence of *A. fumigatus* through gliotoxin secretion.

10. Detection of *Mucor velutinosus* in a Blood Culture After Autologous Peripheral Blood Stem Cell Transplantation: A pediatric Case Report.

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Filamentous fungi were detected in the blood culture of a one-year-old boy after autologous peripheral blood stem cell transplantation. The patient was suspected to have aspergillosis and received micafungin. Fungi were isolated on potato dextrose agar medium and incubated at 37°C for 2-5 days. Grayish, cottony colonies formed. A slide culture showed a spherical sporangium at the tips of the sporangiophores. The fungus could have been a zygomycete. The zygomycete was isolated from three blood cultures. The antifungal drug was changed from micafungin to liposomal amphotericin B, which resulted in an improvement in the patient's symptoms. Growth was observed at 37°C, but not 42°C in a growth temperature test. Gene sequence analysis identified the fungus as *Mucor velutinosus*. To the best of our knowledge, this is the first time *M. velutinosus* has been detected in Japan, and this case is very rare. Zygomycetes

are known to be pathogens that cause fungal infections in immunodeficient patients such as those with leukemia. They are difficult to identify by culture and are identified at autopsy in many cases. Therefore, culture examinations should be performed for immunodeficient patients with the consideration of zygomycetes.

11. *Fusarium napiforme* systemic infection: case report with molecular characterization and antifungal susceptibility tests.

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INTRODUCTION:

During the last decades, *Fusarium* spp. has been reported as a significant cause of disease in humans, especially in immunocompromised patients, who have high risk of invasive life-threatening disease. *Fusarium* species usually reported as cause of human disease are *F. solani*, *F. oxysporum* and *F. verticillioides*.

CASE DESCRIPTION:

We describe the second case in the literature of disseminated fusariosis caused by *Fusarium napiforme*, that occurred in a 60-year-old woman with multiple myeloma after subsequent cycles of chemotherapy.

DISCUSSION AND EVALUATION:

We identified the *F. napiforme* not only by standard morphologic criteria by macroscopic and microscopic

characteristics, but also confirmed by molecular biology methods, including sequencing. The antifungal susceptibility of the *F. napiforme* isolates were tested to seven antifungal drugs; the azoles were the most active drug against all the isolates tested.

CONCLUSIONS:

Fusarium spp. are of relevance in medical mycology, and their profiles of low susceptibility to antifungal drugs highlight the importance for faster and more accurate diagnostic tests, what can contribute to an earlier and precise diagnosis and treatment.

12. Fatal fungemia with *Scedosporium prolificans* in a patient with acute myeloid leukemia.

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13. The role of AtfA and HOG MAPK pathway in stress tolerance in conidia of *Aspergillus fumigatus*.

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Aspergillus fumigatus is a life-threatening pathogenic fungus, whose conidium is the infectious agent of aspergillosis. To better understand the mechanism underlying the long-term viability of conidia, we characterized a bZip transcription

factor, AtfA, with special reference to stress-tolerance in conidia. The *atfA* deletion mutant conidia showed significant sensitivity to high temperature and oxidative stress. The trehalose content that accumulated in conidia was reduced in the mutant conidia. Transcriptome analysis revealed that AtfA regulated several stress-protection-related genes such as *catA*, *dprA*, *scf1*, and *conJ* at the conidiation stage. The upstream high-osmolarity glycerol pathway was also involved in conferring stress tolerance in conidia because *DpbsB* showed stress sensitivity and reduced trehalose in conidia. However, a mutant lacking the SakA mitogen-activated protein kinase (MAPK) produced normal conidia. We investigated another MAPK, *MpkC*, in relation with SakA, and the double deletion mutant, *DsakA*, *mpkC*, was defective in conidia stress tolerance. We concluded that *MpkC* is able to bypass SakA, and the two MAPKs redundantly regulate the conidia-related function of AtfA in *A. fumigatus*.

14. Identification of fungal pathogens by visible microarray system in combination with isothermal gene amplification.

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The increasing incidence of infectious diseases caused by fungi in immunocompromised patients has encouraged researchers to develop rapid and accurate diagnosis methods. Identification of the causative fungal species is critical in deciding the appropriate treatment, but it is not easy to get satisfactory results due to the difficulty of fungal cultivation and morphological identification from clinical samples. In this study, we established a microarray system that can identify 42 species from 24 genera of clinically important fungal pathogens by using a chemical color reaction in the detection process. The array uses the internal transcribed

spacer region of the rRNA gene for identification of fungal DNA at the species level. The specificity of this array was tested against a total of 355 target and nontarget fungal species. The fungal detection was succeeded directly from 10 (3) CFU/ml for whole blood samples, and 50 fg DNA per 1 ml of serum samples indicating that the array system we established is sensitive to identify infecting fungi from clinical sample. Furthermore, we conducted isothermal amplification in place of PCR amplification and labeling. The successful identification with PCR-amplified as well as isothermally amplified target genes demonstrated that our microarray system is an efficient and robust method for identifying a variety of fungal species in a sample.

15. Whole-Genome Comparison of *Aspergillus fumigatus* Strains Serially Isolated from Patients with Aspergillosis.

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The emergence of azole-resistant strains of *Aspergillus fumigatus* during treatment for aspergillosis occurs by a mutation selection process. Understanding how antifungal resistance mechanisms evolve in the host environment during infection is of great clinical importance and biological interest. Here, we used next-generation sequencing (NGS) to identify mutations that arose during infection by *A. fumigatus* strains sequentially isolated from two patients, one with invasive pulmonary aspergillosis (IPA) (five isolations) and the other with aspergilloma (three isolations). The serial isolates had identical microsatellite types, but their growth rates and conidia production levels were dissimilar. A whole-genome comparison showed that three of the five isolates from the IPA patient carried a mutation, while 22 mutations, including six nonsynonymous ones, were found among three isolates from the aspergilloma patient. One aspergilloma isolate

carried the *cyp51A* mutation P216L, which is reported to confer azole resistance, and it displayed an MIC indicating resistance to itraconazole. This isolate harbored five other nonsynonymous mutations, some of which were found in the *afyap1* and *aldA* genes. We further identified a large deletion in the aspergilloma isolate in a region containing 11 genes. This finding suggested the possibility that genomic deletions can occur during chronic infection with *A. fumigatus*. Overall, our results revealed dynamic alterations that occur in the *A. fumigatus* genome within its host during infection and treatment.

16. *Penicilliosis marneffei* complicated with interstitial pneumonia.

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A 71-year-old man with interstitial pneumonia was hospitalized due to a pulmonary infection. He had been living in Thailand and had returned to Japan three months earlier. Antibiotic therapy initially cleared the infection; however, the patient's condition relapsed. *Pseudomonas aeruginosa* and *Penicillium* sp. were both detected in sputum and bronchial lavage fluid cultures and *Penicillium* sp. was identified to be *P. marneffei*. The infiltration observed on chest radiographs improved following treatment with itraconazole and tazobactam/piperacillin, and no relapse occurred. We herein report the first case of a non-HIV patient with *P. marneffei* infection in Japan.

17. *Cryptococcus gattii* genotype VGIIb infection in Japan.

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This report describes a case of *Cryptococcus gattii* VGIIb infection of the pulmonary and central nervous systems in an immunocompetent Japanese man with a travel history, and it hypothesizes the place where he was infected with *C. gattii* using the genotype information.

18. Pulmonary nocardiosis caused by *Nocardia cyriacigeorgica* in patients with *Mycobacterium avium* complex lung disease: two case reports.

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Background

Pulmonary nocardiosis frequently occurs in immunocompromised hosts and in some immunocompetent hosts with chronic lung disease; however, few reports have described pulmonary nocardiosis with nontuberculous mycobacterial lung infection. Here we report for the first time two cases of pulmonary nocardiosis caused by *Nocardia cyriacigeorgica* associated with *Mycobacterium avium* complex (MAC) lung disease caused by *M. avium*.

Case presentation

Case 1 is that of a 72-year-old Japanese man with untreated MAC lung disease, who was diagnosed with rheumatoid arthritis and initiated on methotrexate. After 3 years of methotrexate therapy, the patient remained smear-negative and culture-positive for MAC, but also became smear-positive for *Nocardia* species. He received trimethoprim/sulfamethoxazole, and his symptoms and lung infiltrates improved. Case 2 is that

of an immunocompetent 53-year-old Japanese woman with MAC lung disease, who was treated with a combined therapy of clarithromycin, rifampicin, ethambutol, and levofloxacin. MAC sputum culture was negative after 1 year of combined treatment, which was maintained for 2 years. After four treatment-free years, *Nocardia* species were occasionally isolated from her sputum, although MAC was rarely isolated from sputum cultures over the same period. In both cases, the *Nocardia* species were identified as the recently defined *N. cyriacigeorgica* by 16S ribosomal RNA gene sequencing.

Conclusion

We report two cases of pulmonary nocardiosis caused by *N. cyriacigeorgica* associated with MAC lung disease caused by *M. avium* and suggest that *N. cyriacigeorgica* may be a major infective agent associated with MAC lung disease.

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五ノ井 PI (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、糖鎖と糖鎖受容体を介した菌と宿主の相互作用解明に力を入れている。
- 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興味を持っている。

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 roles of cell surface glycans and their receptors (lectins) of human and fungi in infection.
- 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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|-----------|-------|-------------------------------|---------------|
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| 助 教 | 大荒田素子 | Assistant Professor | Oarada Motoko |
| 特 任 助 教 | 酒井香奈江 | Research Assistant Professor | Kanae Sakai |
| 技 術 補 佐 員 | 川名 直美 | Research Promotion Technician | Naomi Kawana |

1. Refeeding with glucose rather than fructose elicits greater hepatic inflammatory gene expression in mice.

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Objective: We have previously reported that refeeding after a 48-h fast, used as a study model of starvation and refeeding, promotes acute liver inflammatory gene expression, which is at least partly mediated by toll-like receptor 2 (TLR2). We have also demonstrated that dietary carbohydrates play critical roles in this process. In our current study, we compared the outcomes of refeeding with different carbohydrate sources.

Methods: Mice were fasted for 46 h and then refed with 1.5% (w/w) agar gel containing 19% carbohydrate (sources: α -corn starch, glucose, sucrose or fructose). The liver expression of inflammatory genes and other specific genes was then sequentially measured for the first 14 h after refeeding initiation.

Results: Fasting for 46 h up-regulated the liver expression of endogenous ligands for TLRs (HspA5, Hsp90aa1, and Hspd1). Refeeding with agar gel containing α -corn starch or glucose increased the liver expression of *Tlr2*, pro-inflammatory genes (*Cxcl2*, *Cxcl10*, *Cxcl1*, *Nfkb1*, *Nfkb2*, *RelB*, *Sectm1a*, *Il1 β*), stress response genes (*Atf3*, *Asns*, *Gadd45a*, *Perk*, *Inhbe*), detoxification genes (*Hmox1*, *Gsta1*, *Abca8b*), genes involved in tissue regeneration (*Gdf15*, *Krt23*, *Myc*, *Tnfrsf12a*, *Mthfd2*) and genes involved in tumor suppression (*p53*, *Txnrd1*, *Btg2*). This refeeding also elevated the serum levels of alanine aminotransferase moderately but significantly. These effects were attenuated in mice refed with agar gel containing sucrose or fructose.

Conclusion: Dietary glucose, rather than fructose, plays a critical role in refeeding-induced acute liver inflammatory gene expression and moderate hepatocyte destruction. Further

studies should investigate the role of these effects in liver inflammation and, consequently, liver dysfunction.

2. *Aspergillus arcuverdensis*, a new species of *Aspergillus* section *Fumigati* isolated from caatinga soil in State of Pernambuco, Brazil.

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Aspergillus arcuverdensis, a new species isolated from semi-desert soil in a caatinga area, State of Pernambuco, Brazil, and a similar environment in the Xinjiang Uygur Autonomous Region, China, is described and illustrated. It is characterized by relatively long conidiophores for *Aspergillus* section *Fumigati*, and subglobose to broadly ellipsoidal and smooth conidia. The delimitation of this new species is supported further by phylogenetic analyses of the β -tubulin, calmodulin and actin gene sequences.

3. The role of AtfA and HOG MAPK pathway in stress tolerance in conidia of *Aspergillus fumigatus*.

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Aspergillus fumigatus is a life-threatening pathogenic fungus, whose conidium is the infectious agent of aspergillosis. To better understand the mechanism underlying the long-term viability of conidia, we characterized a bZip transcription factor, AtfA, with special reference to stress-tolerance in conidia. The *atfA* deletion mutant conidia showed significant sensitivity to high temperature and oxidative stress. The trehalose content that accumulated in conidia was reduced in the mutant conidia. Transcriptome analysis revealed that AtfA regulated several stress-protection-related genes such as *catA*, *dprA*, *scf1*, and *conJ* at the conidiation stage. The upstream high-osmolarity glycerol pathway was also involved in conferring stress tolerance in conidia because Δ *pbsB* showed stress sensitivity and reduced trehalose in conidia. However, a mutant lacking the SakA mitogen-activated protein kinase (MAPK) produced normal conidia. We investigated another MAPK, MpkC, in relation with SakA, and the double deletion mutant, Δ *sakA*, *mpkC*, was defective in conidia stress tolerance. We concluded that MpkC is able to bypass SakA, and the two MAPKs redundantly regulate the conidia-related function of AtfA in *A. fumigatus*.

4. Whole-genome comparison of *Aspergillus fumigatus* strains serially isolated from patients infected with aspergillosis.

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The emergence of azole-resistant strains of *Aspergillus fumigatus* during treatment for aspergillosis occurs by a mutation selection process. Understanding how antifungal resistance mechanisms evolve in the host environment during infection is of great clinical importance and biological interest. Here, we used next-generation sequencing (NGS) to identify mutations that arose during infection by *A. fumigatus* strains sequentially isolated from two patients, one with invasive pulmonary aspergillosis (IPA) (five isolations) and the other with aspergilloma (three isolations). The serial isolates had identical microsatellite types, but their growth rates and conidia production levels were dissimilar. A whole-genome comparison showed that three of the five isolates from the IPA patient carried a mutation, while 22 mutations, including six nonsynonymous ones, were found among three isolates from the aspergilloma patient. One aspergilloma isolate carried the *cyp51A* mutation P216L, which is reported to confer azole resistance, and it displayed an MIC indicating resistance to itraconazole. This isolate harbored five other nonsynonymous mutations, some of which were found in the *afyap1* and *aldA* genes. We further identified a large deletion in the aspergilloma isolate in a region containing 11 genes. This finding suggested the possibility that genomic deletions can occur during chronic infection with *A. fumigatus*. Overall, our results revealed dynamic alterations that occur in the *A. fumigatus* genome within its host during infection and treatment.

5. Agelamadins C-E, bromopyrrole alkaloids comprising oroidin and 3-hydroxykynurenine from a marine sponge *Agelas* sp.

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Three structurally unique bromopyrrole alkaloids, agelamadins C-E (1-3), were isolated from a marine sponge *Agelas* sp. Agelamadin C (1) possesses a hybrid structure of oroidin and 3-hydroxykynurenine connected through a dihydro-1,4-oxazine moiety. Agelamadins D (2) and E (3) are a C-9/C-10 diastereomer and a 10-epimer of 1, respectively. The structures of 1-3 were elucidated on the basis of spectroscopic analysis as well as application of a PGME method and a TDDFT ECD calculation. Antimicrobial activity of 1-3 was evaluated.

6. Amphidinins C-F, amphidinolide Q analogs from marine dinoflagellate *Amphidinium* sp.

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Four new polyketides, amphidinins C-F (1-4), have been isolated from the culture broth of symbiotic dinoflagellate *Amphidinium* sp. The analysis of their spectral data revealed

that amphidinins C-F (1-4) were 4,5-seco-analogues of amphidinolide Q (5). The absolute configurations of the new compounds were elucidated by the combination of J-based configuration analysis, modified Mosher's method, and chemical derivatization. Amphidinins D (2) and F (4) are the first glycosides related to amphidinolides. Amphidinins C-F (1-4) showed antimicrobial activity against bacteria and/or fungi.

7. Pulmonary nocardiosis caused by *Nocardia cyriacigeorgica* in patients with *Mycobacterium avium* complex lung disease: two case reports.

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Pulmonary nocardiosis frequently occurs in immunocompromised hosts and in some immunocompetent hosts with chronic lung disease; however, few reports have described pulmonary nocardiosis with nontuberculous mycobacterial lung infection. Here we report for the first time two cases of pulmonary nocardiosis caused by *Nocardia cyriacigeorgica* associated with *Mycobacterium avium* complex (MAC) lung disease caused by *M. avium*. Case presentation

Case 1 is that of a 72-year-old Japanese man with untreated MAC lung disease, who was diagnosed with rheumatoid arthritis and initiated on methotrexate. After 3 years of methotrexate therapy, the patient remained smear-negative and culture-positive for MAC, but also became smear-positive for *Nocardia* species. He received trimethoprim/sulfamethoxazole, and his symptoms and lung infiltrates improved. Case 2 is that of an immunocompetent 53-year-old Japanese woman with MAC lung disease, who was treated with a combined therapy of clarithromycin, rifampicin, ethambutol, and levofloxacin. MAC sputum culture was negative after 1 year of combined treatment, which was maintained for 2 years. After four treatment-free years, *Nocardia* species were occasionally isolated from her sputum, although MAC was rarely isolated from sputum cultures over the same period. In both cases, the *Nocardia* species were identified as the recently defined *N. cyriacigeorgica* by 16S ribosomal RNA gene sequencing. Conclusion We report two cases of pulmonary nocardiosis caused by *N. cyriacigeorgica* associated with MAC lung disease caused by *M. avium* and suggest that *N. cyriacigeorgica* may be a major infective agent associated with MAC lung disease.

8. 肺ノカルジア症の12例の臨床的検討—単一施設の後方視的研究—日本呼吸器学会誌.

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背景：肺ノカルジア症の臨床的な特徴についてまとめた報告は限られている。

対象と方法：2000年から2013年に当センターで診療を行った肺ノカルジア症12例を後方視的に検討した。

結果：平均年齢は69.0±12.4歳，男性10例，呼吸器系の基礎疾患は10例，糖尿病1例，ステロイドおよび免疫抑制薬の投与は1例であった。ノカルジアと同時に4例で *Mycobacterium avium* complex, *Streptococcus mitis*, インフルエンザ桿菌 + 緑膿菌 + *Aspergillus fumigatus*, *A. flavus*が⁵

分離された。また，経過中にあらたに *M. avium* complex 症 ($n=1$)，慢性肺アスペルギルス症 ($n=1$)，肺炎 ($n=4$) の発症を認めた。ノカルジアの治療としてST合剤を投与されたのは9例，うち5例では副作用のため他薬への変更を要した。観察期間 [中央値968日 (43-2625日)] 内にノカルジア症1例が再発し，6例が死亡したが，ノカルジア症による死亡はなかった。

結語：肺ノカルジア症には混合感染がまれではない。ST合剤投与中に副作用の頻度が高いことに注意が必要である。肺ノカルジア症の予後については，合併症の影響が無視できない。

9. *Gordonia iterans* sp. nov., isolated from a patient with pneumonia.

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A second novel clinical actinobacterial strain, designated IFM 10348 (T), was isolated from the sputum of the same Japanese patient with bacterial pneumonia from whom the type strain of *Gordonia araii* had been isolated. The strains differed in phylogenetic position and drug-resistance profiles. The taxonomic position of strain IFM 10348 (T) was clarified by phenotypic, chemotaxonomic and phylogenetic studies. Phylogenetic analyses based on 16S rRNA gene sequences clearly demonstrated that strain IFM 10348 (T) occupied a distinct clade within the genus *Gordonia* and

was related closely to *Gordonia malaquae* DSM 45064 (T) and *Gordonia hirsuta* DSM 44140 (T) (97.3 and 97.1% similarities, respectively). Strain IFM 10348 (T) was also clearly differentiated from *G. malaquae* DSM 45064 (T) and *G. hirsuta* DSM 44140 (T) based on *gyrB* and *secA1* gene sequence similarity values. Strain IFM 10348 (T) had MK-9 (H2) as the predominant menaquinone, contained meso-diaminopimelic acid, arabinose, galactose and glucosamine as cell-wall components, and contained C18:1 ω 9c, summed feature 3 (C16:1 ω 7c and/or C16:1 ω 6c) and C16:0 as the major cellular fatty acids. Mycolic acids were present. The DNA G+C content of strain IFM 10348 (T) was 68.0 mol%. DNA-DNA relatedness data coupled with the combination of genotypic and phenotypic data indicated that strain IFM 10348 (T) represents a novel species of the genus *Gordonia*, for which the name *Gordonia iterans* sp. nov. is proposed. The type strain is IFM 10348 (T) (= CCTCC M2011245 (T) = NCCB 100436 (T)).

10. Nakijiquinone S (Ia) and Nakijinol C (Ib), new meroterpenoids from a marine sponge of the family *Spongiidae*.

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New meroterpenoids, nakijiquinone S (1) and nakijinol C (2), have been isolated from an Okinawan marine sponge of the family *Spongiidae*. The gross structures and relative stereochemistries of 1 and 2 were elucidated on the basis of their spectral data. Nakijiquinone S (1) and nakijinol C (2) were new meroterpenoids consisting of a clerodane-type decalin ring connected to a 2-butoxy-5-hydroxy-benzoquinone unit or methyl 2,3,4-trihydroxybenzoate unit through a methylene, respectively. Nakijiquinone S (1) and nakijinol C (2) showed antimicrobial activities against several bacteria and fungi.

11. Agelamadins A and B, dimeric bromopyrrole alkaloids from a marine sponge *Agelas* sp.

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Two structurally unique dimeric bromopyrrole alkaloids, agelamadins A (1) and B (2), were isolated from a marine sponge *Agelas* sp. Agelamadins A (1) and B (2) have a structure consisting of an agelastatin-like tetracyclic moiety and an oroidin-like linear moiety in common. The structures of 1 and 2 were elucidated on the basis of spectroscopic analysis. The antimicrobial activity and cytotoxicity of agelamadins A (1) and B (2) were evaluated.

12. Identification of Fungal Pathogens by Visible Microarray System in Combination with Isothermal Gene Amplification.

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The increasing incidence of infectious diseases caused by fungi in immunocompromised patients has encouraged researchers to develop rapid and accurate diagnosis methods. Identification of the causative fungal species is critical in deciding the appropriate treatment, but it is not easy to get satisfactory results due to the difficulty of fungal cultivation and morphological identification from clinical samples. In this study, we established a microarray system that can identify 42 species from 24 genera of clinically important fungal pathogens by using a chemical color reaction in the detection process. The array uses the internal transcribed spacer region of the rRNA gene for identification of fungal DNA at the species level. The specificity of this array was tested against a total of 355 target and nontarget fungal species. The fungal detection was succeeded directly from 10 (3) CFU/ml for whole blood samples, and 50 fg DNA per 1 ml of serum samples indicating that the array system we established is sensitive to identify infecting fungi from clinical sample. Furthermore, we conducted isothermal amplification in place of PCR amplification and labeling. The successful identification with PCR-amplified as well as isothermally amplified target genes demonstrated that our microarray system is an efficient and robust method for identifying a variety of fungal species in a sample.

13. Genome based analysis of type-I polyketide synthase and nonribosomal peptide synthetase gene clusters in seven strains of five representative *Nocardia* species.

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BACKGROUND: Actinobacteria of the genus *Nocardia* usually live in soil or water and play saprophytic roles, but they also opportunistically infect the respiratory system, skin, and other organs of humans and animals. Primarily because of the clinical importance of the strains, some *Nocardia* genomes have been sequenced, and genome sequences have accumulated. Genome sizes of *Nocardia* strains are similar to those of *Streptomyces* strains, the producers of most antibiotics. In the present work, we compared secondary metabolite biosynthesis gene clusters of type-I polyketide synthase (PKS-I) and nonribosomal peptide synthetase (NRPS) among genomes of representative *Nocardia* species/strains based on domain organization and amino acid sequence homology.

RESULTS: Draft genome sequences of *Nocardia asteroides* NBRC 15531 (T), *Nocardia otitidiscaviarum* IFM 11049, *Nocardia brasiliensis* NBRC 14402 (T), and *N. brasiliensis* IFM 10847 were read and compared with published complete genome sequences of *Nocardia farcinica* IFM 10152, *Nocardia cyriacigeorgica* GUH-2, and *N. brasiliensis* HUJEG-1. Genome sizes are as follows: *N. farcinica*, 6.0 Mb; *N. cyriacigeorgica*, 6.2 Mb; *N. asteroides*, 7.0 Mb; *N. otitidiscaviarum*, 7.8 Mb; and *N. brasiliensis*, 8.9 - 9.4 Mb. Predicted numbers of PKS-I, NRPS, and PKS-I/NRPS hybrid clusters ranged between 4-11, 7-13, and 1-6, respectively, depending on strains, and tended to increase with increasing genome size. Domain and module structures

of representative or unique clusters are discussed in the text.

CONCLUSION: We conclude the following: 1) genomes of *Nocardia* strains carry as many PKS-I and NRPS gene clusters as those of *Streptomyces* strains, 2) the number of PKS-I and NRPS gene clusters in *Nocardia* strains varies substantially depending on species, and *N. brasiliensis* strains carry the largest numbers of clusters among the species studied, 3) the seven *Nocardia* strains studied in the present work have seven common PKS-I and/or NRPS clusters, some of whose products are yet to be studied, and 4) different *N. brasiliensis* strains have some different gene clusters of PKS-I/NRPS, although the rest of the clusters are common within the *N. brasiliensis* strains. Genome sequencing suggested that *Nocardia* strains are highly promising resources in the search of novel secondary metabolites.

14. Taurospingins B and C, new acetylenic fatty acid derivatives possessing a taurine amide residue from a marine sponge of the family Spongiidae.

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Two new acetylenic fatty acid derivatives possessing a taurine amide residue, taurospingins B (1) and C (2), have been isolated from an Okinawan marine sponge of the family Spongiidae. The gross structures of 1 and 2 were elucidated on the basis of their spectral data, especially 2D NMR and FABMS/MS data. The absolute configurations for 1 and 2 were established by chemical means. Taurospingin C (2) showed inhibitory activity against *Cryptococcus neoformans*.

15. Total aflatoxin, fumonisin and deoxynivalenol contamination of busaa in Bomet county, Kenya.

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Mycotoxin contamination is a common problem in developing countries, particularly in cereals, and this poses a serious health risk to its consumers. Busaa is a Kenyan traditional brew whose cereal ingredients are prone to mycotoxin contamination. This study aimed at detecting the presence and subsequently quantifying aflatoxin, fumonisin and deoxynivalenol (DON), in busaa in Bomet county, Kenya. Busaa samples were collected from homesteads involved in brewing in the north eastern part of Bomet East constituency. Mycotoxins were detected in the samples using the Envirologix QuickTox kits and quantified using the QuickScan machine according to the manufacturer's instructions. Among the 61 samples tested, 93, 9.8 and 23% were contaminated with aflatoxin, fumonisin and DON, respectively, (mean: 5.2 ± 0.2 $\mu\text{g}/\text{kg}$, range: 2.8 to 11 $\mu\text{g}/\text{kg}$; mean $1,460 \pm 188$ $\mu\text{g}/\text{kg}$, range 280 to 4,000 $\mu\text{g}/\text{kg}$, mean 259 ± 5.2 $\mu\text{g}/\text{kg}$, range 200 to 360 $\mu\text{g}/\text{kg}$, respectively). Although traditional brews are not directly included in the European law on mycotoxins, it is important to consider their mycotoxin levels. In this study, busaa is a mainly a maize product and also the European Union (EU) guidelines on mycotoxins in maize were used as reference. It was found out that 65.6% of busaa had aflatoxin levels above the limit set in the EU guideline (4 $\mu\text{g}/\text{kg}$). Although, the average levels of fumonisin and DON were within the set limits (fumonisins: 4,000 $\mu\text{g}/\text{kg}$; DON: 1,750 $\mu\text{g}/\text{kg}$), studies have shown that chronic exposure to multiple mycotoxins has detrimental health effects. Therefore, there is need for mycotoxicological quality control of traditionally produced brews for public mycotoxicological safety.

16. Lectin-microarray technique for glycomic profiling of fungal cell surfaces.

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Lectin microarrays are rows of lectins with different carbohydrate-binding specificities spotted on surfaces of glass slides. Lectin microarray technique enables glycomic analyses of carbohydrate composition of fungal cell walls. We will describe an application of the technique in analyzing cell surface glycome of yeast-form fungal cells in the living state. The analysis reveals genus- and species-dependent complex cell surface carbohydrate structures of fungi, and enabled us, therefore, to suggest that cell walls of yeast cells, which have been considered to have relatively simple structures, actually have a more complex structure containing galactose and fucose. This shows that the technique can be used to find new insights into the study of phylogenetic relations and into the classification of cells in the fungal kingdom based on cell wall glycome.

17. Two new species of *Aspergillus* section *Fumigati* isolated from caatinga soil in the State of Pernambuco, Brazil.

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Aspergillus caatingaensis and *A. pernambucoensis*, isolated from semi-desert soil in caatinga area, the State of Pernambuco, Brazil, are described and illustrated. *Aspergillus caatingaensis* is characterized by its white cleistothecia, broadly lenticular ascospores with four equatorial crests and irregularly ribbed to slightly reticulate with aculeate convex surfaces, and ellipsoidal to broadly ellipsoidal conidia with a smooth wall. *Aspergillus pernambucoensis* is characterized by its, white cleistothecia, lenticular ascospores with two equatorial crests and irregularly ribbed with tuberculate to verrucate convex surfaces, and ovoid to broadly ellipsoidal conidia with a smooth wall. The validation of these new species is supported further by analyses of the β -tubulin, calmodulin and actin gene sequences.

18. Hikiokoshins A-I, diterpenes from the leaves of *Isodon japonicus*.

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Diterpenes, hikiokoshins A-I, and twelve known diterpenes were isolated from the leaves of *Isodon japonicus* (Burm. f.) H. Hara (Lamiaceae). The hikiokoshins A-I

possess various skeletons such as ternifonane {hikiokoshin A}, ent-6,7:8,15-diseco-6,8-cyclokauran-7,20-olide {hikiokoshin B}, ent-6,7-secokauran-7,20-olide {hikiokoshin C}, and ent-7,20-epoxykaurane {hikiokoshins D-I}. Their structures were elucidated on the basis of spectroscopic analysis. Antimicrobial activities of hikiokoshins A and B were evaluated.

19. Bromopyrrole alkaloids from a marine sponge *Agelas* sp.

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Five new bromopyrrole alkaloids, 2-bromokeramadine (1), 2-bromo-9,10-dihydrokeramadine (2), tauroacidins C (3) and D (4), and mukanadin G (5), were isolated from an Okinawan marine sponge *Agelas* sp. The structures of 1-5 were elucidated on the basis of spectroscopic data and conformational analysis. Mukanadin G (5) has a tricyclic skeleton consisting of a fused tetrahydrobenzaminoimidazole and 2,5-dioxopyrrolidine moieties. Antimicrobial activities of 1-3, and 5 as well as three related known bromopyrrole alkaloids, keramadine (6), tauroacidin A (7), and taurodispacamide A (8) were evaluated.

20. Hyrtimomines, indole alkaloids from Okinawan marine sponges *Hyrtios* spp.

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Six new indole alkaloids, hyrtimomines F-K (1-6), were isolated from Okinawan marine sponges *Hyrtios* spp. The structures of 1-6 were elucidated on the basis of spectroscopic analysis. Hyrtimomine F (1) is a structurally unique bisindole alkaloid possessing an α -keto- ϵ -caprolactam ring, while hyrtimomine G (2) is a symmetrical bisindole alkaloid. Hyrtimomines H-K (3-6) are indole alkaloids possessing β -carboline skeleton with an imidazolium unit. Antimicrobial activities of hyrtimomines F-K (1-6) were evaluated.

21. Ultra-deep sequencing analysis of the hepatitis A virus 5'-untranslated region among cases of the same outbreak from a single source.

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Hepatitis A virus (HAV) is a causative agent of acute viral hepatitis for which an effective vaccine has been developed. Here we describe ultra-deep pyrosequences (UDPSs) of HAV 5'-untranslated region (5'UTR) among cases of the same outbreak, which arose from a single source, associated with a revolving sushi bar. We determined the reference sequence from HAV-derived clone from an attendant by the Sanger method. Sixteen UDPSs from this outbreak and one from another sporadic case were compared with this reference. Nucleotide errors yielded a UDPS error rate of < 1%. This study confirmed that nucleotide substitutions of this region are transition mutations in outbreak cases, that insertion was observed only in non-severe cases, and that these nucleotide substitutions were different from those of the sporadic case. Analysis of UDPSs detected low-prevalence HAV variations in 5'UTR, but no specific mutations associated with severity in these outbreak cases. To our surprise, HAV strains in this outbreak conserved HAV IRES sequence even if we performed analysis of UDPSs. UDPS analysis of HAV 5'UTR gave us no association between the disease severity of hepatitis A and HAV 5'UTR substitutions. It might be more interesting to perform ultra-deep sequencing of full length

HAV genome in order to reveal possible unknown genomic determinants associated with disease severity. Further studies will be needed.

22. Primary brain abscess caused by *Nocardia otitidiscaviarum*.

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Diagnosing primary cerebral nocardiosis is difficult. This case report describes a 79-year-old immunocompetent Japanese woman with a primary brain abscess caused by *Nocardia otitidiscaviarum* (IFM 11321) and reviews the findings of 11 previous patients with *N. otitidiscaviarum*-induced brain abscesses. Four patients survived, including ours. Beta-lactams were not effective in our patient, and the diagnosis required a pathologic analysis of the surgical specimen. Sulfamethoxazole/trimethoprim (ST) was administered to the patient. On antibiotic susceptibility testing, *N. otitidiscaviarum* (IFM11321) was found to be resistant to amoxicillin-clavulanic acid, ceftriaxone, cefotaxime, cefepime, imipenem and clarithromycin, but sensitive to amikacin, gentamicin, ST and linezolid. Antimicrobial susceptibility patterns differ among *Nocardia* species, making species identification important for treatment. Patients with suspected *Nocardia* infection should therefore be treated empirically with ST and/or amikacin and considered for surgical management.

23. *Fusarium napiforme* systemic infection: case report with molecular characterization and antifungal susceptibility tests.

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During the last decades, *Fusarium* spp. has been reported as a significant cause of disease in humans, especially in immunocompromised patients, who have high risk of invasive life-threatening disease. *Fusarium* species usually reported as cause of human disease are *F. solani*, *F. oxysporum* and *F. verticillioides*.

CASE DESCRIPTION: We describe the second case in the literature of disseminated fusariosis caused by *Fusarium napiforme*, that occurred in a 60-year-old woman with multiple myeloma after subsequent cycles of chemotherapy.

DISCUSSION AND EVALUATION: We identified the *F. napiforme* not only by standard morphologic criteria by macroscopic and microscopic characteristics, but also confirmed by molecular biology methods, including sequencing. The antifungal susceptibility of the *F. napiforme* isolates were tested to seven antifungal drugs; the azoles were the most active drug against all the isolates tested.

CONCLUSIONS: *Fusarium* spp. are of relevance in medical mycology, and their profiles of low susceptibility to antifungal drugs highlight the importance for faster and more accurate diagnostic tests, what can contribute to an earlier and precise diagnosis and treatment.

24. Lung *Nocardia elegans* infection diagnosed on matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS).

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A 73-year-old man with adult-onset Still's disease developed a high fever, coughing, dyspnea and bloody sputum and was therefore admitted to our hospital. Thoracic X-ray and CT scans revealed oval lesions in the bilateral lungs. A bacterial isolate from the sputum was identified to be *Nocardia elegans* (*N. elegans*) on comparative 16S rRNA gene sequencing and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). The patient recovered following treatment with imipenem/cilastatin and amikacin. To the best of our knowledge, this is the first case of nocardiosis caused by *N. elegans* identified on MALDI-TOF MS.

25. *Aspergillus huiyanae* sp. nov., a teleomorphic species in sect. Fumigati isolated from desert soil in China.

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Aspergillus huiyanae, a new teleomorphic species isolated from desert soil in Xinjiang, China, was described and illustrated. *Aspergillus huiyanae* is characterized by its yellowish white to pale yellow cleistothecia, broadly lenticular ascospores with two equatorial crests and irregularly ribbed to slightly reticulate convex surfaces, and subglobose to ovate or broadly ellipsoidal conidia with smooth walls. This species was supported further by the analyses of the β -tubulin, calmodulin and actin gene sequences.

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高橋 PI (微生物創生) プロジェクト

Project for Systems Biology of Microorganisms

研究概要 (Summary)

当分野では、計算機を駆使して新たな生物学的知見の発見を目指しています。一つは、膨大な実験データを対象にデータ処理技術の開発を通じた生命の理解を目指した「バイオインフォマティクス」の研究を行っています。また、生命を真にシステムとして理解することを目的とした「システムズバイオロジー」の研究も進めています。

Our research areas are Systems Biology and Bioinformatics. 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.

准 教 授 高橋 弘喜

Associate Professor

Hiroki Takahashi

1. Whole-Genome Comparison of *Aspergillus fumigatus* Strains Serially Isolated from Patients with Aspergillosis.

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The emergence of azole-resistant strains of *Aspergillus fumigatus* during treatment for aspergillosis occurs by a mutation selection process. Understanding how antifungal resistance mechanisms evolve in the host environment during infection is of great clinical importance and biological interest. Here, we used next-generation sequencing (NGS) to identify mutations that arose during infection by *A. fumigatus* strains sequentially isolated from two patients, one with invasive pulmonary aspergillosis (IPA) (five isolations) and the other with aspergilloma (three isolations). The serial isolates had identical microsatellite types, but their growth rates and conidia production levels were dissimilar. A whole-genome

comparison showed that three of the five isolates from the IPA patient carried a mutation, while 22 mutations, including six nonsynonymous ones, were found among three isolates from the aspergilloma patient. One aspergilloma isolate carried the *cyp51A* mutation P216L, which is reported to confer azole resistance, and it displayed an MIC indicating resistance to itraconazole. This isolate harbored five other nonsynonymous mutations, some of which were found in the *afyap1* and *aldA* genes. We further identified a large deletion in the aspergilloma isolate in a region containing 11 genes. This finding suggested the possibility that genomic deletions can occur during chronic infection with *A. fumigatus*. Overall, our results revealed dynamic alterations that occur in the *A. fumigatus* genome within its host during infection and treatment.

2. Antisense Transcription Regulates the Expression of the Enterohemorrhagic *Escherichia coli* Virulence Regulatory Gene *ler* in Response to the Intracellular Iron Concentration.

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Enteric pathogens, such as enterohemorrhagic *E. coli* (EHEC) O157: H7, encounter varying concentrations of iron during their life cycle. In the gastrointestinal tract, the amount of available free iron is limited because of absorption by host factors. EHEC and other enteric pathogens have developed sophisticated iron-responsive systems to utilize limited iron resources, and these systems are primarily regulated by the Fur repressor protein. The iron concentration could be a signal that controls gene expression in the

intestines. In this study, we explored the role of iron in LEE (locus for enterocyte effacement) virulence gene expression in EHEC. In contrast to the expression of Fur-regulated genes, the expression of LEE genes was greatly reduced in fur mutants irrespective of the iron concentration. The expression of the *ler* gene, the LEE-encoded master regulator, was affected at a post-transcription step by fur mutation. Further analysis showed that the loss of Fur affected the translation of the *ler* gene by increasing the intracellular concentration of free iron, and the transcription of the antisense strand was necessary for regulation. The results indicate that LEE gene expression is closely linked to the control of intracellular free iron homeostasis.

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バイオリソース管理室

Management of Unit of Microbiological Resources

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. New species in *Aspergillus* section *Fumigati* from reclamation sites in Wyoming (U.S.A.) and revision of *A. viridinutans* complex.

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The *Aspergillus viridinutans* complex includes morphologically similar, soil-inhabiting species. Although its species boundaries have not been fully defined, many isolates from the complex have been isolated as opportunistic human and animal pathogens. In the present study, these species were dominant in spoil sites subjected to various types of reclamation management after coal mining. These species were characterised using two different PCR-fingerprinting methods, sequence data from the β -tubulin (*benA*) and

calmodulin (*caM*) genes, macro- and micromorphology (optical and scanning electron microscopy), maximum growth temperatures and mating experiments. In addition, RNA polymerase II gene (*RPB2*), actin (*act1*) and ITS sequences were deposited for the ex-type isolates of newly described species. The mating experiment results, phylogenetic analyses and ascospore morphology suggested the presence of five species in the *A. viridinutans* complex. *Aspergillus aureolus* (syn. *Neosartorya aureola*) was the only homothallic species. Three species, *A. felis*, *A. udagawae* (syn. *N. udagawae*) and *A. wyomingensis* sp. nov., were heterothallic and their morphologically distinguishable teleomorph was induced by systematic mating experiments. *Aspergillus viridinutans* s. str. seems to be a very rare species and was represented only by the ex-type isolate in which the MAT1-1 locus was amplified. *Aspergillus viridinutans* and *A. aureolus* were typified in accordance with the rules of the new botanical code. Other species outside the *A. viridinutans* complex isolated from the reclamation sites were *A. fumigati*affinis and *A. lentulus* as well as two new sister species, *A. brevistipitatus* sp. nov. and *A. conversis* sp. nov. which were closely related each to other and to *N. papuensis*. Both new species are phylogenetically distant from all anamorphic species and resemble *A. brevipes*,

A. duricaulis and *A. unilateralis* in micromorphology and are distinguishable from each other by the slower growth of *A. conversis* on all tested media. Interestingly, no isolate from the reclamation sites represented *A. fumigatus* s. str. which is usually reported as the dominant species from the section *Fumigati* in soil.

2. Occurrence, detection and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health.

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Heat-resistant fungi are often factors causing spoilage of heat-processed products, especially fruit. Contamination of agricultural raw materials is often as a result of their contact with the soil. Materials contaminated by spores of heat-resistant fungi can be a risk to consumers' health by toxic metabolites (mycotoxins) produced by these microorganisms. Due to the resistance of the fungus to high temperatures they are able to survive the industry pasteurization process. Therefore, the only way to prevent the development of these microorganisms in the product is suitable selecting material by conducting tests for the presence of heat-resistant fungi. The use of traditional culture methods is long and, therefore, does not apply in the selecting raw materials for production. However, time is a critical factor in assessing the acceptance or rejection of a given batch of raw material, due to the necessity of processing the raw material fresh, which is very important especially in the case of fruit. Due to the sparse literature on rapid detection techniques for heat-resistant fungi in agricultural raw materials in recent years researchers are looking for methods, effective in the detection of these pathogens. This review includes characterization of occurrence,

detection and molecular and metabolic characterization of heat-resistant fungi and their risk to human health.

3. Risk analysis and rapid detection of the genus *Thermoascus*, food spoilage fungi.

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Recently the numbers of spoilage accidents in food industry by the species of *Thermoascus* are increasing, but the risk of food spoilage have never been evaluated. It became obvious that their heat-resistances were higher than those of other heat-resist fungi, *Byssosclamyces*, *Hamigera* and *Neosartorya* by our analyses. On the other hand, *T. aurantiacus* and *B. verrucosa* had the *idh* gene, but they showed no patulin production in Potato dextrose broth or Czapek-glucose medium. Therefore, *Thermoascus* must be discriminated from other fungi in the food industry. We developed a rapid and highly-sensitive method of detecting *Thermoascus* in the genus level by using PCR. This method is expected to be extremely beneficial for the surveillance of raw materials in the food production process.

4. Phylogeny, identification and nomenclature of the genus *Aspergillus*.

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Aspergillus comprises a diverse group of species based on morphological, physiological and phylogenetic characters, which significantly impact biotechnology, food production, indoor environments and human health. *Aspergillus* was traditionally associated with nine teleomorph genera, but phylogenetic data suggest that together with genera such as *Polyphaecilum*, *Phialosimplex*, *Dichotomomyces* and *Cristaspora*, *Aspergillus* forms a monophyletic clade closely related to *Penicillium*. Changes in the International Code of Nomenclature for algae, fungi and plants resulted in the move to one name per species, meaning that a decision had to be made whether to keep *Aspergillus* as one big genus or to split it into several smaller genera. The International Commission of *Penicillium* and *Aspergillus* decided to keep *Aspergillus* instead of using smaller genera. In this paper, we present the

arguments for this decision. We introduce new combinations for accepted species presently lacking an *Aspergillus* name and provide an updated accepted species list for the genus, now containing 339 species. To add to the scientific value of the list, we include information about living ex-type culture collection numbers and GenBank accession numbers for available representative ITS, calmodulin, β -tubulin and RPB2 sequences. In addition, we recommend a standard working technique for *Aspergillus* and propose calmodulin as a secondary identification marker.

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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、2012年度より第3期が開始された。

NBRP病原微生物中核機関である千葉大学真菌医学研究センター（病原真菌・放線菌）、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, in FY2012, the third phase has started.

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を中心に、上記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、現地食物の多くが、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ました。結果は昨年度、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。また新たに現地で、エイズ患者の命を奪う主な原因である真菌感染症、特にクリプトコッカス属菌による感染を中心に疫学的調査を計画しています。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、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、日本人の医療やQOLの維持にも、将来大きく貢献するはずで

特に平成26年度には、ケニアの地域に伝わり冠婚葬祭時に愛飲される地ビールが、原料となるトウモロコシ、ヒエなどに生息するカビの産生する毒によって、人体に有害なレベルまで汚染されていることを発表しました（論文参照。また地元の新聞に掲載）。同年10月からは、ケニア中央研究所の研究員Olga氏を日本に招き、ケニアの食糧を汚染するカビとカビ毒、皮膚真菌症、エイズ患者のクリプトコッカス症などについて共同で研究を続けています。



2012年2月 ケニア国キスム市の病院・研究施設前

Under assistance of Kenya Research Station, Inst. NEKKEN, Nagasaki Univ., we are analyzing toxins contaminating major local grains (maize, wheat) and milks, and also producer fungi. We found the local foods are contaminated by the toxins at concentrations far above the international standards. The result has been announced in newspapers, and received large attention. A new project for epidemiological study of Cryptococcal fungi in HIV-infected patients is launched in collaboration with Kenya Medical Res. Inst. (KEMRI) and doctors from UCSF, USA.

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.

アスペルギルス症を中心とした新興真菌症制圧プロジェクト

The Project on Controlling Aspergillosis and the Related Emerging Mycoses

アスペルギルス症はわが国を始めとする先進諸国でも多くの患者が失われている真菌症であり、いずれの国でも大きな問題となっている。様々な病型を取るが、侵襲性肺アスペルギルス症と慢性肺アスペルギルス症が、頻度、予後などの点からも早急な解決を求められている疾患といえる。このような病態の相違に加え、同じ慢性肺アスペルギルス症の間でも症例により様々な活動性の違いがあることや同一症例でありながら急速な活動性の増悪を見ることなどの点がアスペルギルス症の大きな特徴であり、これらは本菌による感染機構を理解する上で重要と考えられる。これらの病態における菌株の比較解析や動物モデルによる解析などにより病原因子や感染機構の解明、さらには治療法開発への進展が期待される。

我々は昨年度、慢性肺アスペルギルス症と侵襲性肺アスペルギルス症それぞれの患者（計8人）から単離した原因菌を比較ゲノム学的に解析し、ゲノムには病態にリンクする特徴を見いだせないことを報告した。今回は、さらにこの研究を発展させ、慢性肺アスペルギルス症と侵襲性肺アスペルギルス症各1例の患者から、経時的に単離したアスペルギルス・フミガータス計8菌株を用い、感染中あるいは治療中に同菌に起こる遺伝子突然変異について解析した。今回の我々の症例では、治療期間中に菌の交替が見られなかったにも拘わらず、単離した菌はそれぞれ様々に異なる遺伝子変異を獲得しており、その中には、治療に用いたアゾール薬に対する耐性を与える突然変異なども見いだされた。この結果は、アスペルギルス症原因菌が、感染後も患者体内で様々な遺伝子変異を獲得し多様化して生存することを示唆する。

また、慢性肺アスペルギルス症の経過中に出現することがある急性増悪の原因解明を目的とし動物実験モデルの作成を試みている。マウスに菌体由来物質を経気道的に反復投与を行うことによって投与終了後早期（72時間まで）では肺動脈周囲への炎症細胞の集簇が観察され、

その後（投与終了後7日以降）に肺胞領域の散在性の肉芽腫様病変の形成が認められるなど興味深い知見が得られた。

Aspergillosis takes various forms such as acute invasive aspergillosis and chronic necrotizing pulmonary aspergillosis (CNPA), and is the most serious and important fungal infection in developed countries. However, little is known as to its mechanism of infection.

To learn the mechanism of pathogenicity of *A. fumigatus*, we performed comparative genome analysis of *A. fumigatus* strains isolated from total eight IPA (invasive pulmonary aspergillosis) and CNPA (chronic necrotizing pulmonary aspergillosis) patients, and reported there was no genomic difference found which links to the pathological conditions. In the last year, we developed the research and compared genome sequences of five and three strains isolated sequentially from an IPA and CNPA patients, respectively. Based on MultiLocus Microsatellite typing, we found no alteration of causative agents within the patients during the observation. However, we found the eight isolates have their intrinsic genetic mutations of their own, including a mutation conferring resistance to an azole-drug, which was used in a treatment. Our results indicate *A. fumigatus* strains continuously produce several mutant strains even after dwelling in human body. To better understand the mechanism of the exacerbation frequently seen in CNPA patients, we made an analysis of mice, which were repeatedly given fungal extracts intratracheally. After repeated instillation, pathological examinations showed infiltrate of inflammatory cells around arteries in 72hs, and the development of granulomatous changes in alveolar area in 7days, which may be related to the exacerbation process in patients.

平成 25 年度 共同利用・共同研究報告

2013 Fiscal Year Cooperative Research Program Report

研究課題 '13-1

新規抗真菌薬開発を目指したアスペルギルス属糸状菌の薬剤耐性機構とシグナル伝達機構の解析

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Molecular mechanisms of drug-resistance and signal transduction in *Aspergilli* and their application to development of new antifungal drugs

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研究成果

(1) アスペルギルス属糸状菌のアゾール系薬剤耐性に関する ABC トランスポーターおよびエルゴステロール生合成系を制御する転写因子の機能解析

麹菌において見出されたアゾール系薬剤耐性に関する新規 Zn₂Cys₆型転写因子 AtrR の *Aspergillus fumigatus* のオーソログ破壊株の RNA-seq 解析により, AtrR は薬剤排出 ABC トランスポーターだけでなく, エルゴステロール生合成酵素遺伝子の発現にも関わっていることが示された. 興味深いことに, エルゴステロール生合成酵素遺伝子は bHLH 型転写因子 SrbA 制御下のものと一致していたことから, これらの遺伝子発現が AtrR と SrbA の 2 種類の転写因子によって協調的に制御されている可能性が強く示唆された. 麹菌の atrR 破壊株は srbA 破壊株よりもアゾール系薬剤に対して高い感受性を示し,

atrR/srbA 二重破壊株と同等の感受性を示したことから, AtrR はエルゴステロール生合成のみならず薬剤排出トランスポーターも直接的に制御していることが示された. また, 両末端に GFP を連結させた AtrR と SrbA の細胞内局在解析を行ったところ, AtrR は構成的に核に局在し, SrbA は核膜または小胞体周辺に局在していた.

一方, *A. fumigatus* の AtrR 破壊株では麹菌と同様アゾール系薬剤に超感受性を示し, 標的分子 Cyp51A の発現が著しく低下していることが明らかになった. さらに, アゾール耐性に重要であると報告されている ABC トランスポーター Cdr1B の発現も低下しており, 感染治療において重要なアゾール系薬剤の応答に AtrR が中心的な機能を果たしていることが示唆された. また, AtrR 破壊株の低酸素条件下における生育が著しく悪くなるとともに, マウスを用いた感染実験の結果から病原性も顕著に低くなっていることが明らかとなったことから, 本菌の病原性にも深く関与していると推察された.

(2) アスペルギルス属糸状菌の浸透圧シグナル伝達系の機能解析と浸透圧シグナル伝達系下流致死因子の探索

Aspergillus nidulans を中心に二成分性情報伝達系 (TCS)-HOG MAP キナーゼ (MAPK) 経路が浸透圧応答シグナル伝達系として解析されてきた. ゲノム情報から *Aspergillus* 属糸状菌においては, 浸透圧シグナル伝達系が保存されている. TCS は, ヒスチジンキナーゼ (HK) → リン酸基中間因子 (YpdA) → レスポンスレギュレーター (RR) からなり, 下流 HOG-MAPK 経路を負に調節している. そのうち TCS の YpdA は必須因子であり, 新規創薬標的である. TCS の阻害によるシグナル伝達の遮断は, 下流経路を構成的に活性化して致死性を示すと考えられている. これまでに *A. nidulans* の sskA と srrA 遺伝子の単独および二重欠損株を作出し, さらにそれら欠損株において ypdA 遺伝子の条件発現株の造成を行った. 本年度は, これら ypdA 遺伝子条件発現株の ypdA 発現抑制時の表現型を詳細に解析した. ypdA 遺伝子の発現抑制は死形質を示し, ypdA 遺伝子発現抑制下に srrAΔ と srrAΔ

の単独破壊導入で生育が部分回復し, *sskAΔ srrAΔ* 二重破壊で完全回復した. 転写抑制により *ypdA* 遺伝子転写産物は非抑制時の10%以下に低下し, 抗YpdAペプチド抗体でのWestern解析からもYpdA蛋白質量が10%以下に低下した. YpdA発現量の低下に伴い, HogA MAPKの構成的リン酸化が確認された. *sskAΔ* は隔壁間長が長くなる形質を示すが, *ypdA* 遺伝子発現抑制下での *sskAΔ srrAΔ* 導入では, 隔壁間長が長いままで菌体量が野生型並に回復した.

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研究課題 '13-2

Cryptotoccus neoformans の特異なゲノム安定化機構の分子基盤

—それを標的とした新規治療戦略を目指して

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Molecular basis for specific regulation of genome integrity in *Cryptococcus neoformans* and its application to the development of novel therapeutic strategies

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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, さらにこのような特有のゲノム維持機構が *C. neoformans* の生活環とどのように関連し, あるいはどのように制御されているかを明らかにすることを目的とした.

本年度はまず, *C. neoformans* 一倍体細胞にテロメアDNA伸長酵素テロメラーゼの相同遺伝子 *CnEST2* の遺伝子破壊コンストラクトを導入することで, *CnEST2* 欠損細胞の作製を試みた. 得られた *CnEST2* 欠損細胞では染色体末端の構造が大きく変化していることが見いだされ, このことから, 本菌においても他生物種と同様, 染

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。CnEST2欠損細胞におけるゲノム再編成の分子機構を明らかにするため、真菌医学研究センター所蔵の次世代シーケンサーを用いて全ゲノムシーケンスを行ったところ、野生型株において複数のテロメア末端近傍に特異的に存在するレトロトランスポゾン様配列 *CnII* が、CnEST2欠損細胞で高頻度に増幅し、タンデムに並んで存在することが明らかになった。しかしながら、*CnII* が自身の活性により別の染色体座位に転移したことにより生じる新たな連結点の数は染色体末端の数に比べて少ないため、トランスポゾン様配列がゲノム中でコピー数を増加する要因として、主として組換えを介した機構が関与している可能性が考えられる。

C. neoformans における特異なDNA修復経路選択の分子機構をさらに解析するため、相同組換え、非同相末端結合に関わる進化的に保存された遺伝子の探索を行ったところ、ゲノム中に *RAD52*, *RAD51*, *MRE11*, *DMC1* の相同遺伝子を見いだした。それらのゲノム修復における役割を破壊株を作製することにより検討した結果、出芽酵母の相同遺伝子欠損の場合とは異なる表現型が観察された。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たいと考えている。

研究課題 '13-3

病原真菌における一酸化窒素 (NO) の生成機構と生理的役割

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Synthetic mechanism and physiological role of nitric oxide in pathogenic fungus

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研究成果

病原真菌はヒトに感染する際、自然環境と生体内における劇的な環境変化(温度、酸素濃度、栄養など)によるストレスに曝されている。真菌はこのようなストレスに応答し、耐性を獲得することで、増殖し、病原性を示すことから、一酸化窒素(NO)がシグナル分子として、真菌のストレス耐性や病原性の発揮に関わる可能性が考えられる。本研究では、酵母 *Saccharomyces cerevisiae* に見出したNOの生成機構と生理的役割について、NO生成に関与する酵素(Mpr1, Tah18)のオルソログが存在する病原真菌を用いて解析する。平成25年度には、以下の研究成果が得られた。

まず、*Aspergillus fumigatus* や *Candida glabrata* については、プロモーター部位を改変したTah18オルソログ遺伝子の発現抑制株をそれぞれ作製し、解析を行った。その結果、*C. glabrata* のTah18発現抑制株を用いたカイコ感染実験では、野生型株と比較して毒性が顕著に低下していることを見出した。また、*Cryptococcus neoformans* については、Mpr1オルソログ遺伝子破壊株を作製し、解析を行った。その結果、50℃の熱ショックに対し、野生型株より高い感受性を示すことを見出した。

今後、Mpr1, Tah18の遺伝子破壊株、発現抑制株、過剰

発現株などを用いて、細胞内NOレベルを定量するとともに、ストレス耐性などの表現型を観察することで、病原真菌においてもMpr1やTah18がNOの生成と生理機能に關与するかどうか考察を行う。

研究課題 '13-4

マイコウイルス由来新規抗菌性タンパク質の単離とそれを利用した抗病原性真菌剤の開発

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Screening for antifungal proteins in mycoviruses and development of antifungal agents

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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ORF 4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。

本研究においては、MoCV1-A ORF4完全長(820残基)が、ヒト病原性酵母 *Cryptococcus neoformans* に対しても生育阻害効果を有するか否かの検討を行った。川本進教授の研究室で開発された *C. neoformans* 発現ベクターにMoCV1-A ORF4タンパク質を発現させたところ、パン酵母ADH1プロモーターの共役により、MoCV1-A ORF4タンパク質発現が確認され、ORF4発現の *C. neoformans* では、異常な液胞化や生育速度の減少、そして莢膜多糖形成の抑制が確認することができた(原著論文1)。

現在、MoCV1-A ORF4に関しては、パン酵母遺伝子

発現系を利用して、活性中心領域を明らかにする研究を行っており、今後は、短縮など加工されたORF4タンパク質を *C. neoformans* に発現させたり、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。

アスペルギルス症の原因となり、重篤化をもたらす病原真菌 *Aspergillus fumigatus* に対する新たな薬剤開発を目的として、五ノ井教授の研究グループは、*A. fumigatus* を弱毒化する新規なマイコウイルスの探索とその応用研究を、高橋梓博士を中心として行っているが、本研究課題においては、マイコウイルス探索や同定に関して共同研究を行っている。これまでの探索研究の結果、4種のマイコウイルス由来の2本鎖RNAゲノムが同定されており、このうち、2種に関しては、宿主菌に生育阻害をもたらすことなどが確認できており、マイコウイルスである *Partitiviridae* 属と *Chrysoviridae* 属に分類されるが、既報のウイルスとは類似性は示すが同一ではなく、新種であることが確認されている。マウス感染を用いた実験でも病原性の抑制効果が示されており、今後、宿主菌に対する作用機作などについての検討や、上述したMoCV1-A ORF4の効能などについても、共同研究を継続させることで実施していくことが望まれる。

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研究課題 '13-5

病原性を有する *Aspergillus niger* 及び醸造黒麹菌のアレルゲン遺伝子の検索

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Systematic screening of allergen genes of pathogenic *Aspergillus niger* and domestic *Aspergillus* fungi

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研究成果

発酵や醸造は古来より人類が行ってきたもので、食品の保存性の向上, 味覚の向上, 有用化学物質の生成などをもたらす, 人類の発展, 食文化の発展, また, 食品産業へ非常に大きな貢献をしている。発酵・醸造技術が産業化することは, 発酵・醸造食品由来の真菌の操作が大規模に行われることであり, 作業者が大量の真菌の暴露を受けることを意味する。また, 食品処理施設内に, 当該の真菌が定着することを示す。すなわち真菌を利用する産業分野においては, 単一種の真菌に, 大量かつ長期的に暴露を受けることとなる。アレルギー性疾患が, 我

が国の国民病として認知されている今日, 人に大量かつ長期の感作をもたらす真菌のアレルゲン性の有無を検証する研究は意義深いと考える。現在まで, *Aspergillus fumigatus*によるアレルギー性肺炎が真菌が原因のアレルギーとして広く認知されているが, 上述した産業利用されている真菌群については, 研究がなされていない。本研究では *Aspergillus niger* および醸造黒麹菌が保有する可能性のあるアレルゲン遺伝子を, ゲノム情報を利用して同定するための第一段階として, カビアレルゲンデータベースの作製とゲノム研究への応用手法を検討した。

国際免疫学会が保持するアレルゲンデータベースから, カビアレルゲン部分を抽出し, リスト化した。その際, 各アレルゲンのアレルゲン性の強弱を評価し, リストに追加した。具体的には, アレルゲン遺伝子の同定後, 組換えアレルゲンタンパク質が作製され, あるいは, 粗抽出物から精製され, 当該真菌アレルギーと臨床診断された患者血清中のIgE抗体が結合することが確かめられたアレルゲン, および同アレルゲンタンパク質が臨床診断にまで応用できているアレルゲン, 不純物を含んだ粗抽出標品を用いた臨床成績によってのみ同定されたアレルゲンとして, その強弱をランキングした。これらの情報を総括し, インターネット上での検索に適合するよう, カビアレルゲンデータベースを構築した。*Aspergillus*属真菌については, その2種のゲノム情報が公開されている。作製したカビアレルゲンデータベース中のアレルゲン遺伝子に相同性のある遺伝子群が, 2種の *Aspergillus*属真菌中にあるかどうか, BLAST検索を行い, その結果をデータベース内に追加し, ゲノム中の遺伝子にアクセスできるようにした。構築したカビアレルゲンデータベースは, 一般サーバーを利用した公開を経て, 現在は岩手大学中のサーバー中に保持し, 運営を続けている。

公開データベース

・カビアレルゲンデータベース: <http://fungusallergen.agr.iwate-u.ac.jp/>

研究課題 '13-6

病原真菌の mild heat stress 応答分子の探索と診断マーカーへの応用

長 環 (福岡歯科大学)
永尾潤一 (福岡歯科大学)
中山浩伸 (鈴鹿医療科学大学)
知花博治 (千葉大学真菌医学研究センター)

The search of mild heat shock response molecules and application to a diagnosis marker for the pathogenic fungi

Tamaki Cho (Fukuoka Dental College)
Junichi Nagao (Fukuoka Dental College)
Hironobu Nakayama
(Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science)
Hiroji Chibana
(Medical Mycology Research Center, Chiba University)

研究成果

体外から39℃～40℃の温和な加温 (mild heat stress) をして骨折や癌の治療効果を上げる温熱療法がある。一方で、ヒトが病原体に感染すると体温は39℃～40℃に上昇し、これによる宿主免疫の活性化が亢進されることが知られている。すなわち、mild heat stressはヒト細胞に重要な生物活性を誘導すると考えられる。病原真菌 *Candida albicans* がカテーテルなど医療器具にバイオフィルムを形成しやすく、さらにバイオフィルムという増殖形態により薬剤に抵抗性を示すことが臨床現場で問題視されている。我々は、*C. albicans* のバイオフィルム形成時の抗真菌薬効果を37℃ (通常体温相当) と39℃、41℃ (発熱体温相当) を比較検討した。その結果、細胞壁合成阻害薬のミカファンギンは16倍、エルゴステロール合成阻害薬のフルコナゾールは4倍と37℃と比較して感受性がそれぞれ増加した (Cho T et al, 2012)。さらにこの温度下では菌糸形優位のバイオフィルムを形成する傾向が観察された。*C. albicans* のバイオフィルム形成に対する37℃と39℃の温度の影響を調べるために、マイクロアレイ法による網羅的な遺伝子発現解析を行った。その結果、

37℃でのバイオフィルム形成に比べ39℃のバイオフィルム形成では、2℃の温度差で酵母形増殖にかかわる蛋白遺伝子が強く抑制され、一方で39℃において菌糸に特異的な表層タンパク遺伝子の発現が強くなった。本研究における mild heat stress は宿主の発熱刺激を想定したもので、そのような環境下で変動する病原真菌の細胞表面蛋白質は、宿主応答の有力な候補になると思われる。

研究課題 '13-7

Schizophyllum commune による気管支喘息重症化メカニズムの解明

廣瀬晃一 (千葉大学大学院医学研究院)
渡邊 哲 (千葉大学医学部附属病院)
中島裕史 (千葉大学大学院医学研究院)
豊留孝仁 (帯広畜産大学)
亀井克彦 (千葉大学真菌医学研究センター)

Roles of sensitization to *Schizophyllum commune* in the development of severe asthma

Koich Hirose
(Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University)
Akira Watanabe
(Department of Control and Treatment of Infectious Diseases, Chiba University Hospital)
Hiroshi Nakajima
(Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University)
Takahito Toyotome
(Obihiro University of Agriculture and Veterinary Medicine)
Katsuhiko Kamei
(Medical Mycology Research Center, Chiba University)

研究成果

真菌への暴露、感作は喘息の重症化に関与することが知られている。スエロタケ (SC) は喘息、アレルギー性気管支肺真菌症 (ABPM) の発症に関与することが報告されている真菌だが、これまでにSC特異的抗体の効率

的なスクリーニング方法の報告は無く、喘息患者におけるSC感作率は不明である。我々はこれまでの共同研究によりSCによるABPM患者血清を用いて主要抗原を探索・同定し、この主要抗原を用いてSC特異的抗体測定ELISA法を確立した。このELISA法を用いて喘息患者における感作率を検討した結果、47名の喘息患者（Step2 6名、Step3 29名、Step4 12名）中、4名がSC特異的IgG陽性、6名がSC特異的IgE陽性であることが明らかとなった。さらにSC特異的抗体陽性喘息患者と陰性喘息患者の呼吸機能を比較した結果、SC特異的IgE陽性喘息患者は陰性喘息患者に比較し1秒率（FEV1.0）が有意に低下していることが明らかとなった。

これまでの報告でも真菌感作が生じている喘息患者においては、複数の真菌に感作が生じている傾向が見られたため、本研究ではアスペルギルス、カンジダ、アルテルナリアに対する特異的IgE抗体の有無を検討した。その結果、SC特異的IgE抗体陽性喘息患者は、陰性喘息患者に比較し有意にアスペルギルス感作が高率に成立していることが明らかとなった。今後はSC感作が喘息患者における呼吸機能低下の独立した因子であるか否か、症例数を増やした多変量解析が必要と考えられた。

研究課題 '13-8

海洋生物を素材とした抗真菌剤の開発

五ノ井透（千葉大学真菌医学研究センター）
小林淳一、久保田高明
（北海道大学大学院薬学研究院）

Development of antifungal agents from marine microorganisms

Tohru Gonoï
(Medical Mycology Research Center, Chiba University)
Jun'ichi Kobayashi, Takaaki Kubota
(Graduate School of Pharmaceutical Sciences, Hokkaido University)

研究成果

沖縄で採取した*Agelas*属の海綿より単離したプロモピロールアルカロイドNagelamide類、*Amphimedon*属の海綿

より単離したマンザミンアルカロイドZamamiphidin A、*Hyrtios*属の海綿より単離したインドールアルカロイドHyrtimomine類、*Plakortis*属の海綿より単離したオキシリピンManzamenone類、Spongiidae科の海綿より単離したメロテルペノイドNakijiquinone SおよびNakijinol Cならびにアセチレン脂肪酸誘導体Taurospongins類に、抗菌および抗真菌活性が認められた。

今後は、特異性の高い抗真菌活性を示す化合物の探索を継続して行う予定である。

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平成 25 年度 共同利用・共同研究研究会報告

2013 Fiscal Year Cooperative Research Meetings Report

研究会ー1

感染症研究

グローバルネットワークフォーラム2013

山本友子 (千葉大学大学院薬学研究院)
高屋明子 (千葉大学大学院薬学研究院)
佐藤慶治 (千葉大学大学院薬学研究院)
米山光俊 (千葉大学真菌医学研究センター)
川本 進 (千葉大学真菌医学研究センター)
亀井克彦 (千葉大学真菌医学研究センター)

Infectious diseases research network forum 2013

Tomoko Yamamoto, Akiko Takata, Keiji Sato
(Department of Microbiology and Molecular Genetics,
Graduate School of Pharmaceutical Sciences, Chiba
University),
Mitsutoshi Yoneyama, Susumu Kawamoto,
Katsuhiko Kamei
(Medical Mycology Research Center, Chiba University)

研究成果

千葉大学学内で進められている数多くの感染症研究についてこれらをネットワーク化し、更なる研究のグレードアップを目指して、昨年度から開始された【千葉大学感染症研究ネットワーク】であるが、第2回目となる今年度は平成25年11月30日(土)に千葉大学薬学部120周年記念講堂(医薬系総合研究棟Ⅱ 1階)にて開催された。前回の「学内の研究ネットワーク化」からさらに進展させ、全国さらに国際的な感染症研究のネットワーク化を目指し、会の名称も「感染症研究グローバルネットワークフォーラム」と変更された。内容は学内の感染症研究グループによる一般演題(計8題)に加え、第一線で活躍しておられる国内外の4名の先生方による特別講演が行われ、まさにグローバルの名称にふさわしい内容となった。参加者は118名に達し、全体を通じて非常に

活発な議論が行われた。なお、プログラムは以下のとおりである。

開会の挨拶

山本友子 (薬学研究院 教授)

セッション1

座長: 松田和洋

(エムバイオテック (株) マイコプラズマ感染症
研究センター長)

石和田稔彦 (医学部附属病院 講師)

「小児細菌性髄膜炎予防ワクチンと日本への導入効果に
関する研究」

西村 基 (医学部附属病院/医学研究院 助教)

「臨床細菌検査の動向

—質量分析計による細菌同定など—

野呂瀬一美 (医学研究院 助教)

「トキソプラズマ性網脈絡膜炎の病態解析

—ケモカインとT細胞の動態—

特別講演1

座長: 西城 忍 (真菌医学研究センター 准教授)

金城雄樹 (国立感染症研究所 室長)

「感染免疫におけるiNKT細胞の役割」

特別講演2

座長: 伊藤素行 (薬学研究院 教授)

常世田好司

(German Rheumatism Research Centre Berlin,
Department Head)

「感染を記憶する骨髄」

セッション2

座長: 高橋弘喜 (真菌医学研究センター 准教授)

星野忠次 (薬学研究院 准教授)

「2価金属原子対を標的とした抗ウイルス薬の開発」

神田達郎（医学研究院 講師）

「B型肝炎ウイルスに対する肝細胞自然免疫応答」

特別講演 3

座長：八尋錦之助（医学研究院 准教授）

飯田哲也（大阪大学微生物病研究所 教授）

「次世代DNAシーケンサを用いた感染症研究」

セッション 3

座長：米山光俊（真菌医学研究センター 教授）

西城 忍（真菌医学研究センター 准教授）

「C型レクチンと真菌感染防御機構」

西田篤司（薬学研究院 教授）

「アレルギー性気管支肺真菌症患者から単離された
schizophyllum commune（和名：スエヒロタケ）が
生産する化学物質」

特別講演 4

座長：高屋明子（薬学研究院 准教授）

荒川宜親（名古屋大学大学院医学系研究科 教授）

「新型多剤耐性グラム陰性菌が獲得した耐性機構と
それらの地球規模での拡散」

閉会の挨拶

笹川千尋

（真菌医学研究センター長，東京大学名誉教授，日本
生物科学研究所常務理事）

2014年講演会

2014 Scientific Meetings & Seminars

- 1) 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25年度 成果報告会

【午後の部】

医科学研究所と千葉大学真菌医学研究センターとの 合同成果報告会

特別講演：

亀井克彦
(千葉大学真菌医学研究センター 教授)

Katsuhiko Kamei
“真菌症の現状と展望”

長谷耕二
(東京大学医科学研究所 客員教授)

Hase Koji
“腸内細菌による免疫エピゲノム修飾作用”

千葉大学真菌医学研究センター成果報告：

廣瀬晃一
(千葉大学大学院医学研究院 准教授)

Koichi Hirose
“スエヒロタケ (*Schizophyllum commune*) 特異的IgE 抗体測定ELISA法による喘息患者における感作率の検討”

森山裕充
(東京農工大学大学院 准教授)

Hiromitsu Moriyama
“マイコウイルス由来新規抗菌性タンパク質の単離とそれを利用した抗病原性真菌剤の開発”

松浦 彰
(千葉大学大学院融合科学研究科 教授)

Akira Matsuura
“*Cryptococcus neoformans* の特異なゲノム安定化機構の分子基盤－それを標的とした新規治療戦略を目指して－”

久保田高明
(北海道大学大学院薬学研究院 准教授)
Takaaki Kubota
“海洋生物を素材とした抗真菌剤の開発”

感染症・免疫共同研究領域：

猪腰淳嗣
(北里大学 准教授)
Junji Inokoshi
“新規抗ウイルス剤をめざしたRNA干渉制御物質のスクリーニング”

稲田健一
(藤田保健衛生大学 准教授)
Kenichi Inada
“ウイルス感染に伴い形成される遺伝子制御ネットワークの解明とそれに基づく新規診断マーカーと治療標的の同定”

江下優樹, Lucky Ronald Runtuwene
(大分大学 准教授, 大分大学 大学院生)
Yuki Eshita, Lucky Ronald Runtuwene
“ゲノム情報を利用した蚊媒介性疾患制圧のための網羅的発現遺伝子解析”

小川 遼
(東京大学医科学研究所 大学院生)

Ryo Ogawa
“Molecular Structure of Herpesviruses by Cryo-Electron Microscopy”

呉羽 拓
(沖縄科学技術大学院大学 研究員)

Taku Kureha
“胸腺上皮細胞におけるCCR4-NOT複合体の生理学的意義の解明”

牧野晶子
(京都大学ウイルス研究所 特定助教)
Akiko Makino
“内在性フィロウイルスの機能解析”

小澤 真
(鹿児島大学 准教授)
Makoto Ozawa
“A型インフルエンザウイルス感染動態の生体内ラ
イブイメーキング”

伊藤量基
(関西医科大学 准教授)
Itou Tomoki
“ヒトToll様受容体の発現と機能の解析”

日時: 3月7日(金) 午後の部 13時30分～
場所: 東京大学医科学研究所 講堂

2) 第33回知の拠点セミナー(国立大学共同利用・共同
研究拠点セミナーシリーズ)

講 演:

亀井克彦
(千葉大学真菌医学研究センター 教授)
Katsuhiko Kamei
“生活環境におけるカビと健康被害”

日時: 平成26年6月20日(金) 17:30～
場所: 京都大学東京オフィス

3) 第3回感染症研究グローバルネットワークフォー
ラム2014(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,千葉大学COEスタート
アップ“病原体感染と免疫応答の統合的解析拠点”,
東京大学医科学研究所社会連携研究部門,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”,基盤研究A“エフェクターと宿主標的分子間相
互作用を基盤としたサルモネラ感染分子機構の解
明”,厚生労働科学研究費補助金“肝炎等克服実
用化研究事業”(B型肝炎創薬実用化等研究事業)“B
型肝炎における自然免疫の機能解明とその制御によ
る発癌抑止法開発”)

特別講演:

長谷耕二
(慶応義塾大学薬学部大学院薬学研究科 教授,東
大医科学研究所国際粘膜ワクチン開発研究セン
ター粘膜バリア学分野 客員教授)

Koji Hase
“腸内細菌によるエピジェネティクス制御を介した
Treg分化誘導機構の解明”

脇田隆字
(国立感染症研究所・ウイルス第二部 部長)
Takaji Wakita
“DAA時代におけるC型肝炎ウイルス研究”

山崎 晶
(九州大学生体防御医学研究所感染ネットワーク研
究センター免疫制御学分野 教授)

Sho Yamasaki
“結核菌を認識する受容体と免疫応答”

荒瀬 尚
(大阪大学免疫学フロンティア研究センター免疫化
学研究室 教授,大阪大学微生物病研究所 免疫
化学分野 教授)

Hisashi Arase
“ペア型レセプターを介した宿主病原体相互作用”

中山俊憲
(千葉大免疫発生学 教授)
Toshinori Nakayama
“Pathogenic記憶とTh2細胞の形成と維持機構”

一般講演:

相馬亜希子
(千葉大園芸学研究科 助教)
Akiko Soma
“病原性大腸菌O157株の宿主感染に関わる低分子
RNAの同定と機能解析”

高屋明子
(千葉大薬学研究院 准教授)
Akiko Takaya,
“グラム陽性菌の内因性rRNA修飾と薬剤耐性”

村田武士
(千葉大理学研究科 教授)

Takeshi Murata

“創薬標的膜蛋白質の体系的構造解析技術の確立に向けて”

猪狩英俊

(医学部附属病院感染症管理治療部 部長)

Hidetoshi Igari,

“Non-communicable Oiseases が日本の塗抹陽性結核治療成績へ及ぼす影響について”

加藤直也

(東京大学医科学研究所先端ゲノム医科学分野 准教授)

Naoya Kato

“肝炎ウイルス制御後の肝発がん抑止戦略”

日時：平成26年11月15日(土) 9:30~16:50

場所：千葉大学医学部記念講堂

4) 真菌医学研究センター Monthlyセミナー

(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)

石和田稔彦

(千葉大学医学部附属病院 講師)

Naruhiko Ishiwada

“小児細菌性髄膜炎予防ワクチンと日本への導入効果に関する研究”

日時：6月24日(火) 16時~

場所：真菌医学研究センター 大会議室

5) 真菌医学研究センター Monthlyセミナー

(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)

豊留孝仁

(帯広畜産大学 動物・食品検査診断センター 講師)

Takahito Toyotome

“真菌関連疾患から考える真菌と宿主のかかわり”

日時：7月22日(火) 16時~

場所：真菌医学研究センター 大会議室

6) 真菌医学研究センター Monthlyセミナー

(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)

倉田祥一郎

(東北大学大学院薬学研究科 教授)

Shoichiro Kurata

“ショウジョウバエ自然免疫における病原細菌の認識と排除”

日時：9月30日(火) 15時~

場所：真菌医学研究センター 大会議室

7) 真菌医学研究センター Monthlyセミナー

(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)

平原 潔

(千葉大学大学院医学研究院 准教授)

Kiyoshi Hirahara

“CD4 T細胞を介した免疫恒常性の制御およびその破綻による慢性真菌感染症について”

日時：10月7日(火) 16時~

場所：真菌医学研究センター 大会議室

8) 真菌医学研究センター Monthlyセミナー

(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)

尾野本浩司

(千葉大学真菌医学研究センター 助教)

Koji Onomoto,

“細胞質内ウイルス感染センサー RLRの機能解析”

矢部力朗

(千葉大学真菌医学研究センター 特任助教)

Rikio Yabe

“抑制性C型レクチン受容体による骨代謝機構”

日時：12月9日(火) 10時~

場所：真菌医学研究センター 大会議室

- 9) 真菌医学研究センター Monthlyセミナー
(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)

大荒田素子
(千葉大学真菌医学研究センター 助教)

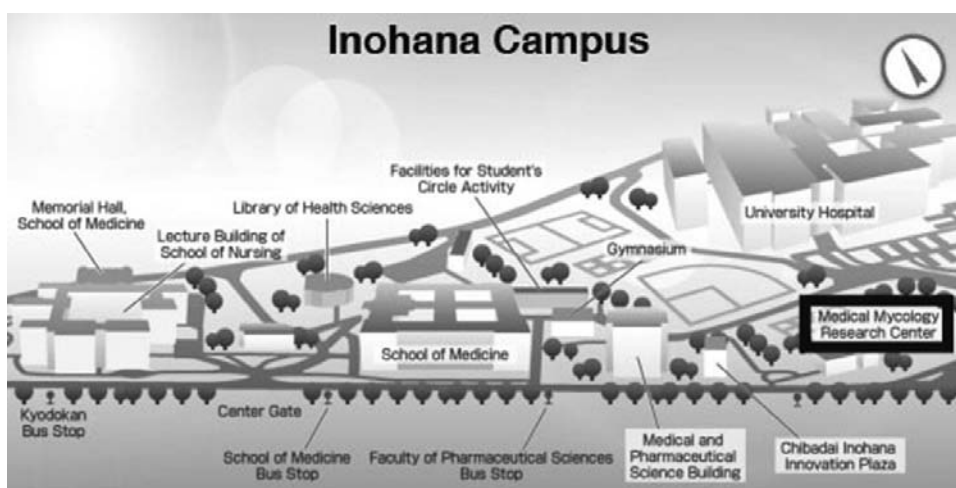
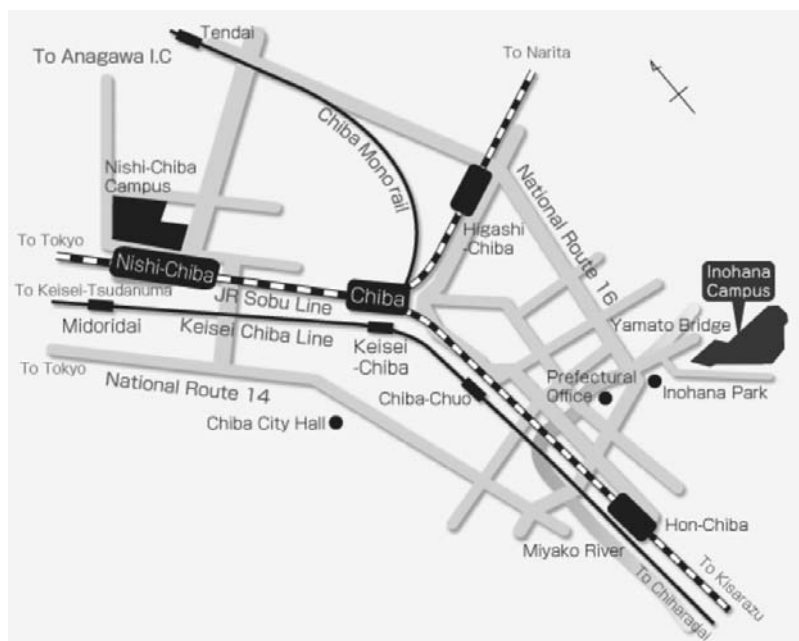
Motoko Oarada
“現代人の食と炎症”

清水公德
(千葉大学真菌医学研究センター 助教)

Kiminori Shimizu
“*Aspergillus niger* のフモニシン産生に関する遺伝子の機能解析”

日時：12月17日（水）10時～

場所：真菌医学研究センター 大会議室



平成 27 年 3 月発行

発行者 千葉大学真菌医学研究センター
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March 2015

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印刷 株式会社 正文社
 Printed by Seibunsha, Ltd. Chiba, Japan



CHIBA UNIVERSITY
2014