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2016 Institut Pasteur Korea Annual Report

2016
Institut Pasteur Korea

Annual Report / 한국파스퇴르연구소 2016년 연간보고서

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2016 French Health Care Day Symposium / 2016 프랑스 헬스케어데이 심포지엄

ANNUAL REPORT CONTENTS

Translational Research Performance in 2016

Achievements in 2016

Mission & Strategy

Research

IPK is a growth engine for Korea's biomedical research industry, working successfully with biotech to develop multi-disciplinary R&D and translate fundamental discovery-based research to enhance human, animal and environmental health.

In keeping with Louis Pasteur's belief, "There is science and the applications of science, bound together as the fruit of the tree which bears it," IPK combines front line biology, IT, and chemistry, with innovative cell-based drug discovery platforms that identify novel molecular targets for potential new therapies to treat diseases. With world-class drug discovery capabilities and a talented team drawn from all over the world, IPK scientists aim to uncover various physiological and pathological processes to devise new strategies for diagnosing, preventing and treating disease. IPK currently focuses on global infectious diseases that represent today's health challenges such as tuberculosis, antibiotic resistance, hepatitis, influenza, cancer, dengue and neglected diseases, as well as preparing for emerging diseases that may impact future health needs.

Additionally, IPK is committed to contributing to Korea's scientific intellectual and technical resources by establishing relevant domestic partnerships with research institutions, academia and industry. IPK recognizes the important role universities and biotech companies' play in research and development of novel drugs and that the future of drug development hinges on the innovative and cooperative efforts of public and private organizations.

To date, the IPK team is approximately 100 strong, working in research laboratories, core technological facilities, project management and administration.

연구

한국파스퇴르연구소는 대한민국 바이오 메디컬 (biomedical) 산업의 성장 동력으로, 바이오 벤처들과 함께 여러 전문 분야에 걸친 R&D 기술을 성공적으로 개발하였으며, 기초 과학을 기반으로 한 응용 연구 기술들을 중개함으로써 인간, 동물, 그리고 환경 보건 증진에 기여하고 있습니다.

"나무에 맺힌 열매와 같이 기초 과학과 응용 과학은 서로 긴밀하게 연결되어 있다"라는 루이 파스퇴르의 믿음에 따라, 한국파스퇴르연구소는 생물학 · 화학 · IT 기술을 결합해 혁신적인 세포 기반의 신약 개발 플랫폼을 구축했습니다. 이 신약 개발 플랫폼을 통해 한국파스퇴르연구소는 잠재적 치료제로 개발 가능성이 있는 새로운 기작을 가진 타깃(target)을 규명할 수 있습니다. 또한 한국파스퇴르연구소 과학자들은 세계 정상급 신약 개발 역량과 전 세계에서 모인 유능한 다국적 연구진들과 함께 새로운 질병 진단법 · 예방법 · 치료법의 초석이 되는 다양한 생리/병리학적 진행 과정을 밝히는 것을 목표로 하고 있습니다. 한국파스퇴르연구소는 오늘날 공중 보건을 위협하는 결핵, 항생제 내성, 간염, 인플루엔자, 암, 뎅기열, 소외질환과 같은 글로벌 감염병 연구에 주력하고 있으며, 동시에 미래 공중 보건에 영향을 미칠 수 있는 새로운 질병에 대비하고 있습니다.

한국파스퇴르연구소는 다른 연구소, 학계 및 산업계와 적절한 국내 파트너십을 체결함으로써 대한민국의 과학 지식 및 기술 자원 축적에 공헌하고 있습니다. 또한 신약 연구 및 개발 분야에서 학계와 바이오 회사들의 중요한 역할을 인지하고 있으며, 미래 신약 개발은 공공 연구기관 및 바이오 산업 전반에 걸친 협력과 혁신이있을 때 가능하다는 것도 이해하고 있습니다.

현재 한국파스퇴르연구소에서는 약 100여 명의 인력이 연구실, 핵심 기술 시설, 프로젝트 관리 및 행정 분야에서 근무하고 있습니다.

Education

Education and teaching are an important part of Louis Pasteur's legacy and the establishment of IPK. The institute contributes to Korea's knowledge economy, fanning the spirit of discovery and innovation by nurturing the development of the next generation of scientists and health professionals.

IPK is committed to sharing scientific expertise and technologies with early career researchers and scientists. IPK provides a variety of educational and training opportunities throughout the year, such as internships for undergraduate and graduate students, international practical courses for select scientists with support from the Institut Pasteur International Network, and a Doctoral program (UST-IPK Campus).

At the community level, IPK's educational programing sparks an interest in science and seeds the importance of research in middle and high school students by offering Life Science Classes, opening IPK to school field trips and mentoring science club students.

교육

교육은 루이 파스퇴르가 남긴 유산이며 한국파스퇴르연구소 설립에도 중요한 역할을 했습니다. 한국파스퇴르연구소는 차세대 과학자 및 공중 보건 전문가 육성을 통해 다음 세대에게 혁신 및 연구 정신을 북돋아 줌으로써 대한민국 지식경제 (knowledge economy)에 공헌하고 있습니다.

한국파스퇴르연구소는 과학 지식과 기술을 젊은 연구원 및 과학자들과 공유하고 있습니다. 대학생과 대학원생을 대상으로 인턴십 프로그램을 제공하며 과학자들에게는 파스퇴르연구소 국제네트워크가 지원하는 연구 기술 교육 과정을 제공합니다. 이외에도 박사과정 (UST– IPK캠퍼스) 등 다양한 교육 및 트레이닝 기회를 연중 제공하고 있습니다.

한편 지역 사회를 위해 생명과학교실, 연구소 현장 견학, 과학자와의 멘토링 시간 등을 중 · 고등학교 학생들에게 제공함으로써 과학에 대한 관심을 불러일으킬 뿐만 아니라 기초과학 연구의 중요성을 알리고 있습니다.

Public Health

IPK is a member of the Institut Pasteur International Network (Network) and actively involved in research on global infectious diseases. IPK stands ready to work in collaboration with national and international health authorities such as the Korea Centers for Disease Control & Prevention (KCDC) and the World Health Organization (WHO), providing expertise in infectious diseases to uncover the basic biology of disease and discover effective countermeasures.

In 2015, when an outbreak of MERS occurred in Korea, IPK brought its expertise in infectious diseases to discover effective countermeasures against the causative virus. With an agreement with the Korean government, IPK uses screening technologies in its high security laboratories to test existing therapeutic molecules already approved by health authorities.

공중 보건

한국파스퇴르연구소는 파스퇴르연구소 국제네트워크의 회원으로 국제적인 감염병 연구 활동에 적극적으로 참여하고 있습니다. 한국파스퇴르연구소는 질병의 생물학적 메커니즘과 효과적인 대응책 등과 같은 감염병에 대한 전문 지식을 제공하며 질병관리본부(KCDC), 세계보건기구(WHO)와 같은 국내 및 국제 보건 전문기관들과 협력할 준비가 되어 있습니다.

2015년 한국에 메르스(MERS) 사태가 발생했을 때 원인이 되는 바이러스에 효과적으로 대응할 수 있도록 감염병에 대한 전문 지식을 제공하였습니다. 또한 대한민국 정부 협조 아래, 한국파스퇴르연구소는 높은 생물 보안 등급 실험실에 구축된 연구소만의 독자적인 스크리닝 기술을 이용해 보건당국이 사전 승인한 치료 물질을 시험하였습니다.

International

Present on five continents, through 33 institutes, the Network plays a major role in research, education and public health via numerous cooperation and training programs and provides a front-line response against emerging, re-emerging and endemic infectious diseases.

As a hub institution, IPK plays a key role in the Network and serves as a bridge between Korea and global bio-pharma science. For over a decade, IPK has forged successful partnerships and expanded Korea's R&D base collaborative research projects with global alliances. Since 2007, IPK has worked in association with Drugs for Neglected Diseases initiative (DNDi), a collaborative, and patients' needs-driven, non-profit organization to develop new treatments for neglected diseases, and has received award recognition multiple times throughout the years for excellence in research and commitment to global health. IPK also promotes scientific exchanges through internships, international seminars and conferences.

국제적 역할

5대륙 33개소에 걸친 파스퇴르연구소 국제네트워크는 다양한 연구 협력과 교육 프로그램을 통해 연구 · 교육 · 공중 보건 분야에서 중요한 역할을 수행하고 있으며, 신종 감염병 및 재유행하는 감염병, 혹은 지역적으로 유행하는 풍토성 감염병에 대응하기 위해 감염 지역 현장에서 적극적으로 연구 활동을 진행하고 있습니다.

한국파스퇴르연구소는 파스퇴르연구소 국제네트워크의 중심 연구소로서 주요한 역할을 하고 있으며 국내와 글로벌 제약 산업을 연결하는 가교 역할을 수행하고 있습니다. 한국파스퇴르연구소는 지난 10여 년간 여러 성공적인 파트너십을 구축해 왔으며, 여러 국제적 연구 제휴를 통해 연구 협력 프로젝트 기반의 한국 R&D 기술을 확장시켰습니다. 2007년부터 한국파스퇴르연구소는 국제적인 비영리 소외질환 신약 개발 연구 기관인 DNDi(Drugs for Neglected Diseases initiative)와 협력하고 있으며, 여러 해에 걸쳐 연구 우수성과 공중보건에 이바지한 공로로 DNDi로부터 여러 상을 수상하였습니다. 이외에도 한국파스퇴르연구소는 인턴십, 국제 세미나, 콘퍼런스 등을 통해 과학 교류 증진에도 힘쓰고 있습니다.

IPK Success Stories

First-in-class TB Drug Discovery (Q203)

IPK identified an innovative drug candidate, Q203, which has a novel mechanism of action and is highly effective against both multi-drug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis (TB). Q203 has successfully advanced through US FDA preclinical testing by Qurient.

결핵 혁신신약 후보물질 도출 (Q203)

한국파스퇴르연구소는 다제내성결핵 (MDR-TB) 및 광범위내성결핵 (XDR-TB)에 뛰어난 치료효능을 보이는 혁신신약 후보물질 Q203을 성공적으로 개발하였습니다. 새로운 작용기전을 가진 이 물질은 현재 신약개발 후속 연구 단계에 있습니다.
(큐리언트가 미국 FDA 임상 1상 시험 중)

Hepatitis C Drug Discovery (TU series)

The well-developed hepatitis C virus (HCV) program at IPK delivered TU series, a highly active lead series that has a novel mechanism of action with therapeutic efficacy against HCV. IPK licensed TU series out to J2H Biotech for further research.

C형 간염 치료제 후보물질 도출 (티오펜 우레아(TU) 시리즈)

한국파스퇴르연구소의 연구진은 자체 혁신 신약개발 연구 프로그램을 통해 C형 간염 바이러스가 간세포로 진입하거나 나가는 기작을 모두 억제하는 새로운 작용기전의 선도화합물 시리즈를 성공적으로 발굴했습니다. 그리고 후속 연구 개발을 위해 J2H 바이오텍에 기술을 이전하였습니다.

Establishment of a Biotech Company (Qurient)

Qurient is a spin-off biotechnology company of IPK dedicated to developing novel therapeutics from late discovery to human proof of concept.
※ Qurient went public on Korea Securities Dealers Automated Quotation (KOSDAQ) in Feb, 2016

바이오 벤처회사 큐리언트 (Qurient) 설립

(주)큐리언트는 한국파스퇴르연구소가 2008년 7월에 설립한 바이오 벤처회사로, 후기 개발 단계의 연구 성과를 임상시험 단계로 연계하는 연구를 진행하고 있습니다.
※ 2016년 2월, 큐리언트 코스닥 (KOSDAQ) 상장

Phenomic Tehcnology (PhenomicScreen™ & PhenomicID™)

IPK developed "phenomic technologies," next-generation drug discovery technology platforms PhenomicScreen™ and PhenomicID™, which combine advances from the latest bio-imaging techniques with high throughput screening technologies, to enable the real-time observation and analysis of cellular disease models in a high throughput mode.

페노믹 (Phenomic) 기술 (PhenomicScreen™ & PhenomicID™)

한국파스퇴르연구소 '페노믹 (Phenomic) 기술'은 세포 이미지 초고속 대용량 스크리닝 기술과 약물표적 규명을 위한 기능성 유전체 연구법을 접목한 차세대 신약 개발 플랫폼으로, 실시간으로 세포 내에서 일어나는 다양한 반응을 초고속 탐색법을 활용하여 관찰 및 분석할 수 있습니다.

IPK History

Research and Partnership
Awards
Events

Oct.2016 Licensed out a hepatitis C virus drug candidate (Thiophene Urea (TU) compound series) to J2H Biotech
J2H 바이오텍에게 C형 간염 치료 후보물질 (티오펜 우레아 (TU) 물질) 기술 이전

Aug.2016 Participated in National Convergent Research of Emerging Virus Infection (CEVI) Program (Development of Anti-Coronavirus small molecules)
미래선도형 융합연구단 (CEVI) 사업 참여 (제4세부 실용화가 가능한 코로나 바이러스 치료제 후보물질 개발)

2016 Awarded DNDi's Project of the Year
※ Drugs for Neglected Diseases initiative (DNDi) is a collaborative, patients' needs-driven, non-profit drug research and development (R&D) organization that is developing new treatments for neglected diseases.
DNDi '올해의 프로젝트 상 (DNDi's Project of the Year)' 수상
※ DNDi (Drugs for Neglected Diseases initiative)는 비영리 신약 연구 개발 조직으로 소외된 열대 질환 치료제를 개발하고 있습니다.

Dec.2015 Q203, a first-in-class (in Korea) tuberculosis drug candidate, designated as an Orphan Drug (development fast track) by US FDA
약제내성 결핵 치료용 화합물 (Q203) 美FDA 희귀의약품 (Orphan Drug) 지정

Jul.2015 Q203, a first-in-class (in Korea) tuberculosis drug candidate, approved for FDA clinical trial phase I
약제내성 결핵 치료용 화합물 (Q203) 美FDA 임상 1상 승인

Dec.2014 Participated in KRICT Convergence cluster (anti-bacterial, antiviral, and diagnosis divisions)
한국화학연구원 주관 융합 클러스터 (Convergence cluster) 참여 (박테리아 분과, 바이러스 분과, 진단 분과)

Dec.2014 10 year anniversary celebration!
창립 10주년 기념식

Jul.2014 Awarded Ministry of Science, ICT, and Future Planning (MSIP)'s 100 Best National R&D Project (Q203)
미래창조과학부 '국가연구개발 우수성과 100선'에 선정 및 수상 (Q203)

May.2014 Designated as a University of Science Technology (UST) member campus
과학기술연합대학원대학교 (UST) 캠퍼스 지정

Apr.2014 Signed a grant agreement with Korea Drug Development Fund (KDDF) for the development of first-in-class hepatitis C virus drug candidate
범부처신약개발사업단과 새로운 작용기전의 C형 간염 치료제 후보물질 개발을 위한 과제 협약서 체결

Dec.2013 Licensed out an anti-inflammatory compound to Qurient for drug development
항염증 화합물 1건 큐리언트에 기술 이전

Aug.2013 Master Agreement with DNDi: collaboration to identify and develop safe, effective and affordable new treatments for patients suffering from neglected diseases including leishmaniasis and Chagas disease
DNDi와 포괄적 연구협력 체결 : 샤가스병과 리슈만편모충증을 포함한 소외질환 치료제 개발을 위한 연구 협력

Aug.2013 Discovered Q203, a first-in-class (in Korea) tuberculosis drug candidate that is highly effective against both multi-drug-resistant (MDR) and extensively drug-resistant (XDR) Mycobacterium tuberculosis. (Results published in *Nature Medicine*)
약제내성 결핵 치료제 혁신 신약 후보 물질 (Q203) 개발
※ Q203 : 다제내성결핵 (MDR-TB) 및 광범위내성결핵 (XDR-TB)에 뛰어난 치료 효능을 보이는 국내 혁신 신약 (First-in-class) 후보 물질. 네이처 誌 논문 게재.

Jan.2012 Selected as a partner in ORCHID alliance, a global project to fight tuberculosis
EU 결핵퇴치 공동연구 프로젝트 (ORCHID) 참여기관으로 선정

2011 Awarded DNDi's Partnership of the Year
DNDi '올해의 파트너십 상 (Partnership of the Year)' 수상

Mar.2010 Licensed tuberculosis compound I series (Q203) to Qurient for drug development
결핵 치료제 1건 큐리언트에 기술 이전 (I 시리즈 – Q203)

Feb.2010 Licensed tuberculosis compound A series to Qurient for drug development
결핵 치료제 1건 큐리언트에 기술 이전 (A 시리즈)

Nov.2009 Ranked top in Lab Safety by Korea's Ministry of Education and Science Technology (MEST)
교육과학기술부 '연구실 우수 안전관리 기관 포상' (교육과학기술부 장관표창) 수상 (안전부분)

2010 Awarded DNDi's Partnership of the Year
DNDi '올해의 파트너십 상 (Partnership of the Year)' 수상

May.2009 New collaboration between GSTEP, INSERM and IPK
경기바이오센터 (GSTEP) – 프랑스 국립보건의학연구원 (INSERM) – 한국파스퇴르연구소 (IPK) 협력연구센터 개소 및 공동연구 협력 체결

Apr.2009 Relocated to Pangyo Techno Valley campus
경기도 판교테크노밸리 내 독립연구시설 이전 및 개소식 (경기도 지원)

May.2008 First Bio Safety Level 3 facility certified by the Korean Government (Certification issued by KCDC)
국내 최초 생물안전 3등급 (BSL) 실험실 국가 인증 (한국질병관리본부 인증)

Jul.2008 Established Qurient an entity for drug development of identified compounds provided by IPK and other research facilities
바이오 벤처기업 (주)큐리언트 (Qurient) 설립

Sep.2007 First screening agreement with DNDi: Technical Agreement for HTS assay for leishmania
한국파스퇴르연구소–DNDi 첫 번째 스크리닝 계약 체결

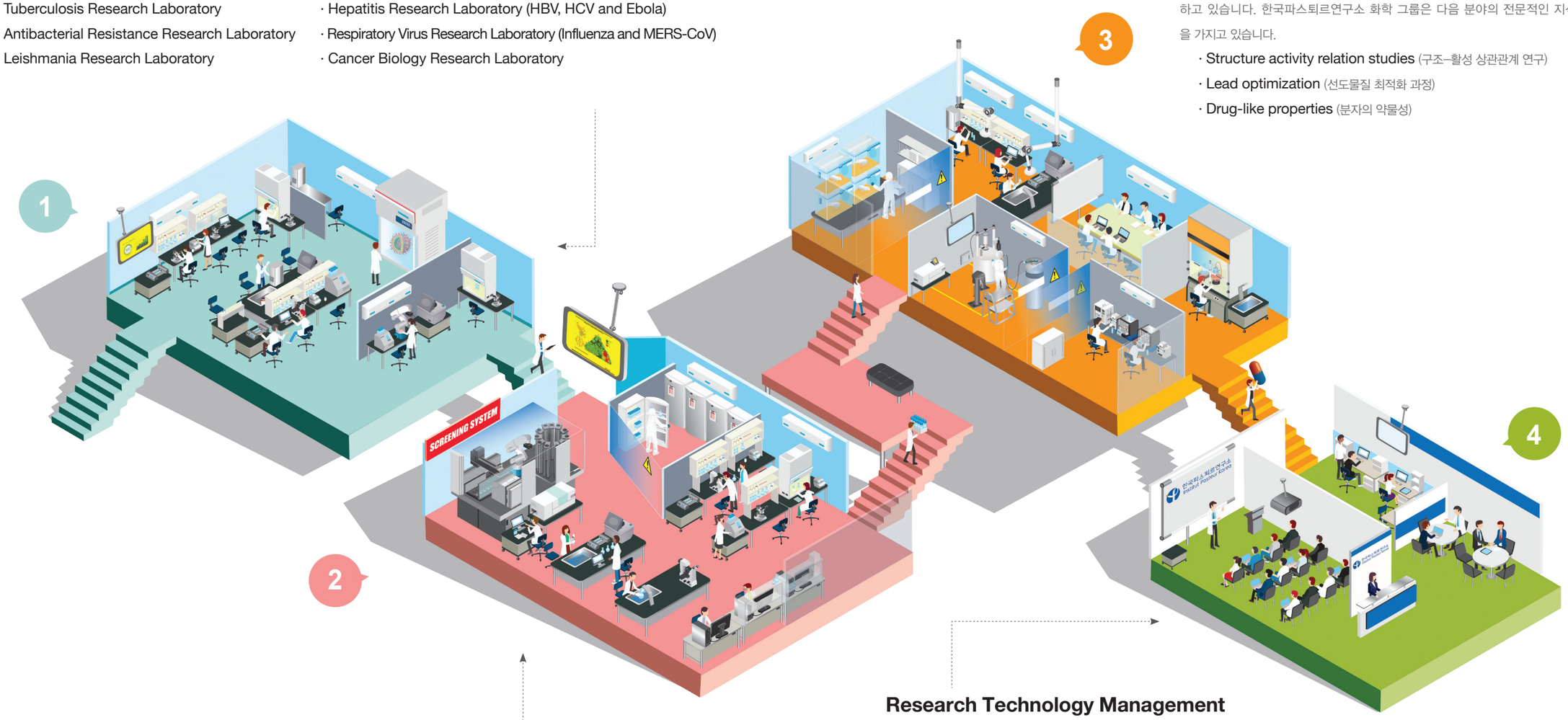
Apr.2004 IPK established on Korea Institute of Science and Technology (KIST) campus in Seoul
한국파스퇴르연구소 설립 (한국과학기술원 KIST 內, 現 미래창조과학부 지원)

Dec.2003 Korea Ministry of Science & Technology (Current: Ministry of Science, ICT and Future Planning), Institut Pasteur and Korea Institute of Science and Technology (KIST) agreed to establish IPK
한국파스퇴르연구소 설립 협정 체결 (과학기술부 (現 미래창조과학부) – (프) 파스퇴르연구소 – 한국과학기술원 (KIST))

IPK Structure

Discovery Biology

- Tuberculosis Research Laboratory
- Antibacterial Resistance Research Laboratory
- Leishmania Research Laboratory
- Hepatitis Research Laboratory (HBV, HCV and Ebola)
- Respiratory Virus Research Laboratory (Influenza and MERS-CoV)
- Cancer Biology Research Laboratory



Discovery Chemistry

Discovery chemistry group supports all IPK translational research programs. The group plays a key role in optimizing hits identified during screening activities. Their expertise cover the following areas:

화학 그룹은 한국파스퇴르연구소의 모든 중개연구 프로그램을 지원합니다. 또한 스크리닝 과정에서 새롭게 발굴된 유효 물질(hit)을 최적화하는 역할을 하고 있습니다. 한국파스퇴르연구소 화학 그룹은 다음 분야의 전문적인 지식을 가지고 있습니다.

- Structure activity relation studies (구조–활성 상관관계 연구)
- Lead optimization (선도물질 최적화 과정)
- Drug-like properties (분자의 약물성)

Assay Development & Screening Platforms

IPK has been building the best High Content Screening platform in both BSL-2 and BSL-3 laboratories in Korea.

한국파스퇴르연구소는 BSL–2 및 BSL–3 실험실에 초고속 대용량 스크리닝 플랫폼을 구축했습니다.

- Technology Development Platform
- Assay Development & Screening
- Computational Biology
- Automation & Logistics Management

Research Technology Management

IPK works closely with industry and academia to increase collaboration and partnership. With these connections, our technologies will be developed into future medical therapies.

한국파스퇴르연구소는 산업계 및 학계와 더 많은 공동연구 및 협력 관계를 만들기 위해 적극적으로 일하고 있습니다. 이런 협력을 바탕으로 한국파스퇴르연구소의 기술력은 미래 치료제로 개발되고 있습니다.

- Invention disclosure
- Knowledge and practice
- Intellectual property
- Consulting
- R&D collaboration
- Patent
- Material transfer agreement
- Licensing

Discovery Biology

Tuberculosis Research Laboratory

Principal Investigator : Vincent Delorme, Ph.D.

Laboratory members: Doyoon Kwon, M.Sc. (Senior researcher); Minjeong Woo, M.Sc. (Junior researcher)

Core support members: Sunhee Kang (CHP), David Shum (ADS), Namyoul Kim (ADS), Jinyeong Heo (ADS), Constantin Radu (ALM)

CHP: Chemistry Platform; **ADS:** Assay Development & Screening; **ALM:** Automation & Logistics management

1. Research Summary

In a context of global emergence of multi and extensively drug-resistant (M-XDR) isolates of Mycobacterium tuberculosis (Mtb), Tuberculosis Research Laboratory (TRL) is conducting drug discovery and optimization programs to deliver new lead compounds against tuberculosis (TB). This work is performed in tight collaboration with the core facility platforms at IPK and aims at translating early discoveries into more advanced projects, ready to enter the clinical phase pipeline. This year, key collaborations were established with Brazil in order to secure a new target-based project, and in house optimizations of a much needed assay enabling the screening of compounds against non-replicating bacteria have been completed. The panel of assays now available at the core screening platform for tuberculosis will allow the selection of the most potent scaffold and increase the translational power of the institute. In parallel, the team is conducting basic research studies, including host-pathogen interactions and compound mechanism of action. Efforts were made to characterize the behavior of a new cell line with respect to Mtb infection, highlighting the role played by alveolar macrophages in this disease. Interesting insights were gained on the mechanism of action of the TTCA family, an asset owned by IPK (a patent application has been submitted) that could lead to the generation of new lead candidates for the treatment of M-XDR TB.

2. Research Progress in 2016

2.1 Summary

In 2016, we completed the study of a library of 11,088 actinomycetes extracts and published the results in collaboration with the screening and compound management teams at IPK. Additionally, the streptomycin auxotrophic 18b strain was used to screen a pilot library of 8,000 molecules in order to investigate its usefulness as a model for the discovery of sterilizing drugs. In collaboration with RVRL team at IPK, we also get insights in the mechanism of action of TTCA molecules, understanding that they could induce a strong down-regulation of the mycobactins synthesis pathway in the bacteria and may thus play a role in the metal homeostasis. Finally, we started a collaboration with the University of Rio Grande do Sul (PUCRS) in Brazil for the study of FolB as a target for anti-TB drug discovery. Tools are being set-up to secure an assay and enable new screening campaigns.

2.2 Natural extract screening

This study has now been completed, after the final assessment of the cytotoxicity of all the extracts against non-infected cells. Results were published in an international, peer-reviewed journal. This collaborative work, conducted with the Myongji University (Korea), opens the door to further studies involving natural extracts and displays our capacity to interact with local partners in order to value their prospecting efforts.

2.2.1 Results

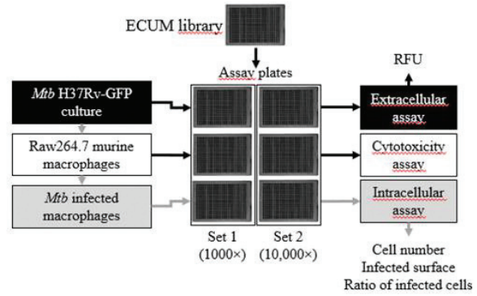


Figure 1. Overall scheme for the multiple screening. In black, steps related to the extracellular assay, yielding as read-out relative fluorescence units (RFU). In grey, steps related to the intracellular assay, yielding as read-out after image analysis a number of cells, an infected surface (expressed in pixels) and a ratio of infected cells. In white, steps related with the cytotoxicity assay, yielding as a read-out a percent of cell survival.

The feasibility and relevance of screening a library of raw actinomycete extracts (ECUM library) for the identification of anti-tuberculosis activities was assessed on 11,088 extracts using a multiple screening approach (**Figure 1**). Each extract was first tested at two concentrations against non-infected macrophages as a control, then against Mtb growing in broth medium as well as infecting murine macrophages. The screening results indicated a library of good quality with an apparent low proportion of cytotoxic extracts (**Figure 2A**). A correlation was found between both bacterial assays (**Figure 2B**), but the intracellular assay showed limitations due to low rates of cell survival. Several extracts of interest were highlighted by this multiple screening. A focus on the strain producing the two most effective revealed similarities with known producers of active molecules, suggesting the possibility of selecting relevant extracts using this strategy.

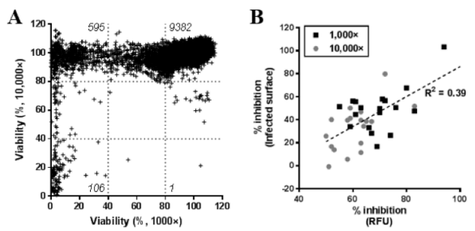


Figure 2. A. Two-dimensional plot of the results of the cytotoxicity assay. Extracts are plotted using their percentage of viability obtained at each dilution. Extracts below 40% and 80% of viability (dotted lines) were considered cytotoxic and cytostatic/partially cytotoxic, respectively. Number of extracts falling in relevant sub-parts of the graph, as delineated by the viability thresholds, are indicated in italic. **B.** Correlation between the extracellular and the intracellular assay results for the 18 extracts selected by the extracellular screening. On the Y-axis, the infected surface was used. On the X-axis, the percentage of inhibition based on the RFU value was used. A statistically significant linear correlation was found ($R^2 = 0.39$; $p < 0.0001$). Values correspond to a single assay.

2.3 Non-replicating bacterial assay for latent TB

The assay using the streptomycin starved 18b strain (SS18b) has been optimized and its robustness tested in an 8,000 compounds pilot screening. The results are satisfying, indicating an assay of good quality. This assay will enable the identification of compounds able to efficiently kill non-replicating bacteria, a step further in the search for new sterilizing molecules against TB.

2.3.1 Results

The principle of the assay is illustrated in **Figure 3A.** The strain is first grown in presence of streptomycin to obtain a sufficient inoculum, then starved for 3 weeks to obtain a static, non-replicating inoculum. The assay read-out is the ability of the strain to reduce resazurin (Alamar Blue) into resorufin, evidencing the activity of its respiratory pathway and the viability of the bacteria. As a pilot screening, 8,000 compounds were tested in duplicate. The Z' values measured along the screening were above 0.5 and the correlation was found to be 0.7, indicating a robust, reproducible assay (**Figure 3B**).

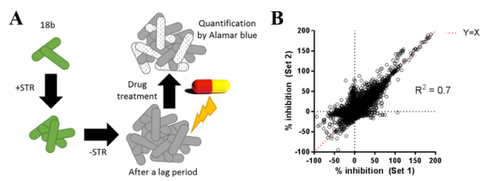


Figure 3. A. General procedure used to obtain and screen compounds against a non-replicating, streptomycin (STR) auxotrophic bacteria. The lag period was 3 weeks and the drug

treatment 5 days. **B**. Correlation between the duplicated screening (Set 1 vs Set 2). Compounds were screened at 10 μM.

2.4 Mechanism of action of the TTCA family

The TTCA family is an important asset of TRL, with a potency to yield a lead candidate for M-XDR TB. Previous results already shown in vivo efficacy, but the mechanism of action of the family is still unknown. Using Transcriptomic analyses, we identified a gene signature indicating the down-regulation of the mycobactin synthesis pathway, involved in iron scavenging.

2.4.1 Results

Whole transcriptome of bacterial cultures exposed to different level of drugs and at different time-points were collected and analyzed. TTCA-01, able to prevent bacterial growth in vitro, shows strong down-regulation of a particular set of genes belonging to the mycobactin synthesis pathway. TTCA-02, which is unable to prevent mycobacterial growth in broth medium, failed to induce a similar response. This suggested that TTCA-01 could act as a growth impairment agent by inducing iron starvation in the bacteria.

This step forward in the understanding of the mechanism of action of TTCA-01 allows us to better explain some specificities of the TTCA family. Firstly, the highest activity seen in macrophages makes sense in view of these results, as iron scavenging is essential in vivo, while its absence in vitro leads to growth arrest but is not detrimental for bacteria survival. Secondly, the absence of similar pattern observed for TTCA-02 suggests the existence of two distinct mechanism of action for this family of molecule. Indeed, TTCA-02 is unable to prevent growth in vitro but is still very active in macrophages, indicating that another target might be involved. Altogether, this points out the uniqueness of the TTCA family as anti-TB agents.

3. Major Accomplishments

3.1 Patents

1. Anti-infective compounds (TB TTCA). Jaeseung Kim, Sunhee Kang, Mooyoung Seo, Jeongjea Seo, Sumi Lee, Juhee Kang. PCT/EP2015/063982

3.2 Publications

1. Singh V, Dhar N, Pato J, Kolly GS, Kordulakova J, Forbak M, Evans JC, Szekely R, Rybniker J, Palčekova Z, Zemanova J, Santi I, Signorino-Gelo F, Rodrigues L, Vocat A, Covarrubias AS, Rengifo MG, Johnsson K, Mowbray S, Buechler J, Delorme V, Brodin P, Knott GW, Ainsa JA, Warner DF, Keri G, Mikušova K, McKinney JD, Cole ST, Mizrahi V, Hartkoorn RC. (2016). Identification of aminopyrimidine-sulfonamides as potent modulators of Wag31-mediated cell elongation in mycobacteria. Molecular Microbiology. Accepted.
2. Heo J, Nam J, Jang J, Shum D, Radu C, Cheng J, Lee H, Suh J-W, Delorme V. (2016). High-Content Screening of Raw Actinomycete Extracts for the Identification of Antituberculosis Activities. Journal of Biomolecular Screening. 1087057116675887.
3. Queval CJ, Song O-R, Deboosere N, Delorme V, Debrie A-S, Iantomasi R, Veyron-Churlet R, Jouny S, Redhage K, Deloison G, et al. (2016). STAT3 Represses Nitric Oxide Synthesis in Human Macrophages upon Mycobacterium tuberculosis Infection. Scientific Reports 6.
4. Nikiforov PO, Surade S, Blaszczyk M, Delorme V, Brodin P, Baulard A, Blundell T, and Abell C. (2016). A fragment merging approach towards the development of small molecule inhibitors of Mycobacterium tuberculosis EthR for use as ethionamide boosters. Organic & Biomolecular Chemistry. 14: 2318-2326.

3.3 Presentations

1. Vincent Delorme. Invited Lectures. Chemical and genetic modulators for the study of Mycobacterium tuberculosis in macrophages, 7th ASEAN Congress of Tropical Medicine and Parasitology (ACTMP), Malang, Indonesia, 17-May-2016.

3.4 Ongoing & new collaborations

1. Investigation of lipolytic enzymes from Mtb as biomarkers to discriminate active and latent TB, Pr. Sunghan Kim, Department of infectious diseases, Asan Medical Center, Seoul, Korea and Dr. Stephane Canaan, EIPL (UMR7282), CNRS, Marseille, France
2. Photodynamic therapy for the treatment of TB, Dr. Ji-Eun Chang, Department of Thoracic and Cardiovascular Surgery, Seoul National University Bundang Hospital, Bundang, korea.

결핵 연구실
(Tuberculosis Research Laboratory)

1. 연구 요약

전 세계적으로 다약제 내성과 광범위 약제내성(M–XDR)을 나타내는 결핵 균주들의 출현에 기인하여 본 연구진(TRL팀)은 이들 결핵에 대한 신약 개발 및 새로운 선도 화합물들을 발굴하고 선도 물질 최적화 프로그램을 수행하고 있다. 본 연구는 한국파스퇴르연구소의 핵심 설비 플랫폼(core facility platforms)과 긴밀한 협력 하에 수행되고 있으며 조기 신약 후보물질 발견을 통해 임상 단계 파이프라인에 진입할 수 있는 보다 발전된 프로젝트로의 전환을 목표로 한다. 올해는 새로운 표적 기반 프로젝트를 확보하기 위해 브라질과의 주요 협력이 이루어 졌으며, 자체적으로 비복제 박테리아에 대한 화합물 스크리닝을 수행하기 위하여 필요한 분석 방법의 최적화를 완료하였다. 이는 한국파스퇴르연구소의 현 핵심 스크리닝 플랫폼에서 가능한 분석방법으로 가장 효과적인 스캐폴드의 선택이 가능할 것이며 또한 이를 통해 한국파스퇴르연구소의 연구 결과 기술 이전 능력을 향상시킬 것으로 기대된다. 이와 동시에 본 연구진은 숙주–병원체 상호 작용 및 복합 작용 메커니즘에 관련된 연구를 수행하고 있다. 결핵에서 폐포대식세포의 역할 규명을 중점적으로 연구하였으며 결핵 감염과 관련된 새로운 세포주의 특성을 규명하기 위한 연구에 매진하고 있다. 또한 한국파스퇴르연구소 소유 자산인 TTCA(특허 출원 제출) 계열의 흥미로운 활성 메커니즘에 기반하여 다약제 내성 /광범위 약제내성 결핵(M–XDR) 치료를 위한 새로운 후보 선도물질을 발굴할 수 있었다.

2. 2016년 연구 성과 요약

2016년 본 연구진은 한국파스퇴르연구소의 스크리닝(screening) 및 화합물 관리팀(compound management teams)과 협업하여 11,088개의 방선균 추출물 라이브러리에 대한 연구를 완료하고 공동 연구 결과를 발표했다. 이와 더불어 결핵에 대한 살균 약물 개발을 위한 모델로서의 가능성을 조사하기 위하여 스트렙토마이신 영양 요구성 균주 SS18b를 이용하여 8,000여 화합물들의 파일럿 라이브러리 스크리닝을 완료하였다. 또한 한국파스퇴르연구소의 RVRL팀과의 공동 연구를 통해 TTCA 화합물들이 박테리아 내에서 마이코백신(mycobactins) 합성 경로의 강력한 하향 조절을 유도할 수 있으며 따라서 금속 항상성에 작용할 수 있다는 결과를 통해, TTCA 화합물들의 활성 메커니즘에 관한 사실을 알 수 있었다. 마지막으로, 본 연구진은 FolB를 표적으로 항결핵 약물의 발견을 위해 브라질의 Rio Grande do Sul 대학(PUCRS)과 공동 연구를 시작하였다. 현재 이를 위한 분석법의 확보 및 새로운 스크리닝 캠페인을 위한 도구(tool)들의 준비를 위한 연구가 진행되고 있다.

Discovery Biology

Hepatitis Research Laboratory

Principal Investigator: Marc P. Windisch, Ph.D.

Laboratory members: Alexander Koenig, Ph.D. (Postdoc. - GG- & IP Ebola Task Force funded, start August 2016); Jaewon Yang, M.Sc. (Researcher); Eunji Jo, M.Sc. (Researcher); MyungEun Lee, M.Sc. (Ph.D. student -left October 2016); Phuong Hong Nguyen, M.Sc, (Ph.D. student -start November 2016); Thoa Thi Than, M.Sc. (Junior researcher - GG-funded, start November 2016)

Core support members: Constantin Radu (ALM), David Shum (ADS), Regis Grailhe (TDP), Sangchul Lee(BIO), Sunhee Kang (CHP)

ALM: Automation & Logistics Management; **ADS:** Assay Development & Screening; **TDP:** Technology Development Platform; **BIO:** Bioinformatics; **DCP:** Discovery Chemistry Platform

1. Research Summary

The main research focus of the Hepatitis Research Laboratory / Applied Molecular Virology (AMV) team has been the molecular virology of **hepatitis B and C viruses (HBV & HCV)**. More than 450 million people worldwide are being chronically infected with viral hepatitis. Either therapies are unable to cure patients, as in case of HBV, or therapies are very costly and thereby limiting access to HCV medications. In order to address this unmet medical needs, IPK devised strategies to screen for novel viral interventions. For both viral diseases cutting edge infectious cell culture systems have been established and developed. Concerning HCV, a first-in-class entry inhibitor was identified, deeply characterized and after further development to a pre-clinical candidate licensed out in October 2016. For HBV a similar strategy is being pursued, IPK developed a phenotypic assay and screened compound libraries with the recently developed HBV infectious cell culture system and selected various promising confirmed hits. Furthermore, together with Kainos Medicine hit-to-Lead studies are being conducted, as well as mechanism of action studies. One hallmark of IPKs infectious HBV cell culture system is that the viral supernatant can be transferred to naive target cells. Thereby, the entire HBV life cycle can be monitored which, to our knowledge, nobody can do at the moment as efficient as IPK. To take advantage of this state-of-the-art system we developed an assay suitable to identify novel biomarker. Using shRNA expressing lenti-viral particle transduced HBV target cells novel marker will be identified crucial to the viral life cycle which can be used for diagnostic purposes or as novel drug targets.

Ebola virus (EBOV) cause severe hemorrhagic fever with fatality rates up to 90%. Neither vaccines nor specific therapies are available, which is mainly due to reason that EBOV exclusively affected the African continent. Furthermore, because of the high pathogenicity of EBOV, laboratory work is restricted to biosafety level 4 laboratories (BSL4) which significantly limits research on these viruses. Recently, an EBOV transcription- and replication-competent virus-like particle (trVLP) system was described, suitable to model almost all aspects of the viral lifecycle under BSL2 conditions. In order to tackle EBOV, IPK devised strategies to identify novel inhibitors by using the state-of-the-art EBOV trVLP cell culture system which enables to target the entire viral life cycle. A screening assay was successfully developed, selected compound libraries have been screened and hits confirmed with the goal to repurpose FDA-approved drugs to EBOV disease.

2. Research Progress in 2016

2.1 Development of a novel hepatitis C virus drug candidate

2.2 Summary

Since recently, chronic hepatitis C is a curable disease. However, viral drug resistance, difficult to treat hepatitis C virus (HCV) genotypes and the high economic burden to therapy are of concern. Furthermore, all marketed direct acting antivirals interfere with HCV RNA replication and are not ideal to treat liver transplantation patients or to prevent vertical virus transmission.

To address these unmet medical needs we devised strategies using the infectious HCV cell culture system to carry out a phenotypic, cell-based target-free screening campaign. By excluding HCV RNA replication inhibitors we focused on compounds interfering with viral entry. Thereby, we identified a potent thiophene urea (TU) scaffold and characterized its mechanism-of-action. TU's putative molecular target is the extracellular loop of glycoprotein E1 as demonstrated by a viral drug resistance study. TU inhibits HCV particle internalization, by preventing fusion of the virion to the endosomal membrane. Furthermore, TU prevents cell-to-cell transmission and inhibits full length HCV genotype 1a and chimeric viruses of all HCV genotypes except of genotype 6. Additionally, TU's antiviral activity mediates synergistic effects when combined with FDA-approved HCV drugs, and TU inhibits pre-existing resistant strains induced by today's therapies. Additionally, in vivo efficacy studies in naive and chronically infected humanized mice using patient derived HCV demonstrated that TU is suitable to prevent a primary infection and viral spread in the liver, respectively. Currently, extensive in vitro ADME/tox, in vivo PK/tox and compound formulation studies are being conducted.

Together, we identified and characterized a novel proprietary small molecule that inhibits HCV entry by targeting glycoprotein E1. TU is a potential HCV drug candidate, blocking a viral envelope protein without affecting viral attachment which is a novel paradigm in antiviral therapy.

2.2.1 Results

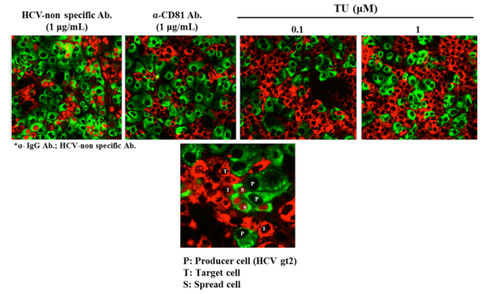


Figure 1. TU inhibits HCV cell-to-cell spread in vitro. Green HCV producer cells (P) and naive target cells (T) with red cytoplasm. After successful spread translocation of red signal into the nucleus (S). An example is shown (lower panel). HCV-nonspecific and anti-CD81 antibodies were used as negative and positive controls, respectively (upper left panel). In the presence of TU at 0.1 μM and 1 μM no HCV spread detectable (upper right panel). TU inhibits HCV cell-to-cell spread which is the major route of viral transmission in the liver.

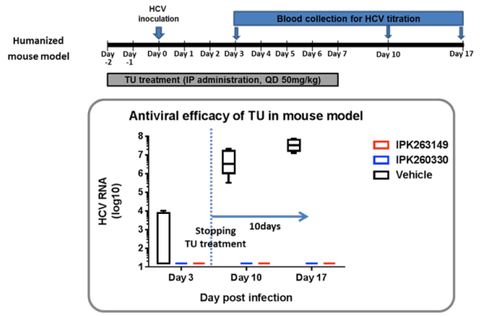


Figure 2. TU inhibits HCV infection in vivo.
Experimental schedule is shown in the upper panel. Groups of 5 humanized mice per study group were pretreated with inhibitors 2 days before inoculation with HCV. Blood was sampled starting from day 3 post inoculation until day 17. Inhibitor treatment was continued until 78 days post inoculation. Determination of HCV RNA load by qRT-PCR is shown in the lower panel. TU derivatives IPK263149 and IPK260330 or with the vehicle control as show in red, blue and black, respectively. HCV RNA load increased in vehicle control (no inhibitor), whereas TU treated mice did not show detectable HCV RNA.

2.3 Development of a hepatitis B virus high throughput screening assay

2.4 Summary

The discovery of human sodium taurocholate transporting polypeptide (hNTCP), a crucial receptor for hepatitis B virus (HBV), enables to study the entire viral life cycle. HBV highly depends on interplay between viral and host cell factors for the generation of new virions. However, knowledge on host factors important for HBV attachment, entry, replication, etc. is limited. In order to identify and characterize novel viral interventions, we devised strategies in 384-well plate format using cell culture derived infectious HBV and hNTCP HepG2 target cells. After successful assay development and adaptation to automation we performed a high throughput screening (HTS) campaign and screened approx. 20,000 small molecule compounds. Identified hits have been confirmed by dose response curve analysis and are being characterized to further develop novel drug candidates.

Additionally, in order to identify and characterize novel cellular restriction factors and potential new drug target for HBV, we devised strategies in 384-well plate format using the infectious HBV cell culture system and developed an HTS assay employing lenti-viral particles expressing selectable shRNAs. Reference genes have been selected and an assay has been developed successfully and is being adapted to automation to screen focus libraries.

Taken together, we have developed a phenotypic HBV infection assay to identify novel HBV interventions, biomarker and drug targets.

2.4.1 Results

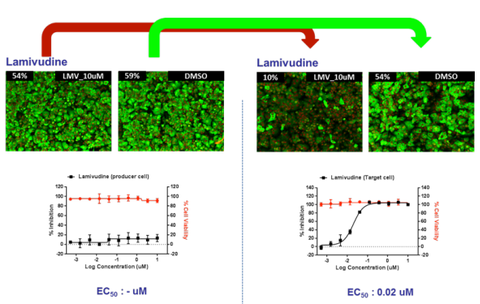


Figure 3. Evaluation of replication inhibitors using the infectious HBV cell culture system.

Naive HepG2-NTCP target cells pretreated with lamivudine, a HBV replication inhibitor, do not show any reduction of HBV antigen expression as shown by green immunofluorescence staining (upper left panel). Cell nuclei are shown in red. Quantification of HBV infection (lower left panel). Supernatant transfer of HBV infected cells to naive target cells (upper right panel). Infected cells treated with lamivudine show a significant reduction in HBV infection, whereas the DMSO control remains unchanged. Quantification of HBV infection (lower right panel) demonstrates that the assay is suitable to monitor the entire HBV life cycle.

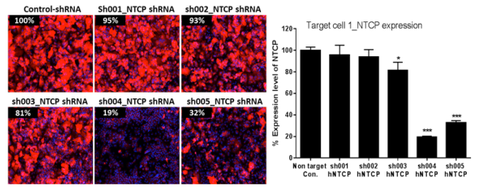


Figure 4. Determination of hNTCP knock-down after transduction with lenti-viral particles expressing shRNAs.

Naive HepG2-NTCP target cells transduced with lenti-viral particle expressions 5 different hNTCP shRNAs or a control shRNA (left panel). hNTCP expression and cell nuclei are shown in red and blue, respectively. Quantification of hNTCP knock-down (right panel). Two shRNAs led to significant reduction of hNTCP levels which resulted in a reduction of HBV infection (data not shown).

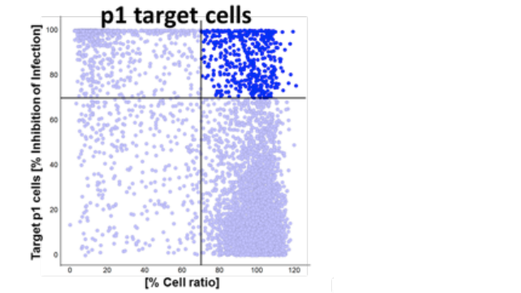
2.5 Characterization of inhibitors and biomarkers identified by screening of the Ebola virus transcription- and replication-competent virus-like particle system - Institut Pasteur International Network/Ebola Task Force

2.6 Summary

Ebola viruses (EBOV) cause severe hemorrhagic fevers with fatality rates up to 90%. Neither vaccines nor specific therapies are available, which is mainly due to reason that EBOV exclusively affected the African continent. Furthermore, because of the high pathogenicity of EBOV, laboratory work is restricted to biosafety level 4 laboratories (BSL4) which significantly limits the research on these viruses. Recently, an EBOV transcription- and replication-competent virus-like particle (trVLP) system was described, suitable to model almost all aspects of the viral life cycle under BSL2 conditions. Using this system, IPK (HRL, RVRL & ADS teams) developed a HTS assay and screened an 8,354 compound collection of FDA-approved and bioactive molecules in duplicates. Identified hits have been confirmed by dose response curve analysis and are being further triaged to select for synergistic drug combinations.

In summary, we developed a novel HTS assay using the recently described EBOV trVLP system, screened and identified novel inhibitors and biomarkers which are being characterized with the goal to reposition drugs for therapeutic use and to select for novel prognosis predicting biomarkers of EBOV disease.

2.6.1 Results



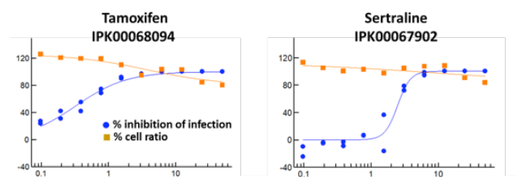


Figure 5. Hit selection and confirmation of EBOV inhibitors.

EBOV inhibitory compounds were selected according >70% EBOV inhibition and >70% cell ratio which is a marker for cytotoxicity. Blue circles represent active compounds (left panel). Examples of drugs inhibiting the EBOV life cycle (right panel). Dose response curve analysis with tamoxifene and sertraline. EBOV inhibition and cell ratios are shown in blue and orange, respectively.

3. Major Accomplishments

3.1 Licensed-out

1. Licensed-out a first-in-class hepatitis C virus drug candidate to J2H Biotech.

3.2 Patents

1. Korean application No. 10-2016-0099581: Micrococcin P1, a naturally occurring macrocyclic peptide inhibiting hepatitis C virus entry inhibitor. Marc Windisch and Myungeun Lee.

3.3 Publications

1. Myungeun Lee, Jaewon Yang, Eunji Jo, Ji-Young Lee, Hee-Young Kim, Ralf Bartenschlager, Eui-Cheol Shin, Yong-Soo Bae, and Marc P. Windisch. "A Novel Inhibitor IDPP Interferes with Entry and Egress of HCV by Targeting Glycoprotein E1 in a Genotype-Specific Manner." Minor revision in Scientific Reports.
2. Senis E, Mockenhaupt S, Rupp D, Bauer T, Paramasivam N, Knapp B, Gronych J, Grosse S, Windisch MP, Schmidt F, Theis FJ, Eils R, Lichter P, Schlesner M, Bartenschlager R, Grimm D. "TALEN/CRISPR-mediated engineering of a promoterless anti-viral RNAi hairpin into an endogenous miRNA locus." Nucleic Acids Res. 2016 Sep 9. [Epub ahead of print]
3. Lee M, Yang J, Park S, Jo E, Kim HY, Bae YS, Windisch MP. "Micrococcin P1, a naturally occurring macrocyclic peptide inhibiting hepatitis C virus entry in a pan-genotypic manner." Antiviral Res. 2016 Aug; 132:287-95.
4. Kim HY, Kong S, Oh S, Yang J, Jo E, Ko Y, Kim SH, Hwang JY, Song R, Windisch MP. "Benzothiazepinecarboxamides: Novel hepatitis C virus inhibitors that interfere with viral entry and the generation of infectious virions." Antiviral Res. 2016 May. 129:39-46.
5. Grunvogel O, Esser-Nobis K, Windisch MP, Frese M, Trippler M, Bartenschlager R, Lohmann V, Binder M. "Type I and type II interferon responses in two human liver cell lines (Huh-7 and HuH6)." Genom Data. 2015 Dec. 28;7:166-70
6. Lee S, Yoon KD, Lee M, Cho Y, Choi G, Jang H, Kim BS, Jung DH, Oh JG, Kim GW, Oh JW, Jeong YJ, Kwon HJ, Bae SK, Min DH, Windisch MP, Heo TH, Lee C. "Identification of a resveratrol tetramer as a potent hepatitis C virus helicase inhibitor." Br J Pharmacol. 2016 Jan; 173(1):191-211.

7. ElHefnawi M, Kim T, Kamar MA, Min S, Hassan NM, El-Ahwany E, Kim H, Zada S, Amer M, Windisch MP. "In Silico Design and Experimental Validation of siRNAs Targeting Conserved Regions of Multiple Hepatitis C Virus Genotypes." PLoS One. 2016 Jul 21; 11(7):e0159211.
8. Yang SH, K R J, Lim S, Choi TG, Kim JH, Akter S, Jang M, Ahn HJ, Kim HY, Windisch MP, Khadka DB, Zhao C, Jin Y, Kang I, Ha J, Oh BC, Kim M, Kim SS, Cho WJ. "Structure-Based Discovery of Novel Cyclophilin A Inhibitors for the Treatment of Hepatitis C Virus Infections." J Med Chem. 2015 Dec 24; 58(24):9546-61.
9. Ryu DK, Ahn Y, Ryu WS, Windisch MP. "Development of a novel hepatitis B virus encapsidation detection assay by viral nucleocapsid-captured quantitative RT-PCR." Biotechniques. 2015 Nov 1; 59(5):287-93.
10. Grunvogel O, Esser-Nobis K, Reustle A, Schult P, Muller B, Metz P, Trippler M, Windisch MP, Frese M, Binder M, Fackler O, Bartenschlager R, Ruggieri A, Lohmann V. "DDX60L is an nterferon-Stimulated Gene Product Restricting Hepatitis C Virus Replication in Cell Culture." J Virol. 2015 Oct 15; 89(20):10548-68.2016 Jan; 173(1):191-211.

3.4 Presentations

1. Windisch MP. Oral. The infectious HBV system, a tool for screening. Heidelberg University, Heidelberg, Germany, 15-April-2016.
2. Windisch MP. Oral. A novel HCV inhibitor interfering with entry and secretion. Twincore enter, Hannover, Germany, 19-April-2016.
3. Windisch MP. Oral. HBV & HCV drug discovery at IPK. Nottingham University, Nottingham, UK. 20-April-2016.
4. Windisch MP. Oral. HBV & HCV cell culture models for HTS campaigns. Friedrich Loeffler Institut, Greifswald, Germany. 29-April-2016.
5. Windisch MP. Oral. HBV infectious cell culture system. Catholic University Hospital, Seoul, South-Korea. 7-June-2016.
6. Windisch MP. Oral. Characterization of a novel HCV entry inhibitor, International Hepatitis Meeting at Catholic University in Seoul, City, South Korea Country, 21-September-2016.
7. Koenig Alexander, Oral. Restriction factors of emerging HBV-related bat viruses - evidence for co-factors restricting orthohepadnaviral entry, 2016 International HBV meeting, Seoul, South Korea, 22-Setember-2016
8. Windisch MP. Oral. Development of a novel HBVscreening assay, HBV Workshop at IPK, Seongnam, South Korea, 26-September-2016.
9. Windisch MP. Oral. Characterization of a novel HCV entry inhibitor, KNIH-IPK workshop, Osong, South Korea, 07-September-2016.
10. Jo Eunji, Quantification of Infectious Hepatitis B Virus by Using an End Point Dilution Assay in Cell Culture, 2016 International HBV meeting, Seoul, South Korea, 22-September-2016
11. Yang Jaewon. Poster. Development of a shRNA-based Screening Assay to Identify HBV Restriction Factor, 2016 International HBV meeting, Seoul, South Korea, 22-September-2016
12. Windisch MP. Oral. Development of a novel HCV inhibitor targeting viral entry, International Hepatitis C Virus Symposium, Kyoto, Japan, 15-October-2016.
13. Windisch MP. Oral. Characterization of inhibitors & biomarkers identified by screening of the Ebola virus transcription- & replication competent virus-like particle system. International Pasteur Network Meeting, Paris, France, 30-November-2016
14. Windisch MP. Poster Characterization of inhibitors & biomarkers identified by screening of the Ebola virus transcription- & replication competent virus-like particle system. International Pasteur Network Meeting, Paris, France, 30-November-2016
15. Yang Jaewon. Oral. Development of a shRNA based high throughput screening assay to discover novel hepatitis B virus biomarkers. International Pasteur Network Meeting, Paris, France, 30-November-2016
16. Yang Jaewon. Poster Development of a shRNA based high throughput screening assay to discover novel hepatitis B virus biomarkers. International Pasteur Network Meeting, Paris, France, 30-November-2016
17. Windisch MP. Poster. The interplay between hepatotropic viruses and hepatic normoxia determines viral levels and response to therapeutics. International Pasteur Network Meeting, Paris, France, 01-December-2016

3.5 Ongoing & new collaborations

1. Evaluation of TU's impact on HCV genotype 5 and 6. Dr. Alex Tarr. University of Nottingham, Nottingham, England.
2. Detection of HDV infection in hepatoblastoma cells. Dr. Mithat Bozdayi, Ankara University, Ankara, Turkey.
3. Evaluation of small molecule inhibitors targeting Zika virus. Dr. Myrielle Dupont-Rouzeyrol Institut Pasteur New Caledonia.
4. A GFP-expressing Zika virus clone to evaluate neutralizing antibody libraries. Dr. Philippe Despres, Institut Pasteur Reunion.
5. Development of a screening assay using Ebola virus trVLP system. Dr. Hoenen, NIH, Rocky Mountain Laboratories, USA.
6. Evaluation of micro RNAs interfering with HCV. Prof. Yoon, Catholic University, Seoul, Korea.
7. Evaluation of nanoparticles in HBV and HCV cell culture systems. Prof. Kattesh, University of Missouri, USA.
8. Determination of ApoE level in TU treated hepatoma cells. Prof. Seungtaek Kim, Yonsei Severance Hospital, Seoul, Korea.
9. Evaluation of protease/polymerase/helicase inhibition of resveratrol tetramer. Prof. Choongho Lee, Dongkuk University, Seoul, Korea.
10. HCV inhibitors MoA study. Prof. Ralf Bartenschlager, University of Heidelberg, Heidelberg, Germany.
11. Evaluation of kinase inhibitors interfering with HCV entry. Prof. Soon Bong Hwang, Ilsong Institute of Life Science, Hallym University, Anyang, Korea.
12. Novel cell culture adapted chimeric HCV to characterize the viral life cycle. Prof. Jens Bukh, Department of Infectious Diseases and Clinical Research Centre, University of Copenhagen, Denmark.
13. Analysis of siRNA targeting conserved RNA regions of HCV. Dr. Mahmoud M. ElHefnawi, American University in Cairo Informatics and systems Department, The National Research Centre, Egypt.
14. Development of a phenotypic HTS using HCV reporter cells. Prof. Charles M. Rice Rockefeller University, New York, USA.
15. HBV infection of NTCP expressing hepatoma cells. Prof. Stephan Urban; Department of Molecular Virology, Heidelberg University, Germany.
16. Development of epsilon HBV Pol and IRF3 nuclear translocation assay. Prof. Wang Shick Ryu, Department of Biochemistry, Yonsei University, Seoul, Korea.

2016 HCA Training / 2016 HCA 교육 프로그램

Discovery Biology

Respiratory Viruses Research Laboratory

Principal Investigator: Ji-Young Min, Ph.D.

Laboratory members: Ji Hoon Park, Ph.D. (Senior researcher); Anne-Laure Pham Hung, Ph.D. (Postdoc.); So Young Chang, M.Sc. (Researcher); Jihye Lee, M.Sc. (Researcher); Dongjo Shin, M.Sc. (Junior researcher); Jinhee Kim, M.Sc. (Junior researcher)

Core support members: Constantin Radu (ALM), Honggun Lee (ALM), David Shum (ADS), Sooyoung Byun (ADS), Inhee Choi (BIO), Yoonae Ko (BIO), Sunhee Kang (CHP), Sunju Kong (CHP), Youngmi Kim (ACP)

ALM: Automation & Logistics Management; **ADS:** Assay Development & Screening; **BIO:** Bioinformatics; **CHP:** Chemistry Platform; **ACP:** Analytical Chemistry Platform

1. Research Summary

The main objective of the respiratory viruses research laboratory is to develop translational disease intervention strategies for emerging and re-emerging respiratory viruses. To this end, we are taking chemical genomics and RNA interference (RNAi) approaches to understand the host-pathogen relationship in an effort to simultaneously identify new targets and potential therapeutic agents. Our research vision is to understand the dynamics of host response to virus infection that will provide new strategies for resolving disease through the development of treatments. We are determining the molecular mechanism of viral proteins contribute to pathogenesis and overcome host defense by inhibiting crucial cellular functions.

Main results during the year and ongoing projects

1. Characterization of newly emerged Middle East respiratory syndrome (MERS)-CoV and development of novel MERS therapeutic agents
2. Development of next-generation influenza therapeutic agents
3. Identification of host pathways and factors regulating replication of influenza viruses via RNAi platform
4. Pathogenesis of H7 avian influenza viruses and its virulence markers

Short outlook

This research will address both the function and mechanism of action of the novel antiviral agents in the context of viral and/or cellular protein interactions and its findings could advance the field of antiviral therapies against emerging and re-emerging respiratory viruses causing significantly high mortality and morbidity in human.

2. Research Progress in 2016

2.1 Summary

2.2 Characterization of newly emerged Middle East respiratory syndrome (MERS)-CoV and development of novel MERS therapeutic agents

The presence of MERS-CoV was confirmed by RT-PCR of viral cultures of four out of seven air samples from two patients' rooms, one patient's restroom, and one common corridor. In addition, MERS-CoV was detected in 15 of 68 surface swabs by viral cultures. IFA on the cultures of the air and swab samples revealed the presence of MERS-CoV. EM images also revealed intact particles of MERS-CoV in viral cultures of the air and swab samples.

These data provide experimental evidence for extensive viable MERS-CoV contamination of the air and surrounding materials in MERS outbreak units. Thus our findings call for epidemiologic investigation of the possible scenarios for contact and airborne transmission, and raise concern regarding the adequacy of current infection control procedures.

In order to develop therapeutic agents against newly emerged MERS-CoV, which showed unique phenotype of persistency in the environmental settings, antibody driven drug development strategy was striven. Four clones of hFc efficiently neutralizing MERS-CoV were identified and will be further optimized and evaluated in preclinical models aiming for the clinical usage in the reoccurrence of MERS epidemics.

2.2.1 Results

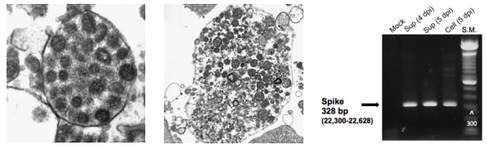


Figure 1. Isolation of MERS-CoV from air and surrounding environments in hospital settings.

(A) Electron microscopy images of Vero E6 cells inoculated with air sampled in Patient's room in Hospital. The core size is 80 nm, which is consistent with MERS-CoV. There are visible spikes on many of the particles. Most of the particles were contained within a membranous vesicle. (B) Detection of MERS-CoV by RT-PCR. Part of the MERS-CoV spike gene (nt 22300 - 22628, black arrow) was targeted for RT-PCRblack arrow) was targeted for RT-PC.

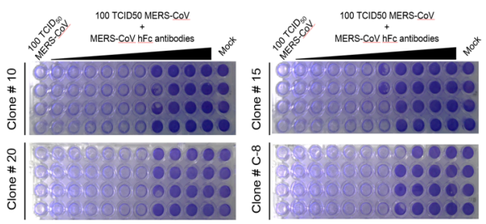


Figure 2. Identification of four clones efficiently neutralizing MERS-CoV.

hFc antibodies are generated from PBMCs of patients recovered from MERS. Its neutralizing activity was evaluated in micro-Neut assay using vero cells infected with MERS-CoV.

2.3 Development of next-generation influenza therapeutic agents

Established a phenotypic high throughput screening (HTS) assay using recombinant influenza virus which is suitable to identify viral inhibitor may target any step during entire virus replication cycle. In a pilot HTS campaign, to evaluate and confirm the reproducibility and robustness of the assay, approx. 20k small molecules have been screened previously and selected hits have been reordered and confirmed in the series of secondary assays for their efficacy against seasonal wild-type influenza viruses and for their ability to block early virus entry process. Afterwards confirmed scaffolds have been clustered according to their chemicals structure and prioritized scaffolds have been subjected to Hit to Lead studies which include structure-property relationship (SPR), in vitro PK/ADME, mechanism of action (MoA) and in vivo efficacy studies (Figure. 3).

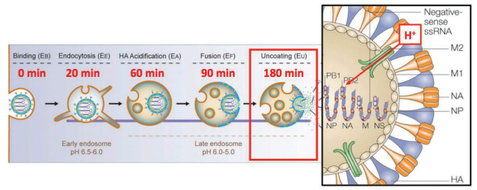
Identified a novel anti-influenza molecule by screening 861 plant-derived natural components using a high-throughput image-based assay that measures inhibition of the influenza virus infection. 1,3,4,6-tetra-O-galloyl-β-D-glucopyranoside (TGBG) from Euphorbia humifusa Willd showed broad-spectrum anti-influenza activity against two seasonal influenza A strains, A/California/07/2009 (H1N1) and A/Perth/16/2009 (H3N2), and seasonal influenza B strain B/Florida/04/2006. Immunofluorescence assay demonstrated that TGBG significantly inhibits nuclear export of influenza nucleoproteins (NP) during the early stages of infection causing NP to accumulate in the nucleus. In addition, influenza-induced activation of the Akt signaling pathway was suppressed by TGBG in a dose-dependent manner (Figure. 4).

Small molecules having the pyrrolopyridinamine (PPA), aminothiadiazole (ATD), dihydrofuropyridine carboxamide (HPC), or imidazopyridinamine (IPA) moiety were selected from a target-focused chemical library for their inhibitory activity against influenza A virus by high-throughput screening using the PR8GFP assay. Among them, PPA showed broad-spectrum activity against multiple influenza A viruses and influenza B virus. PPA was found to block the early stages of influenza virus

infection using a time-of-addition assay. Using additional phenotypic assays that dissect the virus entry process, it appears that the antiviral activity of PPA against influenza virus can be attributed to interference of the post-fusion process: namely, virus uncoating and nuclear import of viral nucleoprotein complexes (Figure. 5).

Extensive MoA studies will include in vitro drug-drug combination evaluation which will enable to select highly synergistic Leads which will be further evaluated in an in vivo activity test thereby increasing the probability to cure seasonal and potential pandemic influenza patients.

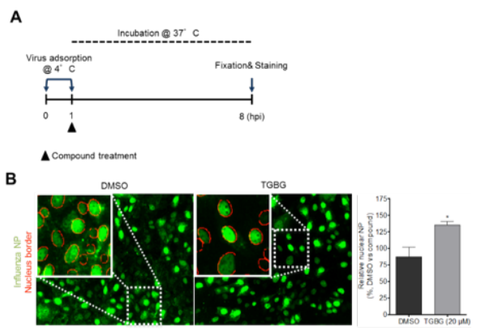
2.3.1 Results



IPK code	B/Florida/04/2006		A/California/07/2009 (H1N1)		A/Perth/16/2009 (H3N2)		MMS T1/2, min		PLS % @2h	
	EC_{50} (uM)	CC_{50} (uM)	EC_{50} (uM)	CC_{50} (uM)	EC_{50} (uM)	CC_{50} (uM)	H	M	H	M
MC_008565	2.52	>50	2.74	>50	1.97	>50	88.1	>60		

Figure 3. Optimization of the lead candidate small molecule, which efficiently inhibits entry of broad-spectrum influenza A and B viruses.

Antiviral activity of lead candidate was confirmed in cells infected with H1N1, H3N2 influenza A viruses and also seasonal influenza B virus. Influenza lead candidate is shown to block uncoating of influenza A and B viruses during its entry.



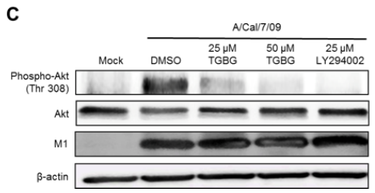


Figure 4. TGBG treatment leads to nuclear retention of viral NP via inactivation of the PI3K/Akt pathway.

(A) Schematic diagram of the experiment. (B) Images and quantification of influenza-infected cells that were treated with TGBG. (C) A549 cells were infected with A/Cal/7/09 (MOI=1) in the absence or presence of TGBG (25 and 50 μM) or the Akt-specific inhibitor LY294002 (25 μM).

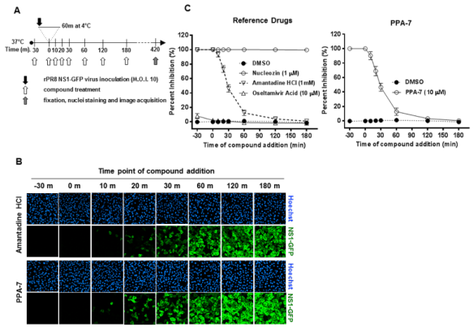


Figure 5. Time-of-addition assay. (A) A549 cells were inoculated with NS1-GFP virus (MOI 10) and treated with the compounds at different time points. (B) The effect of 1 mM amantadine and 10 μM PPA-7, added at different time points, on the infectivity NS1-GFP virus in A549 cells at 7 hpi. (C) Plotted curves showing the inhibitory activity of the compounds against NS1-GFP viral infection relative to the time of compound addition.

2.4 Identification of host pathways and factors regulating replication of influenza viruses via RNAi platform

Influenza viruses exploit host factors to successfully replicate in infected cells. Using small interfering RNA (siRNA) technology, we identified six human genes required for influenza A virus (IAV) replication. Here we focused on the role of acid phosphatase 2 (ACP2), as its knockdown showed the greatest inhibition of IAV replication. In IAV-infected cells, depletion of ACP2 resulted in a significant reduction in the expression of viral proteins and mRNA, and led to the attenuation of virus multi-cycle growth. ACP2 knockdown also decreased replication of seasonal influenza A and B viruses and avian IAVs of the H7 subtype. Interestingly, ACP2 depletion had no effect on the replication of Ebola or hepatitis C virus. Because ACP2 is known to be a lysosomal acid phosphatase, we assessed the role of ACP2 in influenza virus entry. While neither binding of the viral particle to the cell surface nor endosomal acidification was affected in ACP2-depleted cells, fusion of the endosomal and viral membranes was impaired (Figure. 6 and 7).

We found that knockdown of DPF2 in cells infected with influenza virus resulted in decreased expression of viral proteins and RNA. Furthermore, production of progeny virus was reduced by two logs in the multiple cycle growth kinetics assay. We also found that DPF2 was involved in the replication of seasonal influenza A and B viruses. Because DPF2 plays a crucial role in the noncanonical NF-κB pathway, which negatively regulates type I interferon (IFN) induction, we examined the relationship between DPF2 and IFN responses during viral infection. The results showed that knockdown of DPF2 resulted in increased expression of IFN-β and induced phosphorylation of STAT1 in infected cells. In addition, high levels of several cytokines/chemokines (RANTES, IL-8, IP-10, and IL-6) and the antiviral proteins (MxA and ISG56) were produced by DPF2 knockdown cells (Figure. 8).

2.4.1 Results

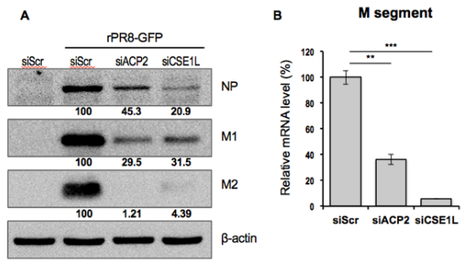


Figure 6. ACP2 depletion inhibits IAV infection.

(A) Viral protein expression in ACP2-depleted cells. A549 cells were transfected with indicated siRNAs for 48 hours. Cells were then infected with the rPR8-GFP virus at an MOI of 1. After 10 hours, cells were lysed and subject to western blot analyses using the anti-ACP2, anti-NP, anti-M2, anti-M1, and anti-β-actin antibodies. The band density was quantified by Image J software. All values are relative to those of cells transfected with siScr. (B) Reduced viral mRNA levels upon ACP2 depletion. A549 cells were transfected with siACP2, siScr, or siSCE1L as indicated prior to infection with rPR8-GFP virus at an MOI of 1.

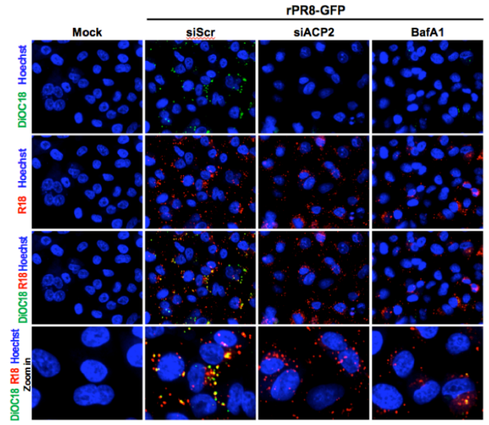


Figure 7. ACP2 is crucial for membrane fusion during entry of influenza A and B viruses.

ACP2 knockdown impaired the fusion step between viral and endosomal membranes. A549 cells were transfected with the indicated siRNAs at 37°C for 48 hours prior to infection with viruses rPR8-GFP

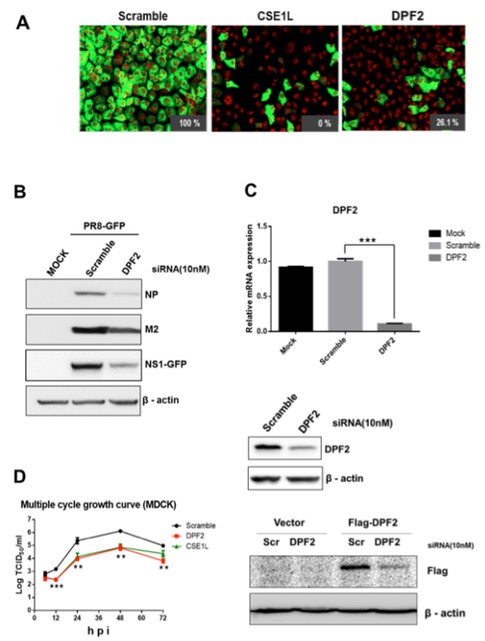


Figure 8. DPF2 plays a crucial role in influenza virus infection.
A549 cells were transfected with 10 nM siRNAs for 48 h, then infected with A/rPR8-GFP-NS1 (MOI=1). Cells were analyzed at 10 hpi. (A) Cells expressing NS1A-GFP (Green) were visualized using the Opera system and analyzed with IM software. Hoechst 33342 (Red) was used for nucleus staining. (B) Detection of viral and cellular proteins was performed by western blotting with corresponding antibodies. (C) Relative expression of DPF2 mRNA was determined by qRT-PCR and normalized with α-tubulin (top). Endogenous DPF2 (middle) and ectopic expression of DPF2 (bottom) were detected by western blotting using corresponding antibodies. (D) Multiple cycle growth curves of A/rPR8-GFP-NS1 in DPF2-knockdown cells. siRNA-transfected A549 cells were infected with A/rPR8-GFP-NS1 at an MOI of 0.01 and the supernatant was collected at the indicated time points. Virus yields were determined by TCID50 assay in MDCK cells. Error bars represent the standard deviation of three independent experiments, **p<0.01, ***p<0.001.

2.5 Pathogenesis of H7 avian influenza viruses and its virulence markers

Using a small interfering RNAs (siRNAs) technology in a cell-based assay, recruitment of those host factors for both the H7N7 and H7N4 strains was assessed. When transfected with siRNAs targeting these host factors, the infectivity of H7N7 and H7N4 differed significantly, except in acid phosphatase 2 (ACP2) depleted cells (Figure.9). ACP2 was recognized as critical for the membrane fusion step during influenza entry. Therefore, we quantified the membrane fusion process for both strains and showed that the H7N4 strain was more efficient in the membrane fusion process than H7N7. Our data demonstrate that, by enhancing the membrane fusion activity, ACP2 is a contributing factor for the high virulence of the H7N4 strain (Figure. 10). Furthermore, this study underlines the importance of host factors recruited during the viral cycle and provides additional evidence for the development of host factors-targeted strategies.

2.5.1 Results

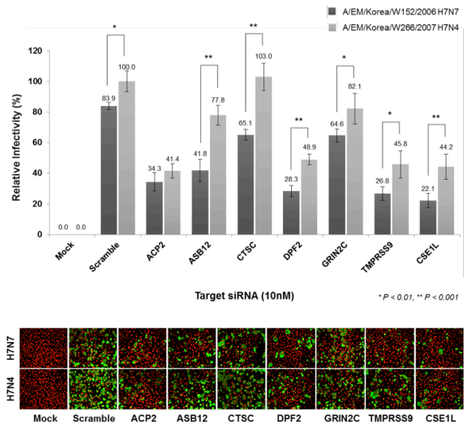


Figure 9. The acid phosphatase 2 knock-down in infected cells alleviates the difference of infectivity observed between the two strains.
A549 cells were transfected with indicated siRNAs 48h prior to infection with A/EM/Korea/W152/2006 (H7N7) or A/EM/Korea/W266/2007 (H7N4) at an MOI of 1.0 for 10h. Confocal images were acquired with a high throughput confocal fluorescence imaging system and analyzed with an in-house image mining platform. Percentage of relative infectivity was normalized to scramble siRNA control with A/EM/Korea/W266/2007 (H7N4) infected group. Values are mean ± standard error of two independent experiments and were statistically analyzed by two-tailed pair t test. *, P<0.01; **, P<0.001.

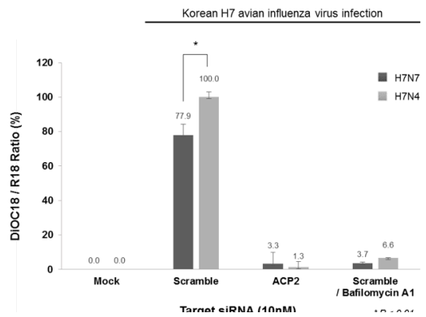


Figure 10. The A/EM/Korea/W266/2007 (H7N4) strain has an enhanced membrane fusion activity during viral entry.
Quantification of membrane fusion activity between A/EM/Korea/W152/2006 (H7N7) and A/EM/Korea/W266/2007 (H7N4) infected cells. Percentage of DiOC18/R18 ratio was normalized to scramble siRNA-transfected cells upon A/EM/Korea/W266/2007 (H7N4) infection. Statistical significance between the indicated groups was tested using the Student's t test with a threshold of p: * <0.01. Values are means of triplicate experiments ± standard error.

2.6 Collaborative research program on the development of MERS therapeutic agents

Established a phenotypic high throughput screening (HTS) assay using 2015 MERS-CoV clinical isolate (Figure. 11) which is suitable to identify viral inhibitor may target any step during entire virus replication cycle. In a pilot HTS campaign, to evaluate and confirm the reproducibility and robustness of the assay, approx. 8000 small molecules have been screened (Figure. 12).

2.6.1 Results

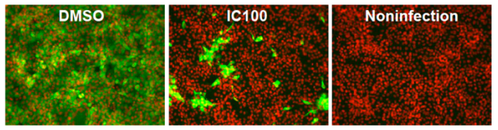


Figure 11. Phenotypic assay development using MERS-CoV clinical isolate.

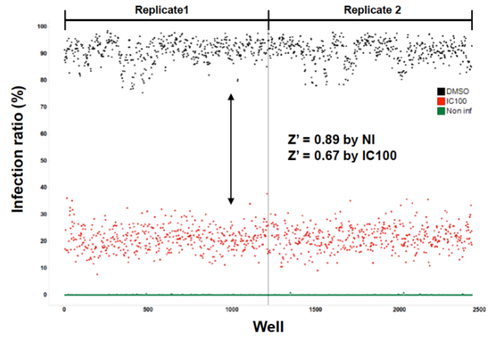


Figure 12. HTS of MERS-CoV inhibitors.
Reproducibility and robustness of the assay, approx. 8000 small molecules have been screened.

3. Major Accomplishments

3.1 Patents

1. Novel human gene crucial for the replication of influenza virus and use thereof (CTSC gene). Min JY, Lee JH, Shin DJ. Filed in Korea. June 3 2016, #10-2016-0069604.
2. Novel human gene crucial for the replication of influenza virus and use thereof (TMPRSS9). Min JY, Lee JH, Shin DJ. Filed in Korea. June 15 2016, #10-2016-0074744.
3. Novel human gene crucial for the replication of influenza virus and use thereof (ASB12). Min JY, Lee JH, Shin DJ. Filed in Korea. June 16 2016, #10-2016-0075138.
4. Novel human gene crucial for the replication of influenza virus and use thereof (DDX19B). Min JY, Lee JH, Shin DJ. Filed in Korea. June 22 2016, #10-2016-0077941.
5. Novel heteroaryl compounds, its enantiomers, its diastereomers or its pharmaceutically acceptable salt and antiviral composition containing the same as an active ingredient (Influenza THO). Min JY, Chang SY, Lee JH, Kang SH, Kong SJ, Jo SY. Filed in Korea. Sep 30 2016, #10-2016-0126997.
6. Novel compounds comprising carbohydrazide scaffold, its enantiomers, its diastereomers or its pharmaceutically acceptable salt and antiviral composition containing the same as an active ingredient (Influenza CHA). Min JY, Chang SY, Lee JH, Kang SH. Filed in Korea. Sep 30 2016, #10-2016-0126989.
7. Novel compounds comprising thionicotinamide scaffold, its enantiomers, its diastereomers or its pharmaceutically acceptable salt and antiviral composition containing the same as an active ingredient (Influenza TNA). Min JY, Chang SY, Lee JH, Kong SJ. Filed in Korea. Sep 30 2016, #10-2016-0127005.

3.2 Publications

1. Chang SY, Cruz DJ, Ko Y, Min JY (2016) Identification of pyrrolo[3,2-c]pyridin-4-amine compounds as a new class of entry inhibitors against influenza viruses in vitro. Biochem Biophys Res Commun.

30;478(4):1594-601.

2. Excler JL, Delvecchio CJ, Wiley RE, Williams M, Yoon IK, Modjarrad K, Boujelal M, Moorthy VS, Hersi AS, Kim JH; MERS-CoV Vaccine Working Group (2016) Toward Developing a Preventive MERS-CoV Vaccine-Report from a Workshop Organized by the Saudi Arabia Ministry of Health and the International Vaccine Institute, Riyadh, Saudi Arabia, November 14-15, 2015. Emerg Infect Dis. 22(8): e1-e7
3. Kim SH, Sung M, Min JY (2016) Reply to Kerhove et al and Oh. Clin Infect Dis. 63(8): 1143-4.
4. Lee J, Park JH, Min JY (2016) A naturally truncated NS1 protein of influenza A virus impairs its interferon-antagonizing activity and thereby confers attenuation in vitro. Arch Virol. [Epub ahead of print]
5. Kim SH, Chang SY, Sung M, Park JH, Bin Kim H, Lee H, Choi JP, Choi WS, Min JY (2016) Extensive Viable Middle East Respiratory Syndrome (MERS) Coronavirus Contamination in Air and Surrounding Environment in MERS Isolation Wards. Clin Infect Dis. 63(3):363-9
6. Chang SY, Park JH, Kim YH, Kang JS, Min JY. (2016) A natural component from Euphorbia humifusa Willd displays novel, broad-spectrum anti-influenza activity by blocking nuclear export of viral ribonucleoprotein. Biochem Biophys Res Commun. 471(2):282-9
7. Park JH, Park EB, Lee JY, Min JY. (2016) Identification of novel membrane-associated prostaglandin E synthase-1 (mPGES-1) inhibitors with anti-influenza activities in vitro. Biochem Biophys Res Commun. 469(4):848-55.
8. Yoon A, Yi KS, Chang SY, Kim SH, Song M, Choi JA, Bourgeois M, Hossain MJ, Chen LM, Donis RO, Kim H, Lee Y, Hwang do B, Min JY, Chang SJ, Chung J. (2015) An Anti-Influenza Virus Antibody Inhibits Viral Infection by Reducing Nucleus Entry of Influenza Nucleoprotein. PLoS One. 10(10):e0141312.

3.3 Presentations

1. Min JY, Oral. Preparedness for the national crisis caused by respiratory viruses. Korean Society of Chemistry, Busan, Korea, Oct 14, 2016.
2. Min JY, Oral. MERS-CoV: Opportunity for drug discovery in Korea. Zoonosis Research Institute International Symposium, Iksan, Korea, Oct 13, 2016.
3. Min JY, Oral. MERS-CoV: Opportunity for drug discovery in Korea. Cambridge Healthtech Institute International Symposium, PEGS Korea, Sep 20-21, 2016.
4. Min JY, Oral. MERS-CoV: Opportunity for drug discovery in Korea. IP-KNIH Workshop, Osong, Korea, Sep 9, 2016.
5. Lee JH, Kim JH, Son KD, Pham Hung d'Alexandry d'Orengiani AL, Min JY. Poster. Acid Phosphatase 2 (ACP2) Is Required for the Membrane Fusion during Influenza Virus Entry, ISIRV OPTIONS IX for THE CONTROL OF INFLUENZA, Chicago, USA, Aug 24, 2016.
6. Shin DJ, Lee JH, Park JH, Min JY. Poster. Double PHD Finger 2 (DPF2) Promotes the Immune Evasion of Influenza Virus by Suppressing Interferon-β Production, ISIRV OPTIONS IX for THE CONTROL OF INFLUENZA, Chicago, USA, Aug 24, 2016.

3.4 Ongoing & new collaborations

1. Characterization of MERS corona- and zika viruses and evaluation of therapeutic antibodies against them, Dr. Junho Jung, Professor, Seoul National University College of Medicine, SNUH.
2. Convergence on the Emerging Viral Infection. Dr. Hyungrae Kim, Principal Researcher, KRICT.
3. 2016 Gyeonggi-IPK Support Program:Development of novel influenza virus entry inhibitors. Dr.Kyungjin Kim, Director, STPharm.
4. Collaborative research program on the development of MERS therapeutic agents. Dr. Guiwan Jeong, Principal Researcher, GSTEP.

Discovery Biology

Antibacterial Resistance Research Laboratory

Principal Investigator: Soojin Jang, Ph.D.

Laboratory members: Yunmi Lee, M.Sc. (Researcher); Hyungjun Kim, M.Sc. (Junior researcher); Hyunjung Lee, Ph.D. (Postdoc.)

Core support members: David Shum (ADS), Nam Youl Kim (ADS), Jinyeong Heo (ADS), Constantin Radu (ALM), Ki Deok Kim (ALM), Honggun Lee(ALM)

ADS: Assay Development & Screening; **ALM:** Automation & Logistics Management

1. Research Summary

Antibiotic resistance is becoming a serious threat to global public health. To fight against antibiotic resistance and save millions of people from the worst crisis of public health, Antibacterial Resistance Research Laboratory (ARRL) has been conducting researches to understand mechanism of antibiotic resistance and discover new antibacterial molecules against superbugs. Major researches of ARRL in three theme are 1) discovery of new antibacterial molecules active against drug resistant strains, 2) investigation of the mechanism of antibiotic resistance development, and 3) surveillance of antibiotic resistance in community. Continuous efforts of ARRL to discover new antibacterial molecules led to successful development of high throughput screening systems against major bacterial pathogens including P. aeruginosa, S. pneumoniae, and S. aureus and have been recognized in the field establishing strategic joint research projects with a research institute and a pharma venture company. Ongoing molecular studies of antibiotic resistance in S. aureus and community-based surveillance of antibiotic resistance are also in progress expected to increase public awareness about the current status of antibiotic resistance as well as uncover new resistant mechanism. Moreover, ARRL is building strong collaborations internationally as well as domestically to conduct successful researches to fight against antibiotic resistance, a major global threat.

2. Research Progress in 2016

2.1 Summary

In 2016 we have conducted three projects that are supported by MSIP/NRF, Gyeonggi-do, and Korean Research Institute of Chemical Technology (KRICT): 1) discovery of antibacterial molecules against Streptococcus pneumoniae, 2) investigation of antibiotic resistance development and development of a screening assay for Staphylococcus aureus, and 3) development of thiamine monophosphate kinase assay for Pseudomonas aeruginosa. A previously developed assay to screen antipneumococcal molecules was optimized for high throughput screening and validated by a pilot screen with ~7800 bioactive compounds creating a new joint research with KRICT. In depth study of the staphylococcal protein (SMP) was conducted. We confirmed that expression of SMP is associated with antibiotic susceptibility of S. aureus and developed a new screening assay as well as a diagnostic biomarker using this unique property of SMP. We have also developed a thiamine monophosphate kinase (ThiL) assay to screen ThiL inhibitors. ThiL is a new target identified from our previous investigation. Successful accomplishment of this project will generate a new antibiotic with a novel mechanism.

Community-based microbiome and antibiotic resistance surveillance was performed with support from an international collaboration (MetsSUB). Currently, we are analyzing metagenome sequences of a pilot study. Along with these research performances, we achieved three

publications, ten invited talks with one poster presentation at various conferences and seminars, and two grant awards in this year.

2.2 Discovery of new antipneumococcal agents

Streptococcus pneumoniae is a major causative agent for pneumoniae that accounts for 15% of all death of children under age 5, killing an estimated 922,000 children in 2015. Despite advances in pneumococcal vaccines which have about 25% global coverage, S. pneumoniae continues to be a major cause for pneumonia and spread of drug resistant strains becomes a serious global concern. In this project, we aim to discovery new antipneumococcal agents active against drug resistant strains.

2.2.1 Results

In 2016 we optimized previously developed S. pneumoniae assay for high throughput screening and successfully performed a pilot screening with about 7,800 bioactive compounds against a wild type and two clinical strains (Figure 1). Using this validated HTS assay, we initiated a collaboration work with Korean Research Institute of Chemical Technology (KRICT) and screened ~ 20,000 compounds of KRICT identifying hit molecules that will be further validated.

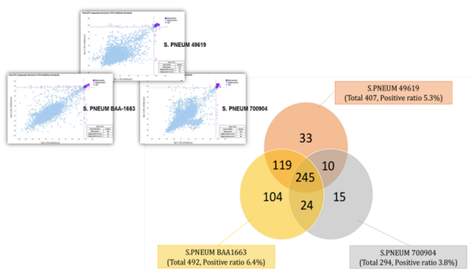


Figure 1. A pilot screening against S. pneumoniae

2.3 Discovery of new antibacterial molecules against S. aureus and P. aeruginosa

Methicillin resistant S. aureus (MRSA) and multidrug resistant P. aeruginosa are categorized as a serious threat by Centers for disease Control and Prevention in US. Infections caused by these bacteria are often life-threatening due to limited treatment options with current available antibiotics. New antibiotics active against these bacteria are urgently demanded. The goal of the project is to discover new antibacterial agents through high throughput screenings developed based on basic researches.

2.3.1 Results

Previously we found that various antibiotics including ciprofloxacin, vancomycin, methicillin, tetracycline, and linezolid trigger induction of a hypothetical protein named as SMP. Based on the result, we hypothesized that SMP induction can indirectly indicate the efficacy of antibiotics against S. aureus. As we anticipated, SMP induction in 5 clinical isolates treated with antibiotics accurately predicted antibiotic resistance profiles of the strains demonstrating its potential as a biomarker for antibiogram of S. aureus (Figure 2). In addition, we developed a new screening assay that measure SMP expression as an indicator of antibacterial effect of compounds.

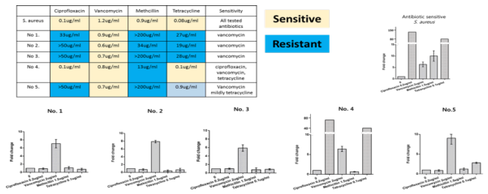


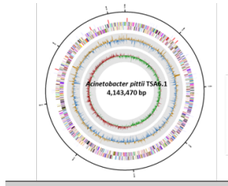
Figure 2. SMP induction in clinical isolates treated with various antibiotics. One pan-drug susceptible strain and five clinical isolates were treated with antibiotics (0.2 ug/ml ciprofloxacin, 2 ug/ml vancomycin, 1.5 ug/ml methicillin, or 0.1 ug/ml tetracycline). After 3 hour incubation, the bacteria were harvested for isolation of total RNA. The protein induction was measured by RTqPCR.

2.4 Surveillance of antibiotic resistance in community (MetaSUB consortium and Bill &Melinda Gates Foundation)

Antibiotic resistance is rapidly spreading around the world. To control antibiotic resistance, it is necessary to understand the current status of antibiotic resistance in all possible reservoirs including community-based microbiome. In this project, we are aiming to investigate microbiome and its antibiotic resistance using samples collected from the community.

2.4.1 Results

Three drug resistant species isolated from our pilot study were characterized by whole genome sequencing. The sequencing results identified genes as well as plasmids likely associated with antibiotic resistance of the strains (Figure 3). In order to investigate community based antibiotic resistance in a larger scale, 12 samples from Seoul Subway stations were collected and their metagenome are currently in the process of analysis.



Genes	BlastP Hit in CARD	Pathogen	Antibiotic Resitance Ontology	BitScore	PercentIdentity
TSA6.1_00833	AdeK	Acinetobacter baumannii AYE		159	31.1
TSA6.1_00834	AdeJ	Acinetobacter baumannii SDF	adeG	734	41.9
TSA6.1_00835	AdeI	Acinetobacter baumannii SDF		154	32.2
TSA6.1_00915	multidrug efflux protein AdeB	Acinetobacter baumannii SDF		622	37.91
TSA6.1_00968	multidrug efflux protein AdeB	Acinetobacter baumannii AB0057	adeB	870	43.93
TSA6.1_01190	ampC protein	Salmonella enterica subsp. enterica serovar Enteritidis		280	37.96
TSA6.1_01190	class C extended-spectrum beta-lactamase ADC-18	Aeromonas dhakensis		345	47.13
TSA6.1_01348	putative Outer membrane efflux protein	Acinetobacter baumannii AYE	adeH	859	92.68
TSA6.1_01349	multidrug efflux protein AdeH	Acinetobacter baumannii SDF	adeG	2007	97.83
TSA6.1_01350	multidrug ABC transporter	Acinetobacter baumannii SDF	adeF	750	95.07
TSA6.1_01356	putative multidrug resistance efflux protein	Acinetobacter baumannii AYE	abeS	206	96.33
TSA6.1_01696	AdeN	Acinetobacter baumannii	adeN	364	78.8
TSA6.1_02202	beta-lactamase OXA-213	Acinetobacter calcoaceticus	OXA-213	500	91.19
TSA6.1_02732	penicillin-binding protein 2'	Staphylococcus aureus		150	25.92
TSA6.1_03368	MacA	Neisseria gonorrhoeae	macA	264	39.34
TSA6.1_03369	MacB	Neisseria gonorrhoeae	macB	700	52.58
TSA6.1_03386	cfr* protein (2;35 rRNA (adenine(2503)-C(2))-methyltransferase RlmN)	Staphylococcus sciuri	cfrA	171	32.87
TSA6.1_03492	multidrug efflux pump AbeM	Acinetobacter baumannii	abeM	872	97.54
TSA6.1_03729	aminoglycoside resistance protein	Acinetobacter baumannii AYE		150	40.36

Figure 3. Genome map of A. pittii TSA6.1 isolated from the pilot study and its genes associated with antibiotic resistance. Four genes that are likely associated with phenotypically confirmed antibiotic resistance were in red.

3. Major Accomplishments

3.1 Patents

1. 10-2016-0039756 (filed): Novel biomarker for predicting susceptibility of Staphylococcus aureus and uses thereof. Soojin Jang, Yunmi Lee, 2016
2. 10-2016-0044958 (filed): Kit and method for screening antibacterial compound using thiamine monophosphate kinase. Soojin Jang, Hyungjun Kim, 2016
3. 10-2016-0136393 (filed): Pharmaceutical composition for prevention or treatment of pneumonia comprisiong quinolin 4-one derivative or pharmaceutically acceptable salt thereof as an active ingredient. Chul Min Park, Jong Hwan Song, Sunkyung Lee, Soojin Jang, Hyungjun Kim, David Shum, 2016

3.2 Publications

1. Lee Y and Jang S (2016) Draft Genome Sequence of the environmentally isolated Acinetobacter pittii Strain IPK_TSA6.1 GenomeA. Sep 29;4(5). pii: e01028-16. doi: 10.1128/genomeA.01028-16.
2. Hwang SH, Jang S, Park WM, and Park JB (2016) Concentrations and identification of culturable airborne fungi in underground stations of the Seoul Metro. ESPR. (DOI: 10.1007/s11356-016-7291-z). [Epub ahead of print]
3. Jang S (2016) Efflux pumps of Staphylococcus aureus and their clinical implication. J. Micro. 54(1):1-8.

3.3 Presentations

1. Yunmi Lee and Soojin Jang, Identification of a staphylococcal protein as a potential biomarker for antibiotic susceptibility test, Institut Pasteur International Network scientific Symposium, Paris, France, 12-1-2016 (Poster)
2. Soojin Jang, Mechanism study: from phenotypes to target identification, Yonsei University, Seoul, Korea, 10-5-2016 (Oral)
3. Soojin Jang, Identification of a staphylococcal protein as a potential biomarker for antibiotic susceptibility test, The 1st symposium on Frontiers for combating antimicrobial resistance, Daejeon, Korea, 9-6-2016 (Oral)
4. Soojin Jang, Discovery of new antibacterials to overcome antibiotic resistance, Global Antibiotic Research and Development Partnership Project Meeting, Geneva, Swiss, 8-25-2016
5. Soojin Jang, Discovery of new antibacterials against Suberbugs, Gyeonggi Pharma IICC seminar, Suwon, Korea, 7-13-2016 (Oral)
6. Soojin Jang, Investigation of community based microbiome and antibiotic resistance, The 2nd Annual conference on MetaSUB, Shanghai, China, 07-01-2016 (Oral)
7. Soojin Jang, Establishment of a smart city by investigation of microbiomes in Subway systems worldwide, the Seoul Green Civic Organization, Seoul, Kora, 6-28-2016 (Oral)
8. Soojin Jang, Bacterial responses to environmental stresses, The 43rd International symposium &

Annual Meeting on The Korean Society for Microbiology and biotechnology, Daejeon, Korea, 06-24-2016 (Oral)

9. Soojin Jang, Investigation of community based microbiome and antibiotic resistance, Institut Pasteur International Network Regional meeting, Shanghai, China, 05-18-2016 (Oral)
10. Soojin Jang, Institut Pasteur Korea "fight against antibiotic resistance", Gangneung-Wonju National University, Gangneung, Korea, 1-29-2016 (Oral)

3.4 Ongoing & new collaborations

1. Investigation of antipseudomonal activity of oxythiamine, Prof. Kevin Pethe, Nanyang technological University, Novena, Shingapore.
2. Development and pre-clinical efficacy evaluation of bacteriophage and synergistic chemicals for transmission control of Acinetobacter species as major cause of death in ICU, Prof. Dong Eun Yong & Prof. Moo Suck Park, Yonsei University, Seoul, South Korea.
3. Screening of small molecules against P. aeruginosa and S. pneumoniae, Dr. Chul Min Park, Korea research Institute of chemical Technology, Daejeon, South Korea.
4. Discovery of new antibacterial agents against Staphylococcus aureus and Pseudomonas aeruginosa, Dr. Jason Kim and Dr. Hyungchul Ryu, J2HBiotech, Ansan, South Korea
5. Development of a new screening assay using 3D culture of gut epithelial cells. Prof. Jong Hwan Sung, Hongik University, Seoul, South Korea.
6. Investigation of ThiL in P. aeruginosa, Prof. Sun-Shin Cha, Ehwa Womans University, Seoul, South Korea
7. MetaSUB project in Korea, Prof. Sung Chul Seo, Korea University, Seoul, South Korea & Dr. Sung Ho Hwang, National Cancer Center, Ilsan, South Korea.
8. MetaSUB project, MetaSUB consortium & Prof. Christopher E. Mason, Cornell University (Weill Cornell Medical College), New York, US

항생제 내성 연구실
(Antibacterial Resistance Research Laboratory)

1. 연구 요약

세계 보건의료는 빠르게 확산되고 있는 항생제 내성 슈퍼박테리아에 의해 심각하게 위협받고 있다. 이에 항생제 내성 연구팀은 항생제 내성 기전에 대한 이해를 넓히고 이를 바탕으로 새로운 항균물질 도출을 꾀하여 인류의 건강을 위협하고 있는 슈퍼박테리아에 대응하고자 한다. 항생제 내성 연구팀의 주요 연구는 1) 내성균에 대응할 수 있는 새로운 항균물질 도출 및 2) 슈퍼박테리아의 내성 발달기전 연구와 더불어 3) 지역사회 내 내성 분포 조사로써, 특히 새로운 항균물질 도출 연구는 주요 병원균인 녹농균, 폐렴구균, 황색포도상구균에 대한 효과적인 약효검색 시스템 개발을 달성하였고 이는 다른 연구기관 및 제약 벤처기업과의 전략적 공동연구를 이끌어내었다. 또한 현재 진행 중인 황색포도상구균의 내성기전 발달 및 지역사회 내성 분포 연구들은 새로운 내성기작의 발견과 더불어 항생제 내성에 대한 대중적 경각심 고취에도 기여할 것으로 기대되며, 이러한 일련의 연구들의 성공적 수행을 위하여 지속적인 국내외 연구자들과의 협력을 도모하고 있다.

2. 2016년 연구 성과 요약

2016년 항생제 연구팀은 미래부 및 한국연구재단, 경기도와 한국화학연구원의 지원 하에 1) 항폐렴구균 물질 도출 및 2) 황색포도상구균의 내성기작 연구와 항균물질 개발 그리고 3) 녹농균의 티아민단인산화 효소반응 에세이 개발의 총 세 가지 연구과제를 수행하였다. 지난해 본 연구팀에 의해 개발된 폐렴구균에 대한 약효검색 방법은 초고속 검색시스템으로의 최적화 단계를 거쳐 7,800여 개의 화합물에 대한 시범 검색을 성공적으로 마쳤으며, 이 결과는 한국화학연구원과의 공동연구를 이끌어 내었다. 황색포도상구균의 특이적 단백질인 SMP에 대한 연구는 세균의 항생제 감수성과 이 단백질 발현과의 상관관계를 밝혀 새로운 항생제 내성 검색을 위한 생물학적 지표로서의 활용 가능성을 보여주었고, 나아가 이러한 특성을 이용한 신개념 약효검색 방법을 개발하였다. 혁신신약 개발을 위한 또 하나의 노력으로써 항생제 내성팀은 새로운 타깃으로 티아민단인산화 효소를 도출하고 이에 대한 효소반응 검색방법을 개발하였다. 새로운 항균물질 도출과 더불어 항생제 내성에 대한 기초연구의 일환으로 국제연구협력단인 MetaSUB consortium에 참여하여 시범연구를 수행하였고, 현재 이에 대한 결과 분석이 진행 중이다. 이러한 2016년도 연구결과는 총 3편의 논문과 다양한 학회 및 세미나에서 포스터 1회와 초청 강연 10회를 통해 발표되었다.

Discovery Biology

Leishmania Research Laboratory

Principal Investigator: Joo Hwan No, Ph.D.
Laboratory members: Kyunghwa Baek, Ph.D. (Postdoc.); Gyongseon Yang, Ph.D. (Senior Researcher - maternity leave)
Core support members: David Shum (ADS), Sooyoung Byun (ADS), Nakyung Lee (ADS), Sun-ju Kong (CHP), Inhee Choi (BIO)
ADS: Assay Development & Screening Group; **CHP:** Chemistry Platform; **BIO:** Bioinformatics

1. Research Summary

Leishmaniasis is one of neglected tropical disease caused by parasitic species of Leishmania. The disease is spread in 98 countries including different parts of Europe, America, Africa and Asia resulting in 12 million current cases and 57,000 death per year. This fatality number presents second largest parasitic killer after malaria, and the disease is considered as a global public health problem. More importantly, linked to global warming, the disease is emerging towards northern part of Europe as well as North part of America. However, there is no available vaccine against the disease and the current treatments including pentavalent antimonials (Pentostam2®) are old as 100 years and toxic, plus, the emergence of drug-resistant Leishmania threatens the efficacy of existing reservoir of antileishmanials leading us to the urgent necessity of developing new treatments. Based on crying needs for novel antileishmanial therapy, Leishmania Research Laboratory (LRL) focuses on developing and delivering next generation interventions for the patient with Leishmaniasis, and also try to understand the underpinning mechanism of the parasite pathogenesis.

2. Research Progress in 2016

The current research area of Leishmania Research Laboratory are 1) discovery of novel leads through phenotypic approach, 2) discovery of novel leads through target-based approach, 3) biology of a unique mitochondria of the parasite called kinetoplast and 4) host-Leishmania interactions.

For the first theme, we have developed phenotypic intracellular Leishmania assay in high throughput screening format to identify first screen bioactive/FDA approved compounds to fast identify drug for repositioning approach and second, to screen large small molecule libraries for identifying hit compounds which can be further optimized for lead compounds. For the second theme, we have chosen Trypanothione synthase (TryS) as a target protein to discover inhibitors which could disrupt the essential pathway of Leishmania survival. Thirdly, we utilize clinically used compounds pentamidine and its analogs to understand the mechanism of action as well as the replication process of Leishmania mitochondria replication that could lead to discovery of novel drug target. The last theme has been recently initiated with an approach of using genome wide siRNA library to identify host factors that are involved in Leishmania infection and intracellular development.

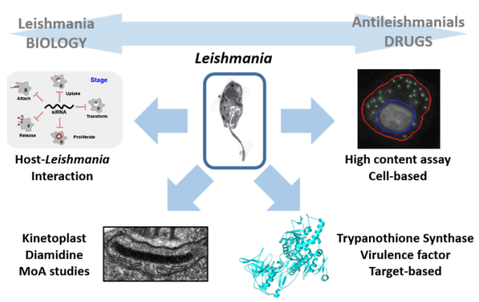


Figure 1. Overall research themes in LRL.

2.1 Discovery of novel leads against Leishmania infection through phenotypic approach

In order to identify lead compounds against Leishmania infection, we have developed intracellular Leishmania assay and screened 1) pilot library composed of bioactive and FDA approved compounds and a diversity set library of ~100,000 compounds. Hits from the pilot screening is subjected to in vivo efficacy test and the hits from large scale screening is under progress for hit to lead optimization. Also we have extended our study to apply the phenotypic assay to Trypanosoma cruzi to identify inhibitors through repositioning strategy.

2.1.1 Results

From the pilot screening of 1,742 bioactive/FDA approved compound library, we have identified a class of compounds, mTOR kinase inhibitors, as potent inhibitors against Leishmania growth in vitro. We have extended the list of inhibitors and come up with a list of antileishmanial mTOR kinst inhibitor (Troin2, BEZ235, NVP-BGT226, INK128, PP242, GDC0032, WYE-125132, and PKI-402) in which the EC_{50} values ranges from 0.040 to 7.4uM. We than check the toxicity in vivo in BALB/c mouse to set the concentration to be tested in vivo for Leishmania infected mouse model. (Figure 2)

Previously, we have identified inhibitor from 50,000 compound screening and recently, we have additionally screened extra 50,000 compounds to increase the number of hits for optimizations. From the previous screening we have optimized imidazopyridine scaffolds to yield an inhibitor with potency of 0.9uM, however a short half-life was observed in microsomal stability. Currently, we are in process of selecting a scaffold (phthalimidethioate, furansulfonamide, pyridazinyl benzene sulfonamide, pyrazolothiazepinone) to optimize for generation of leads. (Figure 2)

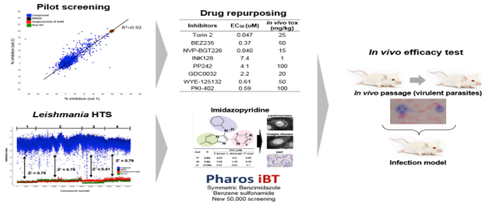


Figure 2. Lead generation through repurposing and hit to lead optimizations approaches.

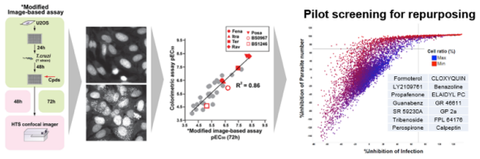


Figure 3. Development and evaluation of intracellular T. cruzi assay and pilot screening for repositioning.

We compare the results of widely used colorimetric and image-based intracellular Trypanosoma cruzi assay protocols by testing 32 reference compounds. The two assays were found to have very poor correlation of R^2 = 0.005 based on EC_{50} values. Further modifications on experimental procedures demonstrate that seeding scheme and incubation time of cells and compounds are the critical factors for determining the potency and efficacy of compounds against T. cruzi. Furthermore, we have screened 8,356 bioactive/FDA approved compound using the assay and identified 53 inhibitors that are active against the parasite but not toxic to the host cells. Currently, we are in process to evaluate their potencies through dose dependent testing. (Figure 3)

2.2 Target-based drug discovery of compounds interfering with trypanothione biosynthesis in trypanosomatids

Trypanothione is an important virulence factor for Leishmania survival and trypanothione synthase (TryS) is considered an attractive drug target due to its absence in human. Therefore, we have developed protein-based biochemical assay in high throughput screening format to identify small molecule inhibitors that interferes the function of TryS and ultimately kill the parasite. Currently, ~50,000 compounds were screened against the enzyme and hits have been identified for optimizations.

2.2.1 Results

In order to identify inhibitors against TryS, we expressed and purified a large quantity (> 300mg) of TryS and developed an assay through the detection of inorganic phosphate by malachite green. The assay was established in 384 plate format and a pilot screening of 4,210 compounds was performed in duplicate (R^2 = 0.96) at 10uM and based on > 90% inhibition threshold 16 hits were identified and furthermore, 47,414 diversity set compounds were screened which identifued 128 hit compounds. Currently, those hit compounds are under optimization with Institut Pasteur de Montevideo. (Figure 4)

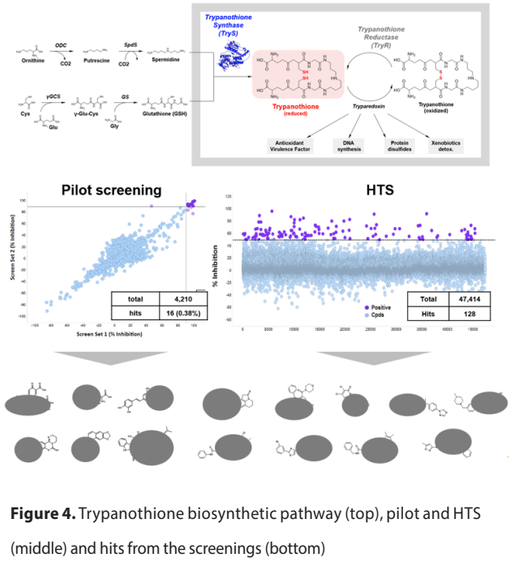


Figure 4. Trypanothione biosynthetic pathway (top), pilot and HTS (middle) and hits from the screenings (bottom)

2.3 Elucidation of mechanism of diamidines in Leishmania and Trypanosoma

Pentamidine is a clinically used drug for Leishmaniasis and Trypanosomiasis, however, the mechanism of action of pentamidine is not well elucidate. Therefore, we have used pentamidine and its related diamidine analogs such as DB75 to decipher the mode of action of their compounds. Based on our study, we have discovered that these types of compounds localize to the mitochondria of Leishmania and selectively inhibit the replication process of mitochondrial DNA which is known as kinetoplast DNA.

2.3.1 Results

Pentamidine, a diamidine type of compound, is one of the treatment, its antileishmanial mechanism of action has not been investigated in-depth. Here we tested several diamidines, including pentamidine and its analog DB75, against Leishmania parasites and elucidate their antileishmanial mechanisms. We identified three promising new antileishmanial diamidine compounds with EC_{50} values of 3.2, 3.4, and 4.5 μM, respectively, while pentamidine and DB75 exhibited EC_{50} values of 1.46 and 20 μM. The most potent inhibitor showed strong DNA binding properties, with a ΔT_m value of 24.4°C, whereas pentamidine had a ΔT_m value of 2.1°C, and DB75 7.7°C. Additionally, DB75 localized in L. donovani kinetoplast DNA (kDNA) and mitochondria, but not in nuclear DNA (nDNA). For 2 new diamidines, strong localization signals were observed in kDNA at 1μM, and at higher concentrations, the signals also appeared in nuclei. All tested diamidines showed selective and dose-dependent inhibition of kDNA, but not nDNA, replication, likely by inhibiting L. donovani Topoisomerase IB. Overall, these results suggest that diamidine antileishmanial compounds exert activity by accumulating towards and blocking replication of parasite kDNA.

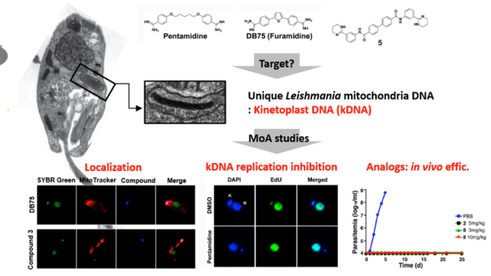


Figure 5. Elucidation of mode of action of pentamidine and related analogs.

2.4 A multilevel systems approach to elucidate the host-Leishmania interactome and to identify host targets for anti-leishmanial drug discovery

Leishmania is an obligated intracellular parasite which resides inside human macrophage. The parasite is known to effectively bypass the host attack by reactive oxygen species and successfully hijack the host for their survival. Therefore, we have established intracellular Leishmania assay using THP-1 as the host, and evaluated siRNA transfection and knock-down efficiency to screen genome wide human gene to identify host factors that are responsible for the parasite invasion and intracellular survival.

2.4.1 Results

In order to establish an assay for human genome-wide siRNA screening, we first evaluated the ability of parasites to invade the host cells and replicate inside. Using L. donovani strain, the parasite were able to invade, however, was not replicating. But by changing the parasite strain to L. amazonenesis, we to verified its ability to replicate through lablelling the replicated DNA with EdU. Next, we have checked siRNA efficiency in THP-1 by transfecting p65 siRNA and observing the expression level of p65 protein by immunofluorescence imaging. Currently, we have optimized the infection condition of the host and siRNA delivery in 384 plate format and preparing for the genome wide screening. (Figure 6)

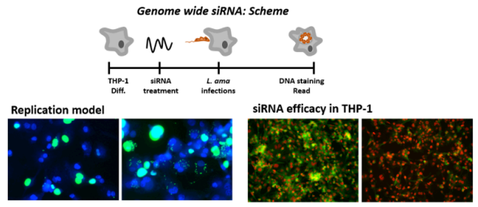


Figure 6. siRNA screening scheme, confirmation of L. amazonenesis replication in THP-1 and validation of siRNA transfection.

3. Major Accomplishments

3.1 Publications

1. Yang G, Lee N, Ioset JR, No JH (2016) Evaluation of Parameters Impacting Drug Susceptibility in Intracellular Trypanosoma cruzi Assay Protocol, J Biomol Screen (Accepted, Front Cover)
2. Yang G, Choi G, No JH (2016) Antileishmanial Mechanism of Diamidines: Targeting Kinetoplasts, Antimicrob Agents Chemother. 60(11): 6828-6836
3. Sun YN*, No JH*, Lee GY, Li W, Yang SY, Yang G, Schmidt TJ, Kang JS, Kim YH. (2016) Phenolic Constituents of Medicinal Plants with Activity against Trypanosoma brucei. Molecules. 21(4):480.
4. Durieu E, Prina E, Leclercq O, Oumata N, Gaboriaud-Kolar N, Vougogiannopoulou K, Aulner N, Defontaine A, No JH, Ruchaud S, Skaltsounis AL, Galons H, Spath GF, Meijer L, Rachidi N. (2016) From Drug Screening to Target Deconvolution: a Target-Based Drug Discovery Pipeline Using Leishmania Casein Kinase 1 Isoform 2 To Identify Compounds with Antileishmanial Activity. Antimicrob Agents Chemother. 60(5):2822-33.
5. No JH. (2016) Visceral leishmaniasis: Revisiting current treatments and approaches for future discoveries. Acta Trop. 155:113-23

3.2 Presentations

1. Drug Discovery for Leishmania infections: Revisiting Current Treatments and Approaches for Future Discoveries. Leishmania and Leishmaniasis workshop. Tehran, Iran. 2016 Nov. (invited)
2. Identification of starting points for antileishmanial drug discovery using high content cell-based assay. Drug Discovery Symposium: See what other don't. Seoul, Republic of Korea. April. (invited)
3. Antileishmanial Mechanism of Diamidines: Targeting Kinetoplasts. Institut Pasteur International Network meeting. Paris, France. 2016. Dec.

3.3 Ongoing & new collaborations

1. Diamidine mechanism of action, Prof. Eric Oldfield, Professor, University of Illinois at Urbana Champaign Department of Chemistry, USA
2. Discovery of inhibitors against TryS, Marcelo Comini, Group head, Institut Pasteur de Montevideo, Uruguay
3. Host-leishmania interactions, Gerald Spaeth, Unit head, Institut Pasteur Paris, France
4. Drug discovery against Leishmaniasis through phenotypic approach, Kiyoub Nam, Director, PharosIBT, Republic of Korea

리슈만편모충 연구실
(Leishmania Research Laboratory)

1. 연구 요약

리슈만편모충증은 열대성 소외질환 중의 하나로 리슈만편모충에 의하여 발병하는 감염성 질환이다. 유럽, 아메리카, 아프리카 및 아시아 등 98개국에 분포되어 있는 리슈만편모충증은 매년 1,200만 명의 감염자와 57,000명의 사망자를 유발하고 있다. 이러한 숫자는 말라리아 다음의 기생충성 질환이며 이로 인하여 세계 공중보건에 심각한 문제를 초래하고 있다. 무엇보다도 전 세계의 열대야 현상으로 인한 북유럽 및 북아메리카로의 확산은 선진국들의 공중보건에 심각한 문제로 대두되고 있는 상황이다. 하지만 현재 본 질환에 대한 백신이 개발되어 있지 않고, Pentostam®을 포함한 일선 치료제는 개발된 지 100여 년이 넘어 독성이 매우 심각할뿐더러, 최근 확산되고 있는 저항성 리슈만편모충으로 인해 기존 치료제들의 효과가 낮아져 새로운 항리슈만편모충 치료제 개발이 시급한 실정이다. 따라서 리슈만편모충팀은 본 팀이 기 확보한 고유의 초고속 리슈만편모충 약효탐색 스크리닝 플랫폼을 활용하여 차세대 리슈만편모충 치료제 개발에 집중하고 있으며 이와 더불어 리슈만편모충의 감염 요인을 분자생물학적 관점에서 연구하고 있다.

2. 2016년 연구 성과 요약

리슈만편모충팀의 연구 분야는 크게 네 가지로 구분된다. 1) 표현형(세포기반)을 기반으로 하는 신규 선도물질 발굴 2) 표적기반 선도물질 발굴 3) 리슈만편모충의 특이적 미토콘드리아인 키네토플래스트의 연구 4) 숙주–리슈만편모충 상호작용. (그림 1)

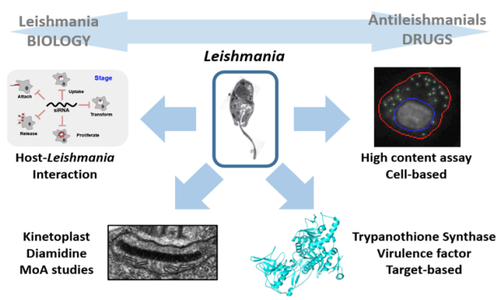


그림1. 리슈만편모충팀의 연구 개요도

1) 본 연구팀은 리슈만편모충 감염 숙주세포 시스템을 이미지 기반의 초고속 약효평가 플랫폼에 적용하여 스크리닝 시스템으로 구축하였다. 이를 이용해 1,742개의 생활성물질/FDA 승인 약물을 스크리닝하였으며 활성이 매우 뛰어난 mTOR kinase 저해제들을 발굴하였다. 이는 현재 리슈만편모충을 표적으로 하는 repurposing이 진행 중이다. 또한 100,000개의 저분자 화합물들을 스크리닝하여 새로운 유효물질을 발굴, 현재 선도물질로의 최적화를 진행 중이다.

2) 리슈만편모충의 생존에 필수불가결한 Trypanothione synthase를 표적으로 하여 단백질 기반 대용량 초고속 스크리닝 시스템을 완비하였으며 4,120개의 파일롯 화합물 스크리닝을 통한 12개의 유효물질과 47,414개의 대용량 스크리닝을 통한 128개의 유효물질을 도출하여 최적화 과정에 착수할 예정이다.

3) 현재 리슈만편모충이나 아프리카 수면병에 사용 중인 pentamidine의 활성 기작은 잘 알려져 있지 않다. 따라서 본 연구팀은 pentamidine과 이의 유도체를 통하여 이러한 종류의 화합물들이 리슈만편모충의 미토콘드리아에 집중적으로 분포되고 리슈만편모충 미토콘드리아의 DNA인 키네토플래스트 DNA(kDNA)에 결합함으로써 kDNA의 자가 복제를 선택적으로 저해한다는 사실을 발힘과 동시에 복제 과정의 필수적 효소인 특이적 Topoisomerase IB가 새로운 표적으로써의 가치가 있다는 사실을 확인하였다.

4) 대식세포 내에서 생존하는 리슈만편모충의 숙주세포와의 상호관계를 연구하기 위하여 본 연구팀은 세포 내 자가 증식이 가능한 리슈만편모충 모델을 개발하였으며 siRNA의 형질주입 조건을 384 well plate에 최적화하였다. 이를 이용하여 human genome–wide siRNA 스크리닝을 실행, 리슈만편모충의 감염 및 증식에 관여하는 숙주인자를 발굴할 계획이다.

Discovery Biology

Cancer Biology Research Laboratory

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ADS: Assay Development & Screening; **ALM:** Automation & Logistics Management

1. Research Summary

Hepatocellular carcinoma (HCC) is the sixth most common malignant tumor and the second leading cause of cancer-related deaths in the world. Chronic infection by hepatitis B and C and alcohol are major causes, as well as metastases from tumors elsewhere in the body.

Most HCCs are resistant to conventional chemotherapeutic agents. Today, Sorafenib, which is a multikinase inhibitor, is approved for the treatment of patients with advanced HCC. Generally, sorafenib treatment costs US$5,400 per month, but it just extends lifespan by an average of only 2.8 months with various side effects. After approval of sorafenib, researchers expected to improve on it quickly and produce many more drugs for HCC that capitalized on the same mechanism. However, several prominent liver-cancer drugs candidates have failed in recent year.

Based on these situations, CBRL should think new strategy for HCC drug discovery. For a long time, oncologists have studied the functions of oncogenes and tumor suppressor genes in tumorigenesis. In the recent years, the concept of cancer biology is changing from the genetics of tumor cells alone to studying the complicated interplay between cancer and the tumor microenvironment (TME) and the role of the TME in HCC has been probed to combat this deadly disease. Particularly, a deeper knowledge of the crosstalk between tumor cells and their tumor microenvironment (TME) is needed to fully understand tumor development, progression and chemo-resistance in HCC, because this cancer develops from chronically damaged tissue that contains large amounts of inflammation and fibrosis.

Based on these backgrounds, here, CBRL established Multicellular tumor spheroid (MTCS) models and co-culture model of HCC cells with hepatocytes for HCC drugs screening. Subsequently, we successfully performed the pilot screening for HCC therapy via utilization of our own drugs screening ways. Our MCTS-based screening system is expected as a powerful method to mirror tumor complexity and heterogeneity enhancement for anticancer research.

2. Research Progress in 2016

Most Hepatocellular carcinoma (HCC) are resistant to conventional chemotherapeutic agents and remain an unmet medical need. Recently, research activities have been focused on the role of tumor microenvironment and its impact on HCC resistance to chemotherapies. Cancer Biology Research Laboratory (CBRL) constructed 3D tumor microenvironment (TME) in vitro to fully understand tumor development, progression and chemo-resistance as well as drug discovery for liver cancer therapy.

In 2016, CBRL focused on three major programs 1) proof of values of multicellular tumor spheroids (MCTS) as miniature systems to mirror human tumors, 2) development therapeutic agents to overcome HCC and 3) find biomarkers for early diagnosis, because international research trends of medical paradigm is converted from treatment to prevention, early diagnosis. Additionally, the recent advancements in the use of liver cancer stem cells (LCSC) to develop efficient and organized means to an antitumor agent is quickly gaining recognition as a novel goal. Hence, we proposed to use non-target based high throughput screening approach to specifically target CD133+ HCC present in mixed populations of HCC cells with hepatocytes.

2.1 Proof of values of MCTS as miniature systems to mirror human tumors

Multicellular tumor spheroid (MTCS) model is expected as a powerful method for anticancer research, because it is able to realize tumor complexity and heterogeneity of tumor tissue, 3-dimensional cellular context and pathophysiological gradients of in vivo tumor. However, the formation of MTCS without considering clinical tumor condition is hard to obtain valuable result. Here, we struggled to provide a proof of concept whether MTCS are in a very close in vivo tumor microenvironments.

2.1.1. Results

In 2016, we struggled to provide a proof of concept whether our MTCS are in a very close in vivo tumor microenvironments. Gene-expression profiling was performed on MCTS to normalize the profiles of tumor spheroids. We first identified 1596 differentially expressed gene features between MCTS and tumor spheroids, with the cut-off of more than 2-fold difference and $P < 0.01$. To demonstrate MCTS specific signature that implicated in phenotype of patient-derived HCC, we performed Gene set enrichment analysis (GSEA) using GEO datasets from two independent HCC cohorts. These results revealed that MCTS specific signature are truly enriched in HCC tumor tissues in comparison to non-tumor tissues.

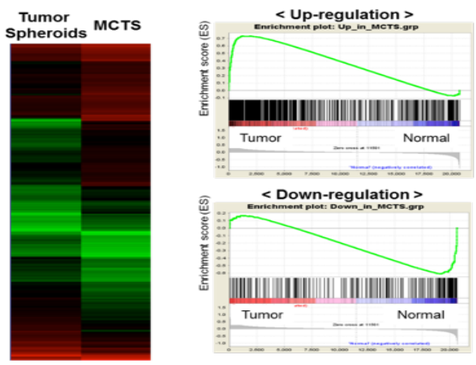


Figure 1. Gene expression profiling and GSEA analysis between MCTS and HCC tissues

2.2 Development therapeutic agents to overcome HCC

We have to start thinking of developing new technologies to allow us for drug screening in a much more realistic way. To apply the MCTS to high throughput screening, first, we should find the most efficient method for conducting of 3D spheroids because formation process of 3D spheroids are expensive, cumbersome, and complicated. In spite of these difficulties, we established and validated highly reproducible MCTS-based high throughput screening (HTS) system for HCC drugs screening through generation of homogenous sized, same configured single spheroids in multi-well plates.

2.2.1 Results

We screened using MCTS-based screening platform against 4,763 compounds in duplicate to confirm the reproducibility of observed effects. A Z' factor of 0.46 and a pearson correlation coefficient of 0.89 for replicate screens indicated that the assay was reliable. 87 compounds that significantly inhibited size of MCTS and intensity of HCC-RFP. To verify hits from the primary screening, dose response studies performed to quantify the potency of selected hits. To confirm selectivity and specificity of the observed response on a HCC population, a counter screen were done various stromal cells such as WI38 cells, LX2 cells and HUVEC. Dose-response curves also used to determine the effective concentration needed to decrease proliferation by 50% (EC_{50}) in HCC cell lines and normal hepatocyte.

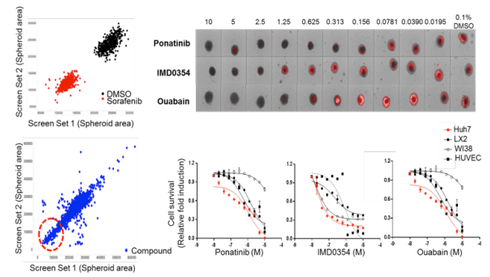


Figure 2. Identification of novel drugs for HCC therapy using MCTS-based screening system

2.3 Secretome analysis of MCTS for the discovery of HCC-specific biomarkers

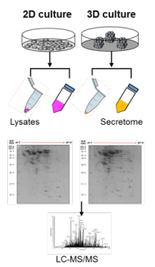
Researchers aim to develop therapeutic agents to overcome HCC and to find biomarker for early diagnosis, because international research trends of medical paradigm is converted from treatment to prevention, early diagnosis. A number of novel biomarkers have now been suggested in the literature, which may reinforce the current surveillance methods. Representative identification methods of biomarkers for cancers are genomics, proteomics and metabolomics.

2.3.1 Results

To find new potential biomarkers for HCC, we identified highly expressed secreted proteins in the 3D-cultured HCC conditioned medium (3D-CM) relative to monolayer-cultured HCC conditioned medium (2D-CM) using 2D-gel electrophoresis (2DE) analysis.

Total of 69 secreted proteins were increased in the 3D-CM compare to 2D-CM. Among these, we selected 6 secreted proteins, which uniquely detected in 3D-CM including PAST1, AKR1C1, YWHAW, TPM4, SORD, AKR1C4. These molecules mainly displayed functional interaction for controlling oxidation-reduction reaction in the pathway of various metabolisms.

The bioinformatics researches showed experimental and literature evidences for AKR1C1, AKR1C4, and SORD to have possibilities of being involved in HCC biological responses. Particularly, AKR1C4 had the most HCC specificity among them on the levels of cells as well as tissues. Hence, these results demonstrated apparent differences in the metabolism between 2D and 3D culture conditions of HCC, and suggested that AKR1C4 is one of the potential HCC markers.



High expressed secreted proteins in the 3D-cultured HCC CM
Pyruvate kinase (PKM)
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
Apolipoprotein E (APOE)
Peroxiredoxin-1 (PRDX1)
Neuropolypeptide h3 (PEBP1)
L-lactate dehydrogenase A chain isoform 1(LDHA)

Uniquely detected secreted proteins in 3D-cultured HCC CM
Phosphoserine aminotransferase isoform 1(PSAT1)
Dihydrodiol dehydrogenase (AKR1C1)
Chain A, 14-3-3 Protein Epsilon Complexed To Peptide (YWHAW)
Tropomyosin alpha-4 chain isoform Tpm4.2cy (TPM4)
L-iditol-2 dehydrogenase (SORD)
Chlordecone reductase (AKR1C4)

Figure 3. Identification of novel biomarkers through secretomics of HCC spheroids

2.4 Identifying specifically compounds targeting CD133+cells

Cancer stem cells (CSCs) are considered the 'Achilles heel' due to their strong resistance to chemotherapy and radiotherapy. Thus, the recent advancements in the use of liver cancer stem cells (LCSC) to develop efficient and organized means to an antitumor agent is quickly gaining recognition as a novel goal. CBRL characterized CSCs in primary hepatocellular carcinoma (HCC) and identified CD133 as a CSC cell-surface marker.

2.4.1 Results

We screened 3,280 compounds selected from compounds libraries (including LOPAC, Prestwick and ChemDiv) in duplicate to confirm the reproducibility of observed effects. We identified 4 compounds, which are -Chloro-L-alanine hydrochloride, LY-294,002, oxytetracycline and fusidic acid, showed significant inhibition activity of CD133+HCC population without damage on hepatocytes. Through secondary assay, we validated that oxytetracycline could suppress expression of CD133 as well as stemness in HCC.

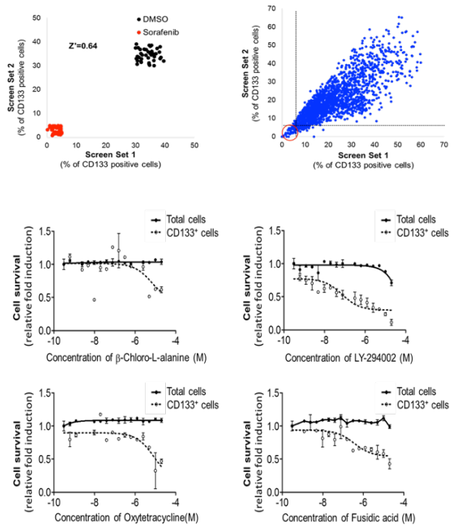


Figure 4. High throughput screening for compounds that regulate CD133+ hepatocellular carcinoma.

3. Major Accomplishments

3.1 Publications

1. Song YH, Kim JS, Choi EK, Kim KM, Seo HR (2016). TGF-independent CTGF induction regulates cell adhesion-mediated drug resistance by increasing collagen I in HCC. Oncotarget. (accepted)
2. Song YH, Kim SH, Kim KM, Choi EK, Kim J, Seo HR (2016) Activated hepatic stellate cells play pivotal roles in hepatocellular carcinoma cell chemoresistance and migration in multicellular tumor spheroids. Scientific reports. 6:36750.
3. Jang JW, Song YH, Kim KM, Kim JS, Choi EK, KimJ,Seo HR (2016) Hepatocellular carcinoma-targeted drug discovery through image-based phenotypic screening in co-cultures of HCC cells with hepatocytes. BMC cancer. 16(1):810

3.2 Presentations

1. Seo HR, Poster. CD133 attenuates ROS accumulation via a steady increase in the expression of the cystine/glutamate transporter xCT: Consequence on chemoresistance in hepatocellular carcinoma, The American Association for Cancer Research Annual Meeting 2016, nest N. Morial Convention Center, New Orleans, Louisiana, 16-April-2016.
2. Song YH, Poster. Establishment of 3D tumor microenvironment in vitro for Hepatocellular Carcinoma Therapy. The American Association for Cancer Research Annual Meeting 2016, nest N. Morial Convention Center, New Orleans, Louisiana, 17- April-2016.
3. Seo HR, Oral. Advances in establishment of 3D tumor microenvironment in vitro for Hepatocellular Carcinoma Therapy. Institut Pasteur International Network Asian Regional Meeting in Shanghai. SIBS conference room, Shanghai, China, 19-May-2016.
4. Song YH, Poster. TGF-independent CTGF induction regulates cell adhesion-mediated drug resistance by increasing collagen I in HCC spheroids. The 42th Annual meeting of Korean Cancer association with international cancer Conference. Seoul, South Korea, 16-June-2016.
5. Kim SH, Oral. Establishment of 3D tumor microenvironment in vitro for Hepatocellular Carcinoma Therapy. The 42th Annual meeting of Korean Cancer association with international cancer Conference. Seoul, South Korea, 17-June-2016.
6. Song YH, Poster. CD133 confers cancer stem-like cell properties by stabilizing EGFR-AKT signaling in hepatocellular carcinoma, KSMCB, Seoul, Korea, 13-October-2016.
7. Kim SH, Poster. Secretome analysis of tumor spheroids for the discovery of HCC-specific biomarkers, KSMCB, Seoul, Korea, 13-October-2016.
8. Seo HR, Poster. Secretome analysis of tumor spheroids for the discovery of HCC-specific biomarkers, International scientific symposium Institute Pasteur International network, Paris, France, 30-November-2016

3.3 Ongoing & new collaborations

1. Establishment of 3D-tumor microenvironment (TME) for liver cancer therapy, Dr. Kang Mo Kim, ASAN Medical Center, Seoul, Korea
2. Development of radiotherapy through regulating radiation-induced endothelial to mesenchymal transition, Dr. Yoon-Jin Lee, KIRAMS, Seoul, Korea
3. Establishment of T2B Infrastructure Center for Advancing Cancer Therapeutics, Dr. Eun Kyung Choi, ASAN Medical Center, Seoul, Korea
4. Identification of new target for liver cancer through elucidation of mechanism of chemoresistance in CD133+ HCC. Joon Kim, Korea University, Seoul, Korea
5. Identification of gastric cancer biomarkers with the final goal to develop non-invasive tests for gastric cancer prevention/detection. Dr. Eliette Touati, Institute Pasteur Paris, Paris, France
6. HCC-spheroid xenograft models; Breakthrough Tools for reappearance of human HCC in mouse models. Dr. Woo Seong Choi, K-bio Cheongju-si, Chungbuk, Korea

종양생물학 연구실
(Cancer Biology Research Laboratory)

대부분의 악성 간세포 암종(HCC)은 기존 항암 화학요법에 대해 강한 내성을 나타내기 때문에 이를 극복할 수 있는 약물개발이 시급한 상황이다. 최근 들어 종양 미세 환경의 역할 및 항암 화학요법에 대한 내성과의 관계를 밝히는 데 많은 연구가 집중되고 있다. 종양생물학 연구팀은 3차원 종양 미세 환경을 구축함으로써 종양의 발달, 진행 및 항암제 내성에 관한 연구를 진행할 뿐만 아니라 간암 치료를 위한 약물 발굴에도 초점을 맞추고 있다.

2016년, 본 연구팀은 다음 3가지 주제 1) 실제 종양을 대변할 수 있는 다세포 종양 구상체의 가치 증명, 2) 간암을 극복하기 위한 치료제 개발, 3) 조기 진단을 위한 바이오 마커 발견을 가지고 연구를 진행하였다.

또한, 효율적이고 체계적인 항암제 개발에 있어 암 줄기세포의 연구가 주목되고 있다. 그러므로, 본 연구팀은 정상 간세포와 간암 세포의 혼합배양을 통해 CD133 발현 세포만을 타깃으로 하는 스크리닝 시스템을 구축하여 스크리닝을 수행하였다.

1. 연구 요약

악성간암종은 세계에서 여섯 번째로 흔한 악성 종양이며, 암 관련 사망의 두 번째 주요 원인이다. 신체의 다른 부위에 있는 종양으로부터의 전이뿐만 아니라, B형 및 C형 간염의 만성 감염과 알코올이 주요 원인으로 알려져 있다.

대부분의 악성간암종은 기존 화학요법에 강한 내성을 갖는다. Multi–tyrosine kinase 억제제인 소라페닙(Sorafenib)은 진행된 간암환자를 치료하는 약물로써 승인 받은 현재까지 유일한 약물이다. 일반적으로 소라페닙을 이용한 치료비용은 월 5,400달러에 이르지만, 다양한 부작용과 함께 평균 2.8개월 만의 수명만 연장할 뿐이다. 소라페닙의 승인 후에, 많은 연구진들은 소라페닙의 개선과 함께 같은 메커니즘을 이용한 더 많은 약물의 발굴이 이뤄질 것이라 기대했지만, 이미 최근 몇몇의 후보 항암제들이 임상시험에서 실패하고 있다.

이런 상황에 맞춰, 종양생물학 연구팀(CBRL)은 간암 신약 개발을 위한 새로운 전략이 필요했다. 오랜기간 동안, 종양학자들은 종양 형성에 있어 종양 유전자 및 종양 억제 유전자의 기능을 연구해왔다. 최근 몇 년간, 종양 생물학의 개념은 종양 세포의 유전학에서 암과 종양 미세 환경 사이의 복잡한 상호작용을 연구하는 것으로 변화되고 있으며, 간암 환자의 종양 미세 환경의 역할에 대한 연구는 간암을 극복하기 위해 연구되어 왔다. 특히, 간암은 다량의 염증 및 섬유화를 보이는 손상된 조직으로부터 발생하기 때문에 종양의 발달 및 진행, 항암제에 대한 내성에 대해 충분히 이해하기 위해서는 암과 종양 미세환경 사이의 상호작용에 대한 전문적인 지식이 필요하다.

이러한 배경을 바탕으로, 종양생물학 연구팀은 간암 약물 스크리닝을 위해 다세포 종양 구상체 모델(MCTS)과 정상 간세포와 암세포의 혼합배양 모델을 구축했다. 그 결과, 본 연구팀은 개발된 약물 스크리닝 방법을 이용하여 간암 치료를 위한 약물 파일럿 스크리닝을 성공적으로 수행했다. 다세포 종양 구상체를 기반으로 한 스크리닝 시스템은 종양의 복합성과 이질성을 반영하여 암 연구에 있어 매우 유용한 방법이 될 것이라 기대된다.

2. 2016년 연구 성과 요약

2.1 실제 종양을 대변할 수 있는 다세포 종양 구상체의 가치 증명

다세포 종양 구상체는 암 치료제 개발 및 연구에 있어서 매우 영향력 있는 기술로 기대되고 있는 모델이다. 실제 종양은 매우 복잡하고, 이질적인 세포들로 구성되어 있을 뿐만 아니라 3차원의 병리, 생리학적 특성을 가지고 있기 때문이다. 그러나 임상적인 종양 상태를 고려하지 않는 다세포 종양 구상체의 형성은 가치 있는 결과를 얻기가 어렵다. 따라서, 본 연구팀은 실제로 생체 내 종양 미세 환경과 가까운 다세포 종양 구상체를 만들기 위해 다세포 구상제의 gene expression profiling 분석을 통한 Gene Set Enrichment Analysis와 In vivo에서의 다세포 구상체의 특이성 등을 밝힘으로써 다세포 종양 구상체의 가치 증명하였다.

2.2 악성간암종을 극복하기 위한 치료제 개발

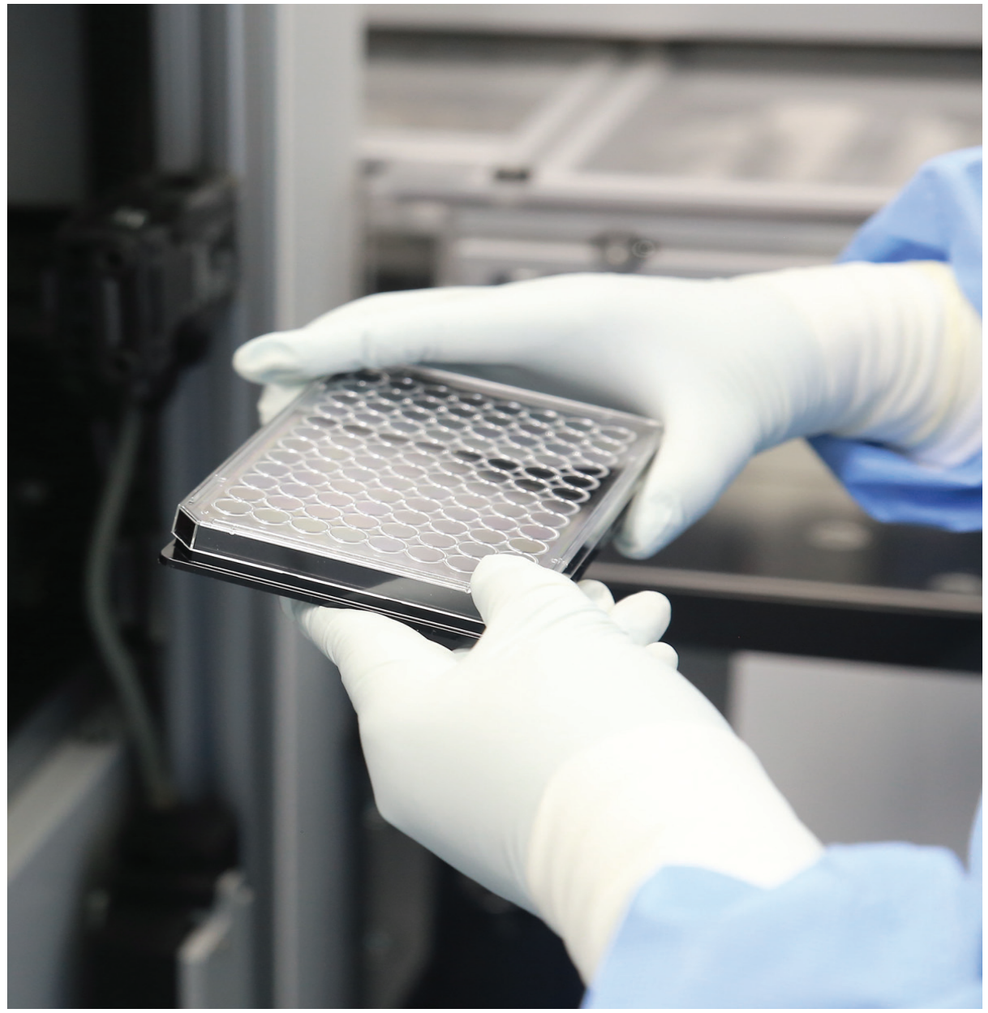
본 연구팀은 좀 더 실질적인 방법으로 약물 스크리닝을 하기 위해 새로운 기술을 개발하고자 하였다. 일반적으로 3차원 구상체는 매우 복잡하고 다루기 어려울 뿐만 아니라 고비용이 드는 방법이기 때문에, 대규모 스크리닝(HTS)에 적용시키기 위해 다세포 종양 구상체를 효율적으로 만들 수 있는 방법을 찾아야만 했다. 많은 어려움에도 불구하고, 본 연구팀은 동일한 크기와 구성의 단일 구상체를 적용한 간암 치료제 스크리닝 시스템을 구축하고 이를 활용하여 성공적으로 pilot 스크리닝을 수행하였다. 이를 기반으로 대용량 스크리닝에 본 플랫폼을 적용하고자 한다.

2.3 간암 특이적인 바이오 마커를 찾기 위한 다세포 종양 구상체의 secretome 분석

현재 세계적인 연구 경향은 질병 치료에서부터 조기 진단으로의 전환이다. 이러한 의학적 패러다임의 변화에 따라 본 연구진은 간암 특이적인 바이오 마커를 찾고자 하였다. 현재 간암 발병을 예측할 수 있는 새로운 바이오 마커들이 제시되고 있으며, 암의 특이적 바이오 마커를 찾는 대표적인 방법으로 유전체학, 단백질체학, 대사체학이 이용되고 있다. 본 연구진은 다세포 구상체에서의 secretome 분석을 통하여 다세포 구상체가 일반적인 세포 배양에 비해 월등히 활발한 신진대사(metabolism) 능력을 보유하고 있음을 확인하였을 뿐만 아니라, 다세포 구상체에서의 특이적인 secretome을 선별하여 새로운 biomarkers로써의 가능성을 연구중에 있다.

2.4 CD133 발현 세포를 타깃으로 한 특이적인 화합물 발굴

암 줄기세포는 화학적 치료법과 방사선 치료법에 강력한 저항성을 가지고 있기 때문에 '아킬레스 건'이라고 여겨진다. 따라서, 효율적이고 체계적인 항암제 개발에 있어 간암 줄기세포를 이용하는 최근 동향은 새로운 전략으로 인식되고 있다. 본 연구팀은 간암에서의 암 줄기세포를 확인 및 분석하였고, CD133을 암 줄기세포 표면 표지자로써 확인하였다. 이를 바탕으로 간암 줄기세포만을 선택적으로 사멸시킬 수 있는 약물을 탐색하기 위한 스크리닝 플랫폼을 구축하였으며, 이를 활용하여 스크리닝 한 결과, 4개의 HIT을 발굴할 수 있었다.



Core Support Groups

Automation & Logistics Management

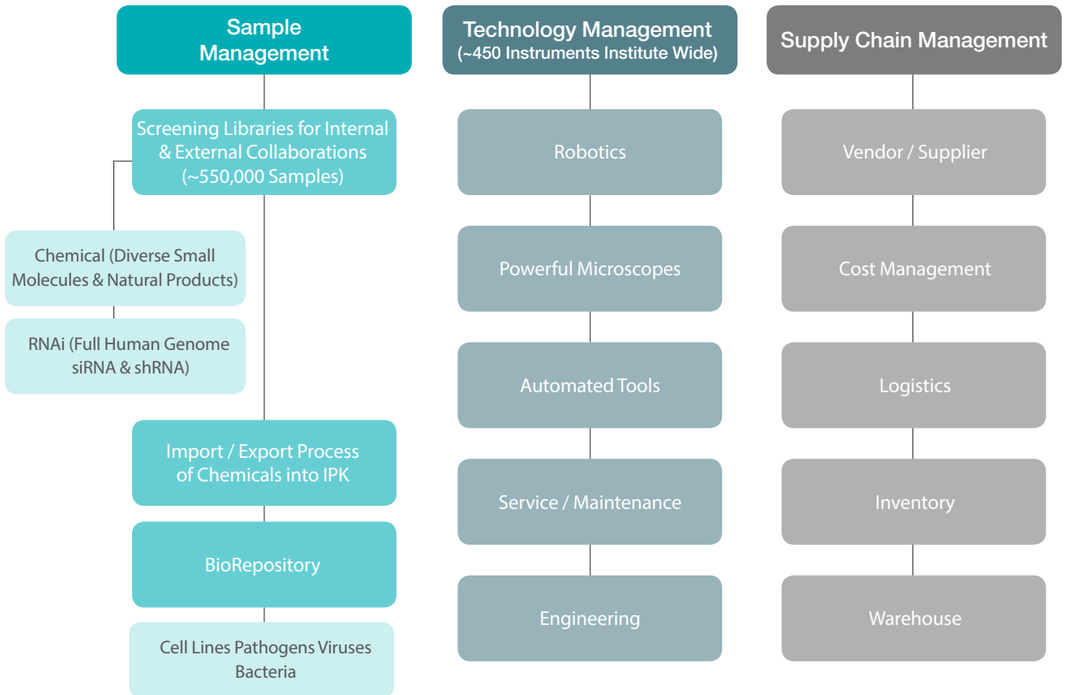
Group Head : Constantin Radu, M.Sc.

Laboratory members: Honggun Lee, M.Sc. (Senior researcher); Kideok Kim, M.Sc. (Researcher); Adrien Mesnard, M.Sc. (Researcher); Jungjin Lee, M.Sc. (Senior assistant); Bumsuk Jean, B.Sc. (Senior assistant); Heeyoung Na, B.Sc. (Senior assistant); Eunhye Kim, B.Sc. (Senior assistant)

1. Research Summary

The Automation & Logistics Management group (ALM) provides expertise in the fields of automation & robotics, engineering, sample management, informatics, supply chain management and logistics. The ALM group includes state-of-the-art robotic systems and an extensive collection of high quality drug-like compounds and Full Human Genome RNAi Libraries.

- The ALM group supports all screening activities on three tiers: 1. Internal for the Assay Development & Screening group, 2. Internal for all IPK biology groups and 3. External collaborations with strategic partners.
- The ALM manages a diverse collection of ~ 400,000 high quality drug-like compounds, including FDA drugs and natural products. Subsets of known drugs and reference compounds for a broad range of biological targets are also available for validation studies. Additionally, it offers the full human genome siRNA & shRNA as well as subsets of Kinase and Phosphatase Libraries.
- The ALM Facility includes a wide range of high performance liquid handling systems for sample management, three robotic systems in BSL 2 and BSL 3 and an open access system for internal and external partners.
- The core facility's mission is to provide dependable services for all research activities for internal and external projects, by managing all IPK equipment and collaborating on engineering projects.
- The ALM Facility manages the distribution and logistics of consumables by consolidating them under a warehouse umbrella for easy distribution. It manages the BioRepository samples of IPK.



2. Research Progress in 2016

2.1 Summary

The Automation & Logistics Management group (ALM) recorded a large number of major achievements. The group help in the screening efforts to complete large screening campaigns and prepared compound confirmation in dose-response for MERS, Ebola, MCTS, HBV, etc.

- In 2016, received all the finished processing ~ 150,000 the NCI Natural Products library to be screened against the IPK panel of various programs as well as for outside collaborations.
- Building up the compounds and RNAi libraries (Enamine, NCI Natural Extracts, Selected FDA Approved Set & Human Genome wide RNAi libraries.)
- Supporting 37 internal and external screening projects using automated systems in BSL 2 and BSL 3 environments.
- Worked with Finance and Purchasing Department to revamp the chargeback system for instruments.
- Addition to the IPK - FDA compounds collection, 180 will be added by November 2016.
- Establishing an organized and transparent platform for instruments scheduling and maintenance for IPK common equipment, including chemistry group.
- Supported engineering projects:
  - Development of an IR camera for Crimson red detection in Mouse Brain
  - Modification of 6 well chamber for using of Cybio chamber in Perkin Elmer Operetta
  - Setup of CBRL lab on the 5th floor
  - Battery replacement for robotic system in BSL2
  - Thermo / Gilson pipettes calibration (Qty. 250) for all IPK labs
- Successfully implemented the Korean Chemical Management Association's importing chemical process to import libraries, resupplies and samples from external collaborators.
- Supported external instrument usage to increase Instruments Recovery budget from:
  - Samjin
  - Kainos
- Conducted visual inspection for all biological samples to manage and identify what is available at IPK. This will be implemented within our database to be managed by ALM with oversight from Safety Department, tentative date of completion end of November.
- ALM Members supported IPK science outreach programs and participated in 46 events.

2.2 Results

	Project Title	Source of funding	Period	Description			
1	Sample Management	IPK	Jan-Feb	Reforamatting NCI Library	153032 compounds	Reformatting 3347 DPLs & IPLs	
2	NTDB	DNDi	Jan	NTDB_S06	381 compounds	Reformatting 3347 DPLs & IPLs	Transferring compounds for 119 assay plates for T.cruzi & Leish screen
3	DNDi	DNDi	Jan	DNDi_Sanofi	5333 compounds	Reformatting 16 DRC plates	Transferring compounds for 41 assay plates for Leish Screen
4	Sample Management	IPK	Feb	SLAS-Poster Presentatiion			
5	NTDB	DNDi	Feb	NTDB_S01&S02IH01	583 compounds	Reformatting 23 DRC plates	Transferring compounds for 162 assay plates for T.cruzi & Leish screen
6	Sample Management	IPK	Mar	Equipment Validation	Apricot/BioMek NX		
7	KRICT_S.pneumoniae	ARRL	Mar	S.pneumoniae screening with KRICT Library	9600 compounds	Reformatting 30 IPLs	Transferring compounds for 34 assay plates
8	Sample Management	IPK	Mar	Reformatting MMV, LOPAC	1680 compounds	Reformatting 20 DPLs & IPLs	
9	NTDB	DNDi	Apr	NTDB_S03IH01	385 compounds	Reformatting 16 DRC plates	Transferring compounds for 104 assay plates for T.cruzi & Leish screen
10	DNDi	DNDi	Apr	DMDi_Daiichisankyo	40000 compounds	Reformatting 125 IPLs	Transferring compounds for 90 assay plates for T.cruzi & Leish screen
11	MCTS	CBRL	Apr	MCTS-Pilot screening	8050 compounds		Transferring compounds for 72 assay plates
12	NTDB	DNDi	Apr	NTDB_S05IH01	366 compounds	Reformatting 16 DRC plates	Transferring compounds for 118 assay plates for T.cruzi & Leish screen
13	KRICT_S.pneumoniae	ARRL	May	S.pneumoniae screening with KRICT Library	9600 compounds	Reformatting 30 IPLs	Transferring compounds for 34 assay plates
14	HCS training	IPK	May	HCS training			
15	T.cruzi screening	LRRL	May	T.cuzi_Combination_Pilot screening	8050 compounds		Transferring compounds for 33 assay plates
16	NTDB	DNDi	May	NTDB_S06IH01	415 compounds	Reformatting 18 DRC plates	Transferring compounds for 116 assay plates for T.cruzi & Leish screen
17	MERS screening	RVRL	Jun	MERS_Pilot screening	8354 compounds		Transferring compounds for 68 assay plates
18	Ebola screening	HRL	Jun-July	Ebola_Pilot screening	8354 compounds		Transferring compounds for 96 assay plates
19	KRICT_S.pneumoniae	ARRL	Jun	S.pneumoniae screening with KRICT Library	9600 compounds	Reformatting 30 IPLs	Transferring compounds for 102 assay plates
20	KRICT_Dengue	Dengue	Jun	Dengue screening with KRICT Library	40 compounds		Transferring compounds for 10 assay plates
21	HBV screening	HBV	July	HBV reference compounds	250 compounds	Reformatting 10 DRC plates	Transferring compounds for 24 assay plates
22	Sample Management	IPK	July	Equipment Validation	Apricot/CyBio		
23	DNDi	DNDi	July	DMDi_MMV_Celgene	684 compounds	Reformatting 12 IPLs & 17 DRC plates	Transferring compounds for 154 assay plates for T.cruzi & Leish screen
24	NTDB	DNDi	July	NTDB_S07	444 compounds	Reformatting 18 DRC plates	Transferring compounds for 116 assay plates for T.cruzi & Leish screen
25	KRICT_S.pneumoniae	ARRL	July	S.pneumoniae screening with KRICT Library	9600 compounds		Transferring compounds for 102 assay plates
26	MERS screening	RVRL	July	MERS_Hit-picking	165 compounds	Reformatting 7 DRC plates	Transferring compounds for 17 assay plates
27	KRICT_S.pneumoniae	ARRL	July	S.pneumoniae screening with KRICT Library	9600 compounds		Transferring compounds for 102 assay plates
28	NTDB	DNDi	July	NTDB_S02IH02	388 compounds	Reformatting 15 DRC plates	Transferring compounds for 100 assay plates for T.cruzi & Leish screen
29	DNDi	DNDi	Aug	DMDi_Daiichisankyo_Hitpick	466 compounds		Transferring compounds for 54 assay plates for T.cruzi & Leish screen
30	BACE1 screening	TDP	Aug	BACE1 Pilot screening	2320 compounds		Transferring compounds for 10 assay plates
31	NTDB	DNDi	Aug	NTDB_S08	448 compounds	Reformatting 18 DRC plates	Transferring compounds for 118 assay plates for T.cruzi & Leish screen
32	KRICT_S.pneumoniae	ARRL	Aug	S.pneumoniae screening with KRICT Library	9600 compounds		Transferring compounds for 102 assay plates
33	CNPM_TB screening	TRL	Sep	TB_CNPM_Counter screening	11088 compounds		Transferring compounds for 70 assay plates for T.cruzi & Leish screen
34	KRICT_S.pneumoniae	ARRL	Sep	S.pneumoniae screening with KRICT Library	9600 compounds		Transferring compounds for 102 assay plates
35	DNDi	DNDi	Sep	DMDi_Eisai-GHIT	3548 compounds	Reformatting 7 IPLs	Transferring compounds for 52 assay plates for T.cruzi & Leish screen
36	NTDB	DNDi	Oct	NTDB_S09-S10	785 compounds	Reformatting 31 DRC plates	Transferring compounds for 13452 assay plates for T.cruzi & Leish screen
37	Ebola screening	HRL	Oct	Ebola_Hit-picking	235 compounds	Reformatting 9 DRC plates	Transferring compounds for 56 assay plates
38	TB SS18b Screening	TRL	Oct	TB SS18b_Pilot screening	8354 compounds		Transferring compounds for 68 assay plates
39	Automation equipment	IPK		Maintenance of automated confocal systems and Reorganization of automation in BSL3			
40	FACS analysis	IPK		FACS analysis for Samjin pharma. (15 times, total 132 samples for analysis)			
41	Chemical Importing	IPK		Organization of chemical importing through KCMA processing (129 events)			
42	MedChem	IPK		Management of MedChem compounds for influenza (151 compounds) and Leishmania project (163 compounds)			

3. Major Accomplishments

3.1 Publications

1. Heo JY, Nam JY, Jang J, Shum D, Radu C, Cheng J, Lee H, Suh JW, Delorme V (2016). High-content screening of raw actinomyceteextracts for the identification of antituberculosisactivities. J BiomolScreen Nov 3. Accepted
2. Shukla N, Somwar R, Smith RS, Ambati S, Munoz S, Merchant M, D'Arcy P, Wang X, KobosR, AntczakC, BhinderB, Shum D, Radu C, et al (2016). Proteasome Addiction Defined in Ewing Sarcoma Is Effectively Targeted by a Novel Class of 19S Proteasome Inhibitors. Cancer Res76:4525-4534
3. Lee H, Radu C, Han J, Grailhe R (2016). Detection and quantification of SOD1 mutant aggregate formation in living cells. BioTechniques, Summited December 2016

3.2 Presentations

1. Honggun Lee, Anticipating the Logistics of Infectious & Neglected Diseases: The Sample Management & Automation Perspective, Society for Laboratory Automation and Screening 2016, San Diego, USA, 01/24/2016, Poster.
2. Kideok Kim, Expanding the Sample & Data Reporting Paradigm, Society for Laboratory Automation and Screening 2016, San Diego, USA, 01/25/2016, Poster.
3. Constantin Radu. Co-Organizer & Speaker. Amplifying the Screening Outcomes Using Diverse Chemical Libraries, 2016 Drug Discovery Symposium, Seoul, South Korea, 04/19/2016, Oral.
4. Constantin Radu, The Institut Pasteur Korea Experience of Logistics in Drug Discovery R&D, Korea Supply Chain Summit of Global Supply Chain Council 2016, Seoul, Korea, 09/08/2016, Oral.
5. Constantin Radu, The IPK Sample Management Experience of The NTD Drug Discovery Booster, International Conference of the Korean Society for Molecular and Cellular Biology 2016, Seoul, Korea, 10/14/2016, Poster.

3.3 Ongoing & new collaborations

1. Fee-for-service: The Drugs for Neglected Diseases Initiative (DNDi) - NTD Booster. A two year project, monthly receiving ~ 400 compounds to be prepared in ten point dilution dose response curve plates that are screened against Leishmania and Tcruzi assays. Compounds arrive from Takeda, Celgene, Eisai, AstraZeneca and Shionogi. Jean-Robert Ioset. Geneva, Switzerland.
2. Fee-for-service: The Drugs for Neglected Diseases Initiative (DNDi) - Sponsored Research. Screening single point in assay plates against Leishmania and Tcruzi assays. Compounds arrive from AnaCor, TB Alliance, Sanofi, Takeda, Eisai and others. Jean-Robert Ioset. Geneva, Switzerland.
3. Fee-for-service: Green Cross, Compound Management and Storage, Dr. Soongyu Choi, Gyeonggi, Rep of Korea
4. Fee-for-service / FACS analysis for protein target. Jonghwan Lee. Pharmacology Toxicology Laboratory. Samjin Pharm Co. LTD, Gyeonggi, Rep of Korea
5. Fee-for-service / Facility and Equipment Use. Sunnam Kim. Medical Chemistry group. Kainos Medicine. Gyeonggi, Rep of Korea
6. Chemical Screening / Management: Korea Research Instutute of Chemical Technology - Convergent Chemistry Division, Sample preparation and screening support for MERS, Jonggeon Jegal. Daejeon, Rep. of Korea
7. BioBank Setup: Biomedical Research Institute at Seoul National University Hospital, Seoul, Rep. of Korea
8. Chemical Screening / Management: Institut Pasteur Montevideo (IP-M), Sample preparation to screen in collaboration with our IPK Leishmania Research Laboratory program. ALM group worked with the IP-M visiting researcher on processing the samples and getting them ready for screening. Montevideo, Uruguay.
9. Chemical Screening / Management: Institut Pasteur Athens (IP-A), Sample preparation to screen in collaboration with our IPK Hepatitis Research Laboratory program. ALM group worked with the IP-M visiting researcher on processing the samples and getting them ready for screening. Athens, Greece.

Automation & Logistics Management

1. 연구 요약

Automation & Logistics Management (ALM) 그룹은 IPK에서 다양한 핵심 업무를 수행합니다. 자동화 및 로봇 공학, 엔지니어링, 생물학, 화학, 정보학, 공급망 관리 및 물류 분야의 전문성을 갖춘ALM 그룹은 다음 3단계의 모든 스크리닝 활동을 지원합니다. 1. 내부 Assay Development & Screening 그룹 지원 2. 모든 IPK 내 연구 그룹 지원 3. 외부 용역 서비스 지원.

ALM 그룹의 주요한 책임은 다음과 같습니다.

1. ALM 그룹은 약물 스크리닝 활동에 필요한 화합물 및 RNAi 라이브러리, 자동화 플랫폼 및 Biorepository 등을 관리합니다.
2. ALM 그룹이 속한 Core facility의 사명은 모든 IPK 장비를 관리하고 엔지니어링 프로젝트에 협력함으로써 내부 및 외부 프로젝트의 모든 연구 활동에 신뢰할 수 있는 서비스를 제공하는 것입니다.
3. ALM 그룹은 경쟁력 있는 가격 협상을 통해 모든 IPK의 소모품 및 물류 관리를 담당하고, 물류 창고를 통해 각 그룹 연구자들에게 쉽게 배포할 수 있도록 하는 책임을 위임받았습니다.

2. 2016년 연구 성과 요약

자동화 및 물류 관리 그룹 (ALM)은 많은 주요 성과를 기록했습니다. 이 그룹은 MERS, 에볼라, MCTS, HBV 등등 다양한 프로젝트에 대한 대규모 스크리닝 캠페인을 완료하고 단계별 농도 반응 확인에서의 화합물 선별 작업에 도움을 주었습니다.

- 2016년, 약 150,000개의 NCI Natural Products 라이브러리를 IPK 내 각 그룹 프로젝트뿐만 아니라 외부 협력 스크리닝 프로젝트에도 적용할 수 있도록 재구성 작업 완료.
- 화합물 및 RNAi 라이브러리 구축 (Enamine, NCI Natural Extracts, 선택된 FDA 승인 화합물 세트 및 Human Genome wide RNAi 라이브러리.)
- BSL 2 및 BSL 3 환경 내 자동화 된 시스템을 활용한 37가지 내외부 스크리닝 프로젝트 지원.
- 재무 및 구매 부서와 협력하여 장비 사용액 지불 시스템을 개선.
- Biorepository의 운영을 위한 한국 라이센스 신청. 그러나 이는 아직 해당 법률이 존재하지 않고 IPK와 같은 형태의 저장소에 대한 레이블 지정 방법이 결정되지 않아 거부되었음. 2017년 해당 법률 개정을 위해 MSIP과 다시 시도할 것임.
- FDA 화합물 구매. 2016년 11월까지 180개가 추가.
- 화학 그룹을 포함한 IPK 공용 장비에 대한 사용 스케줄 관리 및 유지 보수를 위한 체계적이고 투명한 플랫폼 구축.
- 엔지니어링 프로젝트 지원:
  - Mouse Brain 내 Crimson red 검출을 위한 적외선 카메라 개발
  - Perkin Elmer사의 Operetta 내 Cybio chamber 사용을 위한 6 well chamber 개발
  - CBRL 그룹 실험실 구축
  - BSL 2 실험실 내 Robotic 시스템 배터리 교체
  - IPK 모든 실험실 내 Thermo / Gilson 파이펫 보정
  - 약 20개의 초정밀 저울 보정
  - 실험용수 정수
- 얼음생성기 수리 및 문제 해결
- Thermo사의 Multidrop Combi dispenser 보정
- 한국화학물질관리협회의 화합물 수입 절차를 성공으로 수행하여 외부 공동 작업자로부터 화합물 라이브러리 및 샘플 수입.
- 삼진제약, 카이노스메드 등의 외부 업체가 IPK 장비를 사용할 수 있도록 지원하여 해당 장비 유지 보수 예산을 증가시킴.
- 모든 생물학적 샘플에 대해 육안 검사를 실시하여 IPK에서 사용 가능한 샘플을 관리 및 확인함. 이는 IPK 안전팀의 감독 아래 ALM 그룹에 의하여 데이터베이스 내에서 관리될 것이며, 11월 이내에 완성될 예정임.
- ALM 그룹원은 IPK 과학 홍보 프로그램을 지원했으며, 46개의 행사에 참가함.

Core Support Groups

Assay Development & Screening

Group Head: David Shum, M.Sc.

Laboratory members: Jinyeong Heo, M.Sc. (Researcher); Jinyeop Kim, M.Sc. (Researcher); Nakyung Lee, M.Sc. (Researcher); Namyoul Kim, Ph.D. (Researcher); Soonju Park, M.Sc. (Researcher); Sooyoung Byun, M.Sc. (Researcher); Dahae Koh, M.Sc. (Researcher -Temp Relief); Jaewoong Choi, M.Sc. (Researcher - Temp Relief)

1. Research Summary

The Assay Development & Screening (ADS) group provides expertise in the early stages of the drug discovery pipeline for assay development and high-throughput screening. Our research activities enable discovery of small molecules and target identification for drug development, biomarkers, and elucidation of disease mechanisms. ADS works in close collaboration with each investigator, within Institut Pasteur Korea (IPK) and outside collaborators, providing guidance through all stages of the chemical and RNAi screening process: assay development, validation, automation, high-content or high-throughput screening, and data analysis.

Our capabilities/assets include:

- Fully-automated robotic platforms located in BSL-2+ & BSL-3 laboratories for pathogen and biological research
- Validating & conducting screening assays covering a wide range of detection readouts including fluorescence, bioluminescence, absorbance, and imaging in 384-well format
- Chemical library collection of ~400,000 molecules covers diverse sources (synthetic, natural products, extracts, FDA drugs) as starting points for therapeutic development
- Genomic platforms (siRNA and shRNA technology) to identify new targets and signaling pathways as well as uncover mechanism of actions

The ADS group can also customize assays to suit the researcher's needs according to the biological question. Assays can be configured to run as drug synergy and chemo-genomics to assess combinatorial affect or elucidate mechanisms. Furthermore, the ADS group provides expertise in a wide range of disease areas including infectious, pathogen, oncology, and neurodegenerative.

2. Research Progress in 2016

2.1 Summary

As a core support group, ADS optimizes and validates all screening assays for IPK discovery biology programs such as bacterial (S. pneumoniae, S. aureus, TB), parasitic (visceral leishmaniasis), hepatocarcinoma (3D spheroid models) and viral research (hepatitis B, dengue, EBOLA, MERS-CoV). In general for each project, assays are typically streamlined into 384-well format to enable high-throughput screening and then tested against reference compounds to demonstrate robustness. Upon assay validation of the disease models, we screen against our library collection containing FDA approved drugs, known bioactives, and compounds in the clinic. The resulting positives can be used to identify mechanism of action as compounds have known biological target or indications. Promising compounds can also be repurposed for the disease in some cases. Assays are further progressed against larger screening collections to identify potential new scaffolds for development. In total 250,000 data points were screened for IPK and detailed information can be located within the individual biology programs.

In addition to supporting IPK discovery biology programs, ADS performs early drug discovery screening activities for

domestic and international scientists. Depending on the funding opportunities, the screening is collaborative as a means to synergize research or share knowledge. We have performed such activities with KIOST, SKKU, Postech, and Konkuk University. In a sponsored program, our platform is used to process samples from industry or biotech and then screened against a disease model. Such activities include Drugs for Neglected Disease initiative (DNDi) and KRICT.

2.1.1 ADS Collaborative and Sponsored Programs

Within Korea, screening activities amongst collaborative and sponsored research were performed for academic, government, and biotech industries. Through effective outreach and MOU ceremonies, we have worked on a variety of projects that supplemented research, publications, and early drug development. Disease areas included neurodegenerative (SKKU, Postech), oncology (Konkuk), and infectious (KRICT) with nearly 250,000 data points screened (Figure 1).

Neglected Tropical Disease Booster Project or NTD Booster is designed to fast track lead-to-hit optimization by reiterative screening cycles (Figure 2). The idea is used a parental seed compound that is active and then request derivatives from different industrial partners. The derivatives are then screened at IPK against Leishmania and Chagas disease models with more potent compounds being identified. This is performed in monthly cycles and thus far several scaffolds have been found. This multi-collaborative effort was awarded Project of the Year by DNDi's scientific committee. Amongst the international research work, DNDi and Institut Pasteur Network activities were close to 750,000 data points.

2.1.2 Results

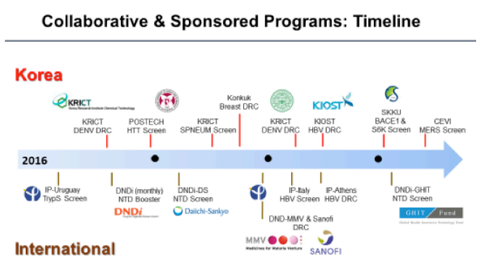


Figure 1. Timeline of ADS screening activities for Korea and International

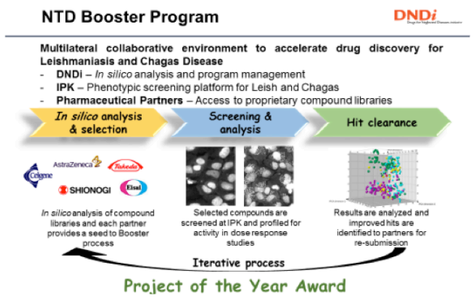


Figure 2. NTD Booster Concept for DNDi. ADS group recognized for screening activities.

3. Major Accomplishments

3.1 Patents

1. 10-2016-0136393: Pharmaceutical composition for prevention or treatment of pneumonia comprising quinolin 4-one derivative or pharmaceutically acceptable salt thereof as an active ingredient; Chul Min Park, Jong Hwan Song, Sunkyung Lee, Soojin Jang, Hyungjun Kim, David Shum; 10/20/2016

3.2 Publications

1. Heo JY, Nam JY, Jang J, Shum D, Radu C, Cheng J, Lee H, Suh JW, Delorme V (2016). High-content screening of raw actinomycete extracts for the identification of antituberculosis activities. J Biomol Screen Nov 3. Accepted
2. Kim HC, Shum D, Seol HS, Jang SJ, Cho SG, Kwon YJ (2016). Development of cell-defined lentivirus-based microarray for mammalian cells. J Biomol Screen October 4. Accepted
3. Shukla N, Somwar R, Smith RS, Ambati S, Munoz S, Merchant M, D'Arcy P, Wang X, Kobos R, Antczak C, Bhinder B, Shum D, et al (2016). Proteasome Addiction Defined in Ewing Sarcoma Is Effectively Targeted by a Novel Class of 19S Proteasome Inhibitors. Cancer Res 76:4525-4534
4. Kim HC, Kim GH, Shum D, Cho SG, Lee EJ, Kwon YJ (2016). The monitoring of gene functions on a cell-defined siRNA microarray in human bone marrow stromal and U2OS cells. Data Brief 7:673-678
5. Shum D (2016). Finding a cure for neglected tropical diseases: Cell-based assays for chemical and functional genomics screening. Drug Target Review 3:50-53

3.3 Presentations

1. Lee NK, Kim HC, Heo JY, Min SH, Lee JH, Koenig A, Choi I, Shum D, et al. Characterization of inhibitors and biomarkers identified by screening of the Ebola virus transcription- and replication-competent virus-like particle system, RIIP International Symposium 2016, Paris, France, 30-November-2016 (Poster)
2. Radu C, Shum D, Perry BG, Mowbray CE, et al. The IPK sample management experience of the NTD Drug Discovery Booster, International Conference of Korean Society for Molecular and Cellular Biology, Seoul, South Korea, 12-October-2016 (Poster)
3. David Shum. Deciphering Fundamental Mechanisms for Dengue Viral Infections, KNIH-IPK Workshop 2016, Osong, South Korea, 7-September-2016 (Oral)
4. Perry BG, Mowbray CE, Shum D, et al. The NTD Drug Discovery Booster: A novel approach for hit to lead chemistry, EFMC International Symposium on Medicinal Chemistry, Manchester, UK, 28-August-2016 (Poster)
5. David Shum. IPK Screening Platform: Tools & Technological Capabilities, Global Antibiotic Resistance & Development Partnership Stakeholders Meeting 2016, Geneva, Switzerland, 25-August-2016 (Oral)
6. Lee NK, Heo JY, Kim HC, Lee JH, Min SH, Kim K, Lee H, Radu C Shum D, et al. Adaptation and screening for small molecule inhibitors using the Ebola virus transcription- and replication-competent virus-like particle system, SLAS High-Content Screening Conference, Dresden, Germany, 27-June-2016 (Poster)
7. Byun SY, Park KP, Kim NY, Lee H, Radu C Shum D. Neglected disease and therapeutic: Revisiting drugs for Dengue using an infectious and release assay system, SLAS High-Content Screening Conference, Dresden, Germany, 27-June-2016 (Poster)
8. David Shum. Deciphering Mechanisms in Infection Diseases: From Pathways to Drug Discovery, KSBMB International Conference, Seoul, South Korea, 19-May-2016 (Oral)
9. David Shum. High Content Screening for Target Discovery Using RNAi Technology, Training Course,Seoul,Korea, 9-May-2016 to 13-May-2016 (Oral)
10. David Shum. Interrogating Genomic Information into Biomarkers & Therapeutics, 2016 Drug Discovery Symposium, Seoul, South Korea, 19-April-2016 (Oral)

3.4 Ongoing & new collaborations

1. Novel anti-TB drug discovery using natural product library and development of latent TB drug discovery model. Vincent Delorme, South Korea
2. Development of next-generation influenza therapeutic agents. Ji-young Min & STPharm, South Korea
3. A multilevel systems approach to elucidate the host-Leishmania interactome and to identify host targets for anti-leishmanial drug discovery. PTR-IP Paris, France
4. Discovery of host cellular factors involved in Lyssavirus infection. Herve Bourhy, IP Paris, France
5. Drug discovery and new agents against relevant therapeutic targets in infective and/or cancer diseases. Istituto Pasteur-Fondazione Cenci Bolognetti, Italy
6. Discovery of new antibacterial agents against Streptococci pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa. Soojin Jang, South Korea
7. Convergent solution on Emerging Virus Infections. KRICT, South Korea
8. Leishmania and Chagas Neglected Disease Research Program & NTD Booster. DNDi, Switzerland
9. Establishment of 3D tumor micro-environment for liver cancer therapy. Haengran Seo & Samjin, South Korea
10. Discovery of HBV inhibitors. Marc Windisch & Kainos, South Korea
11. Discovery of anti-Leishmanial Inhibitors. Joohwan No & Pharos IBT, South Korea
12. High-throughput screening of infectious disease models to identify new chemical scaffolds. KRICT, South Korea
13. Identification of nuclear import inhibitors through synthetic chemical screening. SKKU, South Korea
14. Development of new therapeutic agents against bacterial infections. J2H, South Korea

Assay Development & Screening

1. 연구 요약

본 연구실(Assay Development & Screening)은 초고속 대용량 스크리닝을 활용한 신약개발 파이프라인 초기 단계의 약물개발 연구를 제공한다. 본 연구진의 보유 기술을 이용하여 신약개발, 바이오마커 및 질병기전 연구를 수행한다. 본 연구팀은 한국파스퇴르연구소 내부 연구팀 및 외부 연구팀과의 협력 연구를 진행하며 분석법 개발, 분석법 검증, 분석 자동화, 초고속 대용량 스크리닝, 표현형 정량화 기술을 바탕으로 화합물 스크리닝 및 RNAi 스크리닝을 위한 통합적 스크리닝 파이프라인을 제공한다.

자산 및 보유기술:

· 생물안전 2등급 및 3등급 실험실 내에서 활용가능한 자동화 스크리닝 플랫폼을 활용한 감염체 및 생물학 연구

· 384 well 플레이트를 이용한 분석법 개발 및 스크리닝 수행, 형광(fluorescence), 발광(bioluminescence), 흡광도(absorbance), 이미징 기술을 이용한 결과 측정

· 신약개발 초기 단계의 연구 수행을 위한 40만 개 이상의 화합물 라이브러리 보유(합성신약, 천연물, 천연물 추출물, FDA승인 약물 등)

· 약물기전 연구 및 새로운 약물 표적발굴을 위한 유전체 스크리닝(siRNA & shRNA 기술) 플랫폼

본 연구팀은 또한 각 연구자의 연구 수요에 맞춘 분석법을 제공한다. 약물의 시너지 효과 및 화학–유전체학적 접근을 위한 분석법 개발연구를 통하여 약물 작용기전 및 효력연구를 진행한다. 또한 본 연구팀은 감염성 질환, 암, 신경퇴행성 질환을 포함한 다양한 범위의 질병 연구를 수행하기 위한 풍부한 기술적 역량을 가지고 있다.

2. 2016년 연구 성과 요약

핵심 연구협력 그룹으로서, 본 연구실은 한국파스퇴르연구소의 신약개발 프로그램(박테리아 연구 – S. pneumoniae, S. aureus, TB, 기생충 연구 – visceral leishmaniasis, 간암 연구 – hepatocarcinoma 3차원 세포배양 모델, 바이러스 연구 – B형 간염, 뎅기, 에볼라, 메르스)을 위한 모든 스크리닝법을 최적화하고 검증한다. 각 연구 프로그램에 대하여 개발된 분석법을 384 well 플레이트에 적용하여 초고속 대용량 스크리닝을 가능케 하고, 대조약물 실험을 통하여 분석법을 검증한다. 다양한 질병 모델 연구를 위해 개발한 분석법 검증을 위하여 본 연구소가 보유하고 있는 FDA승인 약물, 작용기전이 알려진 약물, 임상사용 약물 라이브러리를 스크리닝한다. 이를 통해 나온 결과는 약물의 표적이나 적응증이 알려져 있는 만큼 즉각적인 약물기전 연구로 적용할 수 있으며, 나아가 약물의 새로운 적응증 개발을 위한 신약재창출 연구(drug repositioning)를 진행할 수 있다. 대용량 스크리닝을 통하여 신약개발을 위한 새로운 스캐폴드(scaffold)를 제시한다. 현재까지 스크리닝을 통하여 25만 개의 데이터를 도출하였으며, 이를 통하여 각 신약개발 프로그램의 초기 연구를 진행한다.

본 연구실은 본 연구소(IPK) 내부의 연구 프로그램뿐만 아니라, 국내외 과학자들과의 협력 연구도 진행한다. 연구비 확보의 측면에서 스크리닝 기술은 다양한 협력연구를 통한 시너지 효과 및 지식의 교류를 위한 중요한 분야다. 본 연구진은 한국해양과학기술원, 성균관대학교, 포항공대, 건국대와의 협력연구를 수행했다. 또한 본 연구실의 스크리닝 플랫폼을 통하여 기업체나 바이오텍의 연구비 출자를 통한 협력연구를 진행할 수 있으며, 현재까지 DNDi, 한국화학연구원의 연구비 지원을 통한 연구를 수행하고 있다.

국내에서 스크리닝 연구는 학교, 정부 산업체의 협력 및 지원을 통하여 이루어진다. 교류협력 및 양해각서(MOU) 체결을 통하여 지원 연구사업, 논문 출간, 초기신약개발 연구 등 다양한 연구 프로그램을 진행했다. 신경퇴행성질환(성균관대, 포항공대), 암(건국대), 감염성질환(한국화학연구원) 등 다양한 질병 분야의 연구를 수행하였으며, 스크리닝을 통하여 25만 개의 데이터를 도출했다(Figure 1 / 그림 1).

소외질환 연구를 위한 Neglected Tropical Disease Booster 프로그램은 신약개발 초기 단계인 선도물질과 유효물질(hit to lead) 최적화 단계의 시간 및 비용 절감을 위해 고안된 스크리닝 프로그램이다(Figure 2 / 그림 2). 국외 제약사의 화합물을 바탕으로 라이브러리를 구성하여 본 연구소의 리슈마니아 편모충 및 샤가스질환 분석법 모델에 적용하여 유효한 물질을 도출한다. 이 과정을 매달 반복함으로서 효율적으로 유효 물질 및 구조를 찾고 신약개발의 시간을 절약한다. 이 프로그램은 DNDi로부터 올해의 연구상을 수상한 바 있다. 이러한 국제협력 연구를 통하여 DNDi와 파스퇴르 연구소 네트워크는 75만 개의 데이터를 도출했다.

Core Support Groups

Technology Development Platform

Principal Investigator: Regis Grailhe Ph.D.

Laboratory members: Hyeju Eun, M.Sc. (Junior researcher); Seonhee Kim, M.Sc. (Researcher)

1. Research Summary

The maintenance of Institut Pasteur Korea's phenotypic screening facility requires a steady and consistent improvement of advanced cutting-edge screening technologies. We aim to further expand high content screening technologies to investigate the relationship between cell and pathogen. Taking advantage of our multi-disciplinary team composed of biologist, biophysicist, and microscopy specialists, we are developing three open platforms to quantify infectious disease process from the nano-scale to the macro-scale respectively in cellular and animal models.

(1) Using novel imaging resonance energy transfer techniques, we are scrutinizing protein-protein interactions occurring between pathogen and host protein in infected living cells. Our study focus on the multiple strategies leading viruses to evade and manipulate the innate immunity of their hosts.

(2) We are adapting more physiologically relevant and predictive cell-based assays adapted to high-content screening, using differentiated human brain cells derived from induced pluripotent stem cells (iPSCs). This platform is currently used for the study of neuron, astrocyte and microglia cells vulnerability to neurotropic viruses and neurodegenerative pathologies.

(3) We are seeking for methods to visualize and quantify biology using novel fluorescence probes. In particular taking advantage of near infrared fluorescent probes for their low absorption coefficient and auto-fluorescence to generate novel transgenic reporter mice reporting in-vivo inflammation in various tissues. We are aiming using such mouse models to accelerate preclinical drug development targeting at infectious and chronic diseases.

2. Research Progress in 2016

2.1 Summary

In 2016, we followed three research activities.

(1) We showed that the Nanoluciferase signal brightness using furimazine substrate enable visualization and quantification of such reporter expressed in living cells using entry-level widefield microscopes. Furthermore, taking advantage of Nanoluc system properties, we were able to quantify protein-protein interaction, using bioluminescence resonance energy transfer (BRET) technique adapted to our high content screening platform. With such technology platform, we characterized the protein network occurring between NFKB and Jak/Stat host cell pathway and rabies virus proteins (M & P) in collaboration with the group of Herve Bourhy from the Institut Pasteur.

(2) Taking advantage of our multi-color image-based screening capacity, we build a small library of fluorescent compounds. With this initial set of molecules, we found unique organelle-specific dyes applicable for fluorescence cell imaging.

(3) We generated a transgenic expressing a novel near infrared fluorescent protein under the control of a brain specific promoter targeting astrocyte cell population. We showed that the expression of our reporter can be detected in freely moving animal through-skull and report brain-inflammation. Importantly, we were able to use the same probe to perform live small animal imaging and histology studies.

2.1.1 Development of a platform to quantify protein-protein interaction in living cells

Fluorescence and bioluminescence resonance energy transfer (FRET, BRET) techniques are powerful tools for studying protein-protein interactions in cellular assays. In contrast to fluorescent proteins, chemiluminescent proteins do not require excitation light, known to trigger autofluorescence, phototoxicity and photobleaching. Regrettably, low signal intensity of luciferase systems restrict their usage as they require specialized microscopes equipped with ultra-low light imaging cameras. In this study, we report that bioluminescence quantification at a single cell level using a standard widefield automated microscope dedicated to screening and high content analysis is possible with the newer luciferase systems, Nanoluciferase (Nluc). With such equipment, we showed that it is possible to measure robust intramolecular BRET using a combination of Nluc and yellow fluorescent protein (YFP). Using the human Superoxide Dismutase 1 (SOD1) dimer model, we next validated that intermolecular BRET could be quantified at a single cell level. The enhanced signal brightness of Nluc enabling BRET imaging to widefield microscopy, show strong potential to open up single cell protein-protein interactions studies to a wider audience (Jiho et al).

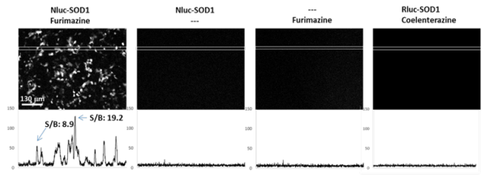


Figure 1. Nanoluc based bioluminescence and BRET imaging. HEK-293 cells show robust bioluminescence signal in the presence of Nluc-SOD1 and furimazine. In contrast, in the absence of Nluc or furimazine or in presence, no localized bioluminescence signal was detectable. Renilla luciferase tagged SOD1 protein (Rluc-SOD1) in presence of coelenterazine showed no detectable bioluminescence signal. A plot of the luminescence intensity along the length of each image was measured for all three conditions. The signal-to-background ratio (S/B) of the two peaks (arrows) corresponding to the bioluminescent cells was calculated as described in the study method.

2.1.2 Development of a synthetic organic dye library

Taking advantage of our multi-color image-based screening capacity, we build a small library of fluorescent compounds, for the discovery of selective fluorescent probes. With this initial set of molecules, we evaluate their fluorescence properties as well as sub-cellular staining and cellular toxicity properties. As a result, we found a unique dye which stain cells but more selectively stain cell plasmalema. The dyes stain plasmalema in live cells, and show no toxicity for period of 72 hours. A selection of a subset of derivative molecules will be selected for developing compound derivatives by collaboration with IP-K Discovery Chemistry group. Last, the characterized fluorescent compounds will be tested on IP-K cellular models to seek for biomarkers relevant to infectious or chronic disease.

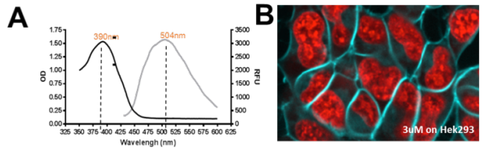


Figure 2. Bright, photostable and non-toxic fluorescent dye selective to cell plasmalema for long term staining. A. Absorption and emission spectra of our new dye. B. Dye plasmalema sub-cellular staining properties.

2.1.3 Development of small animal optical imaging technology to track inflammation

We generated a transgenic expressing a near infrared fluorescent protein under the control of a brain specific promoter targeting the expression of the transgene in the astrocytes population. We show for the first time that the expression of infrared fluorescent protein can be detected in living animal through-scalp and through-skull of freely moving mouse. Furthermore, we validate that near infrared monitoring is possible for quantifying acute neuro-inflammation induced with Kainic acid. Last, this new approach shows the possibility to use the same probe to perform histological analysis at the cellular level with high signal/noise ratio, using our inflammatory reporter on post mortem animal brain tissues.

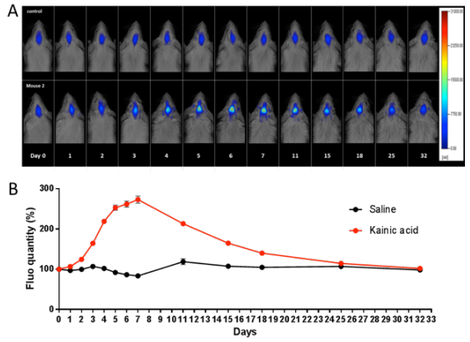


Figure 3. Longitudinal study using in vivo imaging post Kainic acid induced injury. A. Live optical imaging of a FVB transgenic mouse expressing infrared protein reporter in the brain after Kainic acid injection (30mg/kg), using FMT2500 system. B. The expression of infrared protein was found to increase from the second day post injection (3) of Kainic acid injection (30mg/kg) as reported.

3. Major Accomplishments

3.1 Publications

1. Kim J, Grailhe R (2016). Nanoluciferase signal brightness using furimazine substrates opens bioluminescence resonance energy transfer to widefield microscopy. Cytometry A. Aug;89(8):742-6. doi: 10.1002/cyto.a.22870.

3.2 Presentations

1. R Grailhe. Invited lecture. Title: Elucidating protein interaction network in living cells Invited speakers for the 2016 Functional High Throughput Technologies Australia meeting located at the Victorian Comprehensive Cancer Centre in Melbourne, Australia. October 21
2. R Grailhe. Oral. Title: Elucidating protein interaction network in living cells. Seminar at Monash Institute of Pharmaceutical Sciences hosted by Cameron Nowell, Australia. October 19
3. R Grailhe. Oral. Title: Elucidating protein interaction network in living cells. Seminar at the University of Melbourne hosted by Paul McMillan, Australia. Thursday, October 19 afternoon.
4. G. Dumas, N. Antoniou, S. Kim, R. Matsas, R. Grailhe. Poster. Title: Network Biomarker Extraction with High-Throughput calcium Imaging Microscopy on hiPSC-Derived Human Neurons. RIIP, France. November 29
5. AV. Komarova, A Meignie, C Combrede, T Douche, A Zhukova, M Matondo-Bouzanda, B Schwikowski, R Grailhe, and Frederic Tangy. Poster. Title: Measles virus C protein interplay with cellular apoptotic pathways RIIP, France. November 29
6. J Kim, H Eun, Y Zhenzhen, M Vandenberghe, T Delzescaux3, and R Grailhe. Poster. Title: Three-dimensional histological imaging of mouse brain using High Content Microscope Analysis and 3D-HAPi. KSBMB, Korea. July
7. F. Sonthonnax, B. Besson F. Larrous, J. Chamot-Rooke, R. Grailhe, H. Bourhy.. Poster. Title: Cooperation of Lyssavirus P and M proteins on Jak/Stat and NF-kB pathways regulation. 7th International Symposium on Emerging Viral Diseases. Wuhan, China. October 21

3.3 Ongoing & new collaborations

1. Measles virus C protein interplay with key protein in the cellular apoptotic pathways.
   Anastassia V. Komarova. Institut Pasteur, Paris France
2. Lyssavirus infection properties in neural tissues and lyssavirus P and M proteins. Dr. Herve Bourhy. Institut Pasteur, Paris France
3. Technology development for High-Throughput calcium imaging on hiPSC-Derived Human Neurons. Guillaume Dumas. Institut Pasteur, Paris France
4. Application of High Content imaging on hiPSC-Derived Human Dopaminergic Parkinson Patient Neurons. Rebecca Matsas. Institut Pasteur, Greece France
5. Application of High Content Imaging on hiPSC-Derived Human Glutamic Alzheimer's neurons. Dr. Jihwan Song Cha Institut Pangyo, Korea

Technology Development Platform

1. 연구 요약

한국파스퇴르연구소의 phenotypic 스크리닝 시설 유지를 위해서는 꾸준하고 지속적인 진보된 최첨단 스크리닝 기술의 개발이 필요하다. 본 연구팀의 목표는 초고속 대용량 스크리닝 기술을 더욱 확장하여 세포와 병원균 간의 연관성에 대해 연구하는 것이다. 생물학, 생물물리학 그리고 현미경에 능통한 전문가들로 구성된 본 연구팀의 이점을 활용하여 세포와 동물모델에서 나노 입자 매크로 수준의 감염 연구를 위해 세 가지 기술을 개발하고 있다.

(1) 새로운 이미지 기반의 공명 에너지 전달(RET) 기술을 이용하여, 살아있는 세포 내에서 숙주 세포와 병원체 간의 단백질-단백질 상호작용을 면밀히 관찰, 연구 중이다. 보다 구체적으로, 숙주의 선천적 면역력을 파괴하도록 이끄는 바이러스의 다양한 작용경로에 초점을 맞추어 연구를 진행하고 있다.

(2) 유도만능줄기세포(iPSC)로부터 분화된 인간 뇌 세포를 이용하여 생리학적으로 더 적합하고 예측 가능한 세포 기반의 어세이를 초고속 대용량 스크리닝 기술에 적용할 수 있도록 연구 중이다.

(3) 새로운 형광 probe를 이용하여 생물학을 시각화하고 정량화하는 방법을 연구하고 있다. 특히 근적외선 형광단백질의 낮은 흡수 계수 및 낮은 자가 형광의 이점을 활용하여 다양한 조직에서 생체 내 염증반응을 관찰할 수 있는 새로운 동물모델을 개발하고자 한다. 이러한 마우스 모델을 이용하여 전염성 및 만성 질환을 타깃으로 하는 전임상 약물 개발을 목표로 연구 중이다.

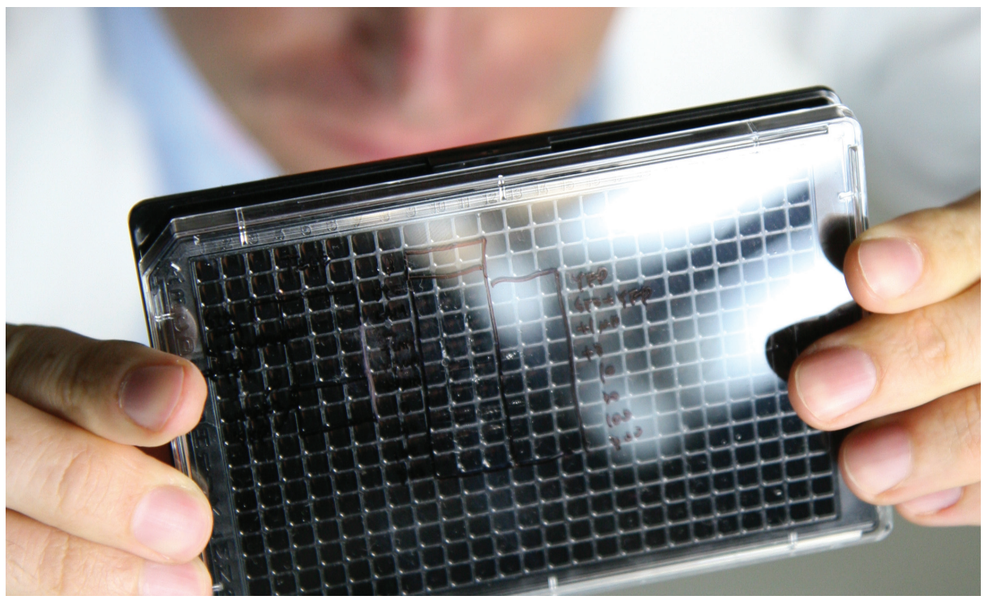
2. 2016년 연구 성과 요약

2016년에 우리는 주로 세 가지의 주제에 초점을 맞추어 연구를 진행하였다.

(1) 살아있는 세포 내에서 furimazine substrate를 이용하여 Nanoluciferase에서 유도되는 신호를 widefield microscope를 이용하여 시각화 및 정량화 할 수 있음을 확인하였다. 그리고 이러한 Nanoluc 시스템의 이점을 활용하여 개발된 생물발광 공명 에너지 전달 기술을 대용량 초고속 스크리닝 기술에 적용하여 단백질-단백질 간 상호작용(PPI)을 정량화할 수 있다. 실제로 프랑스 파스퇴르연구소의 Herve Bourhy 박사와의 공동연구에서 NFKB / TNF pathway에 관계된 단백질들의 상호작용을 측정하는데 사용하기도 하였다.

(2) 우리가 가지고 있는 다채널 이미징 스크리닝 능력을 십분 활용하기 위하여 fluorescent compound 라이브러리를 개발하였다. 초기 테스트에서 세포 내 소기관을 선택적으로 염색하는 독특한 dye들을 발견하였고 이는 세포 형광이미징에 유용하게 쓰일 수 있다.

(3) 우리는 아교세포(astrocyte)에 특이적인 프로모터를 이용하여 새로운 근적외선 형광단백질을 발현하는 동물모델을 개발하였다. 자유롭게 움직이는 동물에서 두개골을 투과하는 형광단백질의 발현을 확인할 수 있었고 뇌의 염증반응에 따른 변화 또한 관찰하였다. 나아가 같은 형광단백질을 이용하여 살아있는 소동물 이미징 및 조직학적 이미지를 연구하였다.



Bio-Imaging Platform

Principal Investigator: Jiho Kim, Ph.D.

Laboratory members: I-Seul Park, M.Sc. (Junior researcher)

1. Research Summary

Proteins interact with each other in a highly specific manner, and protein interactions play a key role in many biological processes; in particular, the distortion of protein interfaces may lead to the development of many diseases. Therefore, protein interaction networks can elucidate the molecular basis of diseases, which can inform methods for prevention, diagnosis and treatment. To address this issue, it is important to understand various molecular interactions within cells that translate genomic information into functional networks to drive biological or pathophysiological outcomes, for instance protein-protein interactions (PPIs).

In cancer, PPIs form signaling nodes and hubs to transmit pathophysiological cues along molecular networks to achieve an integrated biological output, thereby promoting tumorigenesis, tumor progression, invasion, and/or metastasis. Thus, pathway perturbation, through the disruption of PPIs critical for cancer, offers a novel and effective strategy to curtail the transmission of oncogenic signals. As the understanding of cancer biology has significantly increased in recent years, interest in targeting PPIs as anticancer strategies has increased as well.

In case of infectious diseases, it caused by pathogens, including viruses, bacteria and parasites, pose a serious threat to human health worldwide. Molecular interactions between pathogens and their hosts are the key parts of the infection mechanisms. Novel antimicrobial therapeutics to fight drug resistance is only possible in case of a thorough understanding of pathogen-host interaction (PHI) systems.

Due to the higher interests on PPIs, our group have already established various kinds of techniques that could monitor the protein-protein interaction in live cells such as bioluminescence resonance energy transfer (BRET), intensity based fluorescence resonance energy transfer (FRET), lifetime based FRET, and bimolecular fluorescence complementation (BiFC).

This year, we applied BRET/FRET platform to develop the assay system for finding inhibitors for cancer and Leishmania disease. We successfully developed the assay system for Axin2-Gsk3b interaction and hetero-dimerization of L.donovani Topoisomerase I.

During 2017, we plan to validate our BRET/FRET platform for small molecule/peptide inhibitor screening for cancer and leishmania and expand this platform to other disease models such as cancer related to apoptosis and hippo signaling and bacteria (Staphylococcus aureus). Additionally, we will extend our PPI techniques to develop the new methods for determination of dissociation constant (Kd) of protein-protein interaction and target-drug interaction based on the fluorescence polarization (FP) and BRET based techniques.

2. Research Progress in 2016

2.1 Summary

In 2016, we focused on application of protein-protein interaction techniques to development of BRET/FRET assay for cancer and infectious diseases which are main research fields of IPK.

First, we applied our Nanoluc/YFP based bioluminescence resonance energy transfer (BRET) and CFP/YFP based fluorescence resonance energy transfer (FRET) to develop the screening platform to find inhibitor of

Leishmania donovani topoisomerase I dimer in live cells in collaboration with Leishmania Research Laboratory group. Based on the heterodimeric properties of Topoisomerase I in Leishmania, we developed the BRET/FRET assay system and monitor the dimerization of L.donovani Topoisomerase I.

Second, we applied BRET/FRET techniques to develop the screening platform for identification of epithelial-mesenchymal transition (EMT) inhibitor through regulation of Axin2-Gsk3β-Snail 1 axis in collaboration with Cancer Biology Research Laboratory group. Interaction between Axin2 and Gsk3β plays a key role on inhibition of Snail 1 degradation which is regulated by Gsk3β-dependent Snail 1 phosphorylation. Based on this background, we established the BRET/FRET platform and monitored the strong increase of BRET ratio as increasing the Gsk3β-YFP/Axin2-Nanoluc ratio.

Based on this successful assay development, we will validate and apply this platform to inhibitor screening to find the novel drugs.

2.2 Development of BRET/FRET platform to find an inhibitor of Leishmania donovani topoisomerase I dimer

2.2.1 Results

Starting from bacteria to human to viruses, topoisomerase I are encoded by a single gene that contains the highly conserved DNA biding and catalytic domains on single peptide. But in kinetoplastid parasites, topoisomerase I is encoded by two genes, which associate with each other to form a heterodimeric topoisomerase I enzyme within the parasite.

This heterodimeric topoisomerase I is exclusive to Leishmania, thus known as an attractive drug target. Based on this properties, we prepared the FRET/BRET construct for the LdTOP1L and LdTOP1S (Figure 1A, B). I grafted the LdTOP1L and LdTOP1S with cyan fluorescent protein (CFP) or Nanoluc luciferase (Nluc) as an energy donor, and with yellow fluorescent protein (YFP) as an energy acceptor. Four different donor and acceptor construction combinations were tested and the best FRET/BRET pairs were selected upon transient transfection on the HEK cell.

On the spectral analysis (Figure 1C), the BRET platform showed the distinctive shoulder at the YFP emission peak which means the direct interaction between two subunits of Topoisomerase I. On the BRET saturation assay (Figure 1D), we could clearly see the increase of BRET ratio as increasing the acceptor/donor ratio. In the FRET lifetime analysis, the decrease of CFP lifetime also supported the heterodimerization of L.donovani Topoisomerase I. We successfully developed the assay system based on FRET/BRET techniques and plan to apply this assay for finding the inhibitor of dimerization.

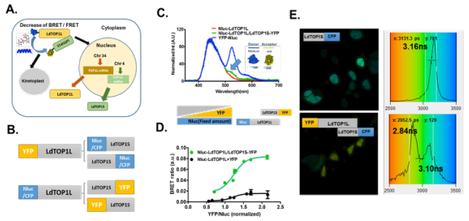


Figure 1. BRET/FRET assay system for L.donovani Topoisomerase I. A. Schematic diagram of BRET/FRET assay system in the cells. B. BRET/FRET constructs for TOP1L and TOP1S. C. Spectral analysis for LdTOP1L and LdTOP1S combination. D. BRET saturation for Nluc-TOP1L and TOP1S-YFP on dependence of YFP/Nluc ratio. E. Decrease of fluorescence lifetime in LdTOP1S-CFP and YFP-LdTOP1L co-expressed cells.

2.3 Development of BRET/FRET platform for identification of EMT inhibitor through regulation of Axin2-Gsk3ß-Snail 1 axis

2.3.1 Results

Strong Axin2-Gsk3β interaction in cytoplasm can enhance the expression of Snail, which is inducible protein of EMT as E-box binding transcription factor. Overexpression of nucleus Snail 1 increases EMT via decreasing of the E-cadherin expression. Interaction between Axin2-Gsk3β plays a key role on inhibition of Snail degradation which is regulated by Gsk3β-dependent Snail1 phosphorylation (Figure 2A). Based on the background, we established the BRET/FRET platform which enables to screen candidate drugs targeting EMT regulators through regulation of Axin2-Gsk3β-Snail1 axis. Among the variable BRET/FRET combinations, the best BRET combination was selected based on the BRET ratio. On the spectral analysis, we confirmed that the direct interaction between Axin2 and Gsk3β from the YFP fluorescence signal without direct excitation source. On the BRET saturation assay, we clearly monitored the BRET ratio was saturated as increasing the relative expression ratio of acceptor/donor. Therefore, we successfully established the BRET/FRET platform for Axin2-Gsk3β interaction and we plan to validate this assay system and apply to the small molecule inhibitor screening. As an alternative, we will also develop the assay system based on the Snail expression on the nucleus using Nluc or E2-Crimson.

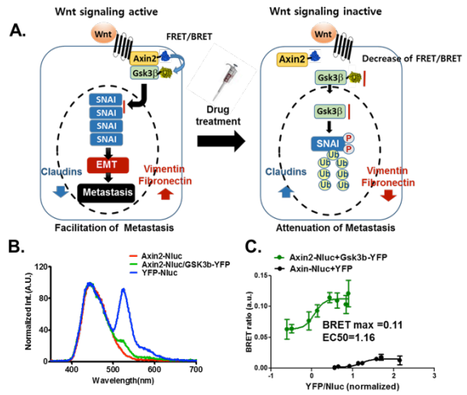


Figure 2. BRET/FRET assay system for Axin2-Gsk3β interaction. A. Illustration of regulation model of Snail 1 by Axin2-Gsk3β interaction. B. Spectral analysis for Axin2-Nluc and YFP-Gsk3β combination. C. BRET saturation for Axin2-Nluc and YFP-Gsk3β on dependence of YFP/Nluc ratio

3. Major Accomplishments

3.1 Publications

1. Kim J and Grailhe R (2016). Nanoluciferase signal brightness using furimazine substrates opens bioluminescence resonance energy transfer to widefield microscopy. Cytometry A. 89.(8.):742- 746.

3.2 Presentations

1. Kim J., Eun H, Zhenshen Y, Vandenberghe M, Delzescaux T, and Grailhe R., Three-dimensional histological imaging of mouse brain using High Content Microscope Analysis and 3D-HAPi, 2016 KSBMB, Seoul, Korea, 19-May-2016(poster)
2. Park I-S, Yang G, No J and Kim J., Development of live cell FRET/BRET screening platform to find an inhibitor of Leishmania donovani topoisomerase I dimer, 2016 KSMCB, Seoul, Korea, 14-Oct-2016. (poster)

3.3 Ongoing & new collaborations

1. Development of Heterodimeric Topoisomerase I of Leishmania donovani PPI assay for PPI inhibitor screening. Dr. Joohwan No, Leishmania Research Laboratory, Institut Pasteur Korea (Internal)
2. Development of BRET/FRET platform for identification of EMT inhibitor through regulation of Axin2-Gsk3β-Snail 1 axis. Dr.Haengran Seo, Cancer Biology Research Laboratory, Institut Pasteur Korea (Internal)

Bio-Imaging Platform

1. 연구 요약

단백질은 매우 특정한 방식으로 서로 상호작용하는데, 이는 많은 생물학적 과정에서 중요한 역할을 한다. 특히, 단백질 상호작용면의 왜곡은 많은 질병을 발달시킨다. 따라서 단백질 상호작용 네트워크는 예방, 진단 및 치료 방법에 대한 정보를 제공할 수 있는 질병의 분자 기반을 밝힐 수 있다. 이 문제를 해결하기 위해서는 생물학적 또는 병태 생리학적 결과를 유도하기 위해 게놈 정보를 기능 네트워크로 변환하는 단백질–단백질 상호작용과 같은 세포 내의 다양한 분자 상호 작용을 이해하는 것이 중요하다.

암에서 단백질 간 상호작용은 종양 형성, 종양 진행, 침입 또는 전이를 촉진하여 통합된 생물학적 산출물을 달성하기 위해 분자 네트워크를 따라 병태생리학적 단서를 전달하는 신호 노드 및 허브를 형성한다. 따라서, 암에 중요한 단백질 상호작용의 저해와 전달 경로의 간섭은 종양 발생 신호의 전달을 억제하는 새롭고 효과적인 전략을 제공한다. 최근 몇 년 동안 암 생물학에 대한 이해가 크게 증가하면서 단백질 상호작용을 항암제 발굴의 타깃으로 사용하는 것과 관련된 관심 또한 증가하였다.

전염병의 경우 바이러스, 박테리아 및 기생충을 비롯한 병원균에 의해 발생하며 전 세계적으로 인체 건강에 심각한 위협이 되고 있다. 병원체와 그 숙주 사이의 분자 상호 작용은 감염 메커니즘의 중요한 부분이다. 따라서 약제 내성과 싸울 수있는 새로운 항균제 치료법은 병원체–숙주 상호작용 시스템에 대한 철저한 이해를 바탕으로만 가능하다.

이처럼 단백질 간 상호작용에 대한 관심이 높아짐에 따라 본 연구 그룹은 생물발광 공명 에너지 전달, 형광 세기 및 형광 수명을 기반으로 한 형광 공명 에너지 전달, 이분자 형광 상보법과 같이 살아있는 세포에서 단백질 간 상호작용을 모니터링할 수 있는 다양한 종류의 기술을 이미 수립하였다.

2016년 본 연구팀은 BRET/FRET 플랫폼을 적용하여 암과 리슈마니아증에 대한 저해제를 발굴하는 분석 시스템을 개발하였다. Axin2–Gsk3β 단백질 간 상호작용 및 리슈마니아 도노바니 위상 이성질화 효소의 이종 이합체화에 대한 분석 시스템을 성공적으로 개발하였다.

2017년에는 기존에 개발한 BRET/FRET 플랫폼에서 암 및 리슈마니아증에 대한 저분자/펩타이드 저해제 스크리닝을 수행하고, 이러한 플랫폼을 자가 세포 사멸 및 히포 신호 전달과 관련된 암 및 박테리아(황색포도상구균)와 관련된 다른 질병 모델로 확장할 계획이다. 또한, 본 연구팀은 단백질–단백질 상호작용 연구 기술을 확장하여, 형광 편광 및 BRET기술을 기반으로 하여 단백질–단백질 상호작용 및 표적–약물 간 상호작용의 해리 상수(Kd)를 결정하기 위한 새로운 방법을 개발할 것이다.

2. 2016년 연구 성과 요약

2016년, 본 연구팀은 단백질–단백질 상호작용 기술을 한국파스퇴르연구소의 주요 연구 분야인 암 및 감염성 질환에 적용하여 BRET/FRET 분석 시스템 개발에 중점을 두었다.

첫째, 본 연구팀은 리슈마니아증 연구 그룹과 협력하여 Nanoluc/YFP 기반 생물 발광 공명 에너지 전달 및 CFP/YFP 기반 형광 공명 에너지 전달 기술을 살아있는 세포 내에서 리슈마니아 도노바니 위상 이성질화 효소의 이합체 형성 억제제를 발굴할 수 있는 스크리닝 플랫폼을 개발하는데 적용하였다. 리슈마니아 위상 이성질화 효소의 이종 이합체 형성 특성에 기반하여, 본 연구팀은 BRET/FRET 분석 시스템을 개발하였고, 리슈마니아 위상 이성질화 효소의 이합체 형성을 모니터링하였다.

둘째, BRET/FRET 기법을 적용하여 종양 생물학 연구 그룹과 공동으로 Axin2–Gsk3β–Snail 1축의 조절을 통해 상피 간엽 전환 저해제를 발굴하기 위한 스크리닝 플랫폼을 개발하였다. Axin2와 Gsk3β 간의 상호작용은 Gsk3β에 의존한 Snail 1의 인산화에 의해 조절되는 Snail 1의 분해를 억제함에 있어 중요한 역할을 한다. 이러한 사실을 바탕으로 BRET/FRET 플랫폼을 확립하였고, Gsk3β–YFP/Axin2–Nanoluc 비율이 증가함에 따라 BRET 비율이 크게 증가함을 확인하였다. 성공적인 분석법 개발을 바탕으로, 본 연구팀은 이러한 플랫폼을 검증하고, 새로운 약물을 발굴하기 위해 저해제 스크리닝에 이 플랫폼을 적용할 것이다.

Core Support Groups

Bioinformatics

Principal Investigator: Sangchul Lee, Ph.D.

Laboratory members: Inhee Choi, Ph.D., R.Ph. (Senior researcher); Yoonae Ko, M.Sc. (Resarcher)

1. Research Summary

At the beginning of 2016, the computational biology group was reorganized to reinforce bioinformatics functionality to the existing cheminformatics function, while image-mining group being discontinued. As a result, the newly named "bioinformatics" team is partly cheminformatics for drug discovery process and partly bioinformatics for other related researches. The team continues to be a part of the core supporting groups to help with drug discovery process such as hit compound clustering, compound information search, patent search, compound library analysis, and integrative database implementation. In addition, a new role was assigned to respond to increasing demands of analyzing bioinformatics data to facilitate mechanism-of-action studies or target identification research of biology teams. Research teams in IPK requested analysis for their own data such as next-generation sequencing (NGS) data, microarray data or siRNA screening data, which requires computational or statistical expertise.

To deal with new types of data, we focused on establishing infrastructure of hardware, software and protocols in the new area of analysis through trainings, workshop and internal benchmark tests. With such background effort, we collaborated with various teams in IPK. Bioinformatics projects in 2016 include RNA-Seq transcriptomic analysis of TB drug treatment, microarray transcriptomic analysis of lung cancer cell culture, protein interaction analysis from hepatocellular carcinoma 2D-gel analysis, metagenome analysis of environmental samples, and systemic siRNA screening analysis. We provided key information to each team for following experiments or publications.

In the field of cheminformatics, in addition to several hit compound clustering tasks and structure-based patent search, we endeavored to establish integrative database platform for the drug screening system in IPK. With collaboration of ADS, ALM and IT teams, we registered all IPK compounds along with their legacy screening data. As part of the IPK-Gyeonggido-Pharmaceutical company project, we have started a virtual screening of Pseudomonas aeruginosa protein against IPK library collection in order to find compounds binding with target protein.

2. Research Progress in 2016

2.1 Summary

As a core support team, we analyzed different data from various demands. Those tasks are summarized as the following. They all are completed projects except mentioned as on-going:

- **Bioinformatics**
  - Transcriptomics: TB, Lung cancer, Liver cancer (on-going), P. aeruginosa
  - Proteomics: Liver cancer
  - Metagenome: Environmental samples (on-going)
  - siRNA screening: Rabies screening, Software development (on-going)
- **Cheminformatics**
  - Data registration in Integrative database: IPK compounds, FDA approved compounds, bioactive compound, RNAi library (on-going), Inventory data (on-going)
  - Hit compound clustering: S. pneumonia, Leishmania, Cancer, Ebola, HBV, Influenza, MERS

- Patent search: MERS, S. pneumonia, HBV
- Compound library characterization

• **Molecular modeling**

- Drug-Target protein binding modeling: TB
- Virtual screening: P. aeruginosa (on-going)

2.2 Bioinformatics

The team was funded as part of the "Project management on the operation of IPK" grant, as one of the technology development group. Thus, we served to facilitate drug discovery and mechanism studies in IPK with many different kinds of chemical and biological data analysis.

2.2.1 Results

Individual cheminformatics tasks are not represented as figures in below results. There were too many of them and listed in the summary in above section 2.1 . Examples of bioinformatics analysis and integrative database are depicted as follows.

Figure 1. RNA-Seq transcriptomic data analysis. (left) Time-dependent pathway enrichment change in Pseudomonas drug treatment (right) Enriched pathway by drug treatment in Mycobacterium

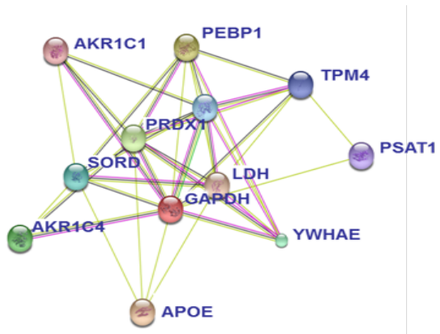


Figure 2. Biological network analysis of secreted proteins in spheroids of hepatocellular carcinoma. Those differentially expressed proteins were selected from 2D-gel electrophoresis analysis.

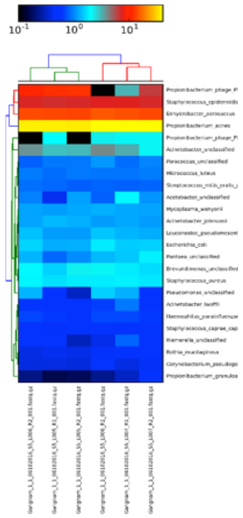


Figure 3. Species abundance heatmap from shotgun metagenome sequencing data of environmental samples (on-going). Proportion of top25 species of 6 samples are presented with heat map.

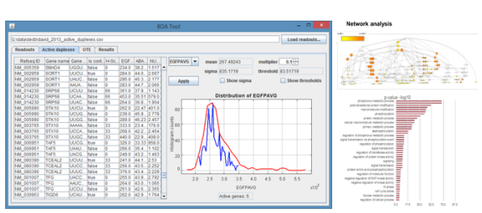


Figure 4. siRNA screening data analysis (left) Internally developed software for hit gene selection. There have been improvements in functionalities (right) Network analysis from selected hit genes of siRNA screening against rabies virus

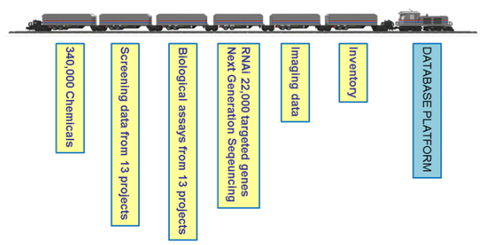


Figure 5. Overview scheme of integrated information system database: Registered 349,296 IPK compounds, 2,608 FDA approved compounds info, 8,356 FDA-approved and bioactive compound library info. RNAi library (on-going), Inventory of cell lines and pathgens (on-going)

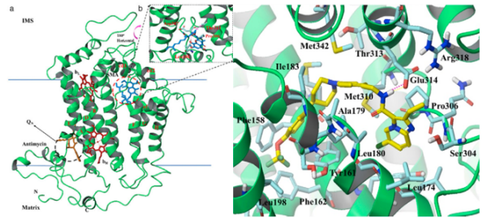


Figure 6. Molecular model of drug-target binding (Q203 to QcrB of M. tb) (published in Bull Kor Chem Soc 2016)

2.3 Bioinformatics support

Five IPK-Gyeonggido-pharmaceutical company projects began in late 2016. With each project team's support in software license, we participate in virtual screening research as well as cheminformatic tasks.

2.3.1 Results

The projects have only recently started. The team is expected to be involved in next year for tasks related to compound clustering and information search, in several projects.

Along with those compound screening, we initiated a virtual screening project targeting a Pseudomonas protein, previously identified as a good target from ARRL team of IPK. Since 3D x-ray structure of the protein from P. aeruginosa is not available yet, we have selected Acinetobacter baumannii protein structure as the template. Homology modeling and reference compound docking showed good representation the original binding environment. We completed ligand conformer preparation for the pilot screening collection. The actual virtual screening will continue throughout 2017.

3. Major Accomplishments

3.1 Publications

1. Ko Y, Choi I (2016). Putative 3D Structure of QcrB from Mycobacterium tuberculosis Cytochrome bc1 Complex, a Novel Drug-Target for New Series of Antituberculosis Agent Q203. Bull Kor Chem Soc 37: 725-731
2. Kim H, Kong S, Oh S, Yang J, Jo E, Ko Y, Kim S, Hwang J, Song R, Windisch M (2016). Benzothiazepinec-arboxamides: Novel hepatitis C virus inhibitors that interfere with viral entry and the generation of infectious virions. Antiviral Research 129: 39-46
3. Chang SY, Cruz DJ, Ko Y, Min JY (2016). Identification of pyrrolo[3,2-c]pyridin-4-amine compounds as a new class of entry inhibitors against influenza viruses in vitro. Biochem Biophys Res Commun. 478(4):1594-601

3.2 Presentations

1. Inhee Choi, Oral. The locomotive of discovery explorer database, 2016 Dotmatics User Group Meeting & Open Seminar, Tokyo, Japan, 17-May-2016 & Osaka, Japan, 20-May-2016.
2. Se-Hyuk Kim, Yeonhwa Song, Sangchul Lee, Kang Mo Kim, Inhee Choi, Haengran Seo. Secretome analysis of tumor spheroids for the discovery of HCC-specific biomarkers, The Korean Society for Molecular and Cellular Biology (KSMCB) 2016, South Korea, 13-Oct-2016 (Poster)
3. Yoonae Ko, Invited lecture, RNAi screening data analysis and controlling off-target effect, 2016 Institut Pasteur Korea Training Course, High Content Screening for Target Discovery Using RNAi Technology, Seongnam, Korea, 9-May to 13-May, 2016.
4. Na Kyung Lee, Hee Chul Kim, Jinyeong Heo, Sae Hong Min, Jihye Lee, Alexander Koenig, Inhee Choi, David Shum, Kideok Kim, Hong-gun Lee, Constantin Radu, Ji-Young Min, Marc P. Windisch, Characterization of inhibitors and biomarkers identified by screening of the Ebola virus transcription- and replication-competent virus-like particle system, Institut Pasteur International Network Scientific Symposium, Paris/France, 29-Nov to 2-Dec, 2016

3.3 Ongoing & new collaborations

All collaborations during 2016 were with internal teams of IPK. What is still on-going are as follows.

1. Integrative Database: Automation & Logistics Management (ALM), Assay Development and Screening (ADS) and IT team
2. Virtual screening: Antibacterial Resistance Research Lab (ARRL)
3. Functional analysis of cancer proteins with microarray data: Cancer Biology Research Lab (CBRL)

Bioinformatics

1. 연구 요약

한국파스퇴르연구소(IPK)의 생물정보학팀은 2016년 1월에 새로 구성된 조직으로 기존의 화학정보학팀에 생물정보학 기능과 인원이 추가된 것이다. 이렇게 만들어진 '생물정보학 팀'은 여전히 화학정보학적인 작업인 유효화합물 클러스터링, 화합물 정보 수집, 특허 검색, 화합물 라이브러리 분석 등의 신약 개발 과정에 필요한 연구지원 조직으로의 역할을 수행하고 있다. 여기에 생물학 연구팀들의 작용기전 연구와 표적단백질 연구를 지원하기 위해 생물정보학적 데이터 분석의 요구 증가에 대처하는 새로운 역할이 추가되었다. IPK의 연구팀들이 보유한 데이터의 종류에는 RNA-Seq, Microarray, Whole genome sequencing, protein 2D-gel, siRNA, metagenome 등이 있다.

새로운 종류의 데이터 타입에 대응하기 위해 여러 교육, 워크숍, 자체 소프트웨어 평가 등을 통해 우선적으로 기반이 되는 하드웨어, 소프트웨어, 프로토콜을 갖추는 데 집중하였다. 이를 바탕으로 연구소 내부의 여러 팀과 협력하여 다양한 여러 프로젝트를 수행하고 있다. 2016년에 수행한 프로젝트에는 결핵균 약물처리 후 RNA-Seq 발현 분석, 폐암세포주의 배양환경에 따른 microarray 발현 분석, 간암세포주의 3차원 배양에서의 발현 단백질 분석, 환경시료에서 metagenome 분포 분석, siRNA 스크리닝 결과 분석 등이 있다. 이는 해당 연구팀의 향후 연구나 논문 작성의 중요 데이터로 사용되었다. 여러 유효화합물 클러스터링, 특허 검색 등 일반적인 스크리닝 결과 분석 외에도 ADS, ALM, IT 팀과의 협력으로 연구소 내의 모든 화합물 정보와 기존 스크리닝 결과를 통합 분석할 수 있는 데이터베이스 구축작업 또한 계속 진행 중이다. IPK-경기도-제약기업 공동연구 프로젝트의 지원을 이용해 P. aeruginosa의 단백질을 타깃으로 하는 가상 스크리닝 프로젝트도 진행하고 있다.

2. 2016년 연구 성과 요약

연구지원팀으로서 각 연구팀의 필요와 요청에 따라 다양한 종류의 데이터 분석을 수행하여 아래 리스트로 정리하였다. '진행중'으로 표시된 것 이외에는 모두 2016년에 완료한 과제들이다.

- **생물정보학 분석**
  - mRNA 발현 분석: 결핵, 폐암, 간암(진행중), 녹농균(Pseudomonas)
  - 단백질 발현 분석: 간암
  - Metagenome: 환경 샘플(진행중)
  - siRNA 스크리닝: 광견병 바이러스, 결과분석 소프트웨어 개발(진행중)
- **화학정보학 분석**
  - 통합 데이터베이스에 데이터 등록: IPK 화합물 라이브러리, FDA 승인 약물, 생물활성이 알려진 화합물, RNAi 라이브러리(진행중), 세포주, 바이러스 등 인벤토리 정보(진행중)
  - 유효화합물 클러스터링: 연쇄상구균(Streptococcus), 리슈마니아, 암, 에볼라, B형 간염 바이러스, 인플루엔자, 메르스
  - 특허 정보 검색: 메르스, 연쇄상구균, B형 간염 바이러스
  - 화합물 라이브러리 특성 분석
- **분자 모델링**
  - 신약 후보물질-단백질 결합 모델링: 결핵
  - 신약 가상 스크리닝: 녹농균(진행중)

Core Support Groups

Chemistry Platform

Group Head : Sunhee Kang, M.Sc.

Laboratory members: Youngmi Kim, M.Sc. (Senior researcher); Sunju Kong, M.Sc. (Senior researcher); Junghwan Choi, M.Sc. (Researcher)

1. Research Summary

Main research of discovery chemistry focuses on the drug discovery program. In this year, we involved in the early stage of leishmania, HBV, Ebola and MERS programs for hit selection and early structure-activity relationship (eSAR) studies. Especially, hit-to-lead and lead optimization were intensively proceeded for influenza drug discovery program. As a results, we successfully obtained potential leads which have not only potent activities against 3 different seasonal influenza viruses, H1N1, H3N3 and B but also orally available good in vivo PK properties. With this promising results, additional optimization is ongoing and in vivo efficacy and toxicity evaluation is in due course.

At the same time, we discovered novel plasmalema selective fluorescent dyes. Based on the structure of compound S which has specific membrane staining property, a set of analogues were designed and synthesized. The representative compounds showed improved fluorescence intensity without cytotoxicity even at high concentration as well as they had good solubility against aqueous media.

In addition, we performed quality control for IPK compounds which were synthesized on drug discovery program and we provided fee-based open NMR service to K-Pharma to expand collaborations.

2. Research Progress in 2016

2.1 Summary

Discovery chemistry focused on the hit-to-lead and lead optimization for influenza drug discovery program. Among screening hits, THO (Thio-oxadiazole) scaffold was identified and we performed hit-to-lead and lead optimization. Through the structural modification, the metabolic instability and cytotoxicity of scaffold were dramatically improved and we could finally obtained potential candidates for in vivo experiment. Current leads showed potent antiviral activity against H1N1, H3N2 and B influenza viruses as well as orally available in vivo PK properties.

In addition, we develop a series of fluorescence dye which shows plasmalema selective staining properties by collaboration with TDP group. They displayed improved staining properties with strong fluorescence intensity as well as they are non-cytotoxic even at high concentration and soluble in aqueous. These analogues could therefore be promising novel fluorescent dye which have specific staining properties.

2.2 Influenza drug discovery program (collaboration with RVRL group)

Through the 110,000 library screening with 'target-free' strategy, many hits were identified and early structure-activity relationships (eSARs) were studied for four selected scaffolds. After hit validation with eSAR study, promising scaffold, THO (THio-oxadiazole) was finally selected and an extensive optimization was performed. In the meantime, low metabolic stability of the scaffold was improved in human and mouse liver microsomes ($t_{1/2}$ > 60 min for both) by strategic modification. The THO leads exhibited not only potent activity spectrum against three different seasonal influenza virus strains (H1N1, H3N2 and B viruses) without cytotoxicity but also promising in vivo PK values with high drug exposure

level and good bioavailability after oral administration. With the promising results of in vitro potency and in vivo PK results, we are intensively progressing lead optimization and in vivo efficacy experiment as POC is in due course. (3 scaffold was patented in Korea.)

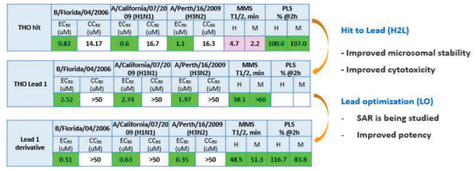


Figure 1. Improvement of activity and in vitro ADME of THO scaffold during optimization

Compd	Pharmacokinetics (i.v. 2mg/kg)			Pharmacokinetics (p.o. 10mg/kg)				
	$t_{1/2}$ (h)	Cl (mL/min/kg)	Vd_{ss} (mL/kg)	C_{max} (ng/mL)	$t_{1/2}$ (h)	T_{max} (h)	$AUC_{0\text{-}inf}$ (ng·h/mL)	F (%)
Lead 1	2.14	16.5	1640	4573	1.98	0.50	16120	160

Table 1. Pharmacokinetic values of lead 1

2.3 Development novel plasmalema selective fluorescent dye (collaboration with Technology development Platform (TDP))

Compound S has specific property that stains selectively plasma membrane with fluorescence. However, the fluorescence intensity of compound S is not strong and it has low solubility not only in aqueous media but also in DMSO. To develop plasmalema selective dye for biological uses, a set of compound S analogues were designed and synthesized. As a result, the staining property was improved with strong fluorescence and the modified analogues showed good solubility in DMSO and aqueous solution. In addition, they didn't show cytotoxicity even at high concentration. With the promising results of derivatives and obtained structural novelty, filing of patent is currently ongoing.

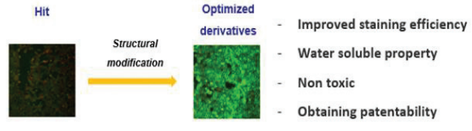


Fig. 2. Improved staining property and fluorescent intensity of derivatives

2.4 Other research progress

- Thiophene urea (TU) scaffold was successfully optimized in drug potency as well as ADMET by supporting KDDF grant last year and it was licensed out to J2H biotech for further development this year.
- Supporting target identification: Designed and synthesized probe molecules such as conventional biotinylated probes, photo-reactive probes and click probes to support target identification for TB and HCV program (retained potency with no cytotoxicity)
- Supporting hit selection from library screening for HBV, EBOLA and MERS and eSAR study for leishmania program
- Quality control of IPK chemical libraries and synthesized compounds on drug discovery program
- Open NMR service to K-Pharma to expand collaboration

3. Major Accomplishments

3.1 Patents

1. 10-2016-0127005 (filed): Novel compounds comprising thionicotinamide scaffold, its enantiomers, its diastereomers or its pharmaceutically acceptable salt and antiviral composition containing the same as an active ingredient (Influenza)
2. 10-2016-0126989 (filed): Novel compounds comprising thionicotinamide scaffold, its enantiomers, its diastereomers or its pharmaceutically acceptable salt and antiviral composition containing the same as an active ingredient (Influenza)
3. 10-2016-0126997 (filed): Novel compounds comprising thionicotinamide scaffold, its enantiomers, its diastereomers or its pharmaceutically acceptable salt and antiviral composition containing the same as an active ingredient (Influenza)
4. 10-1646833 (issued): Compounds for Treatment of Inflammatory Diseases (Inflammation)
5. 10-1646833 (issued): Compounds for Treatment of Inflammatory Diseases (Inflammation)
6. 2011229423 (issued): Anti-infective compounds (TB)
7. 5944837(issued): Anti-infective compounds (TB)
8. 2012/06814 (issiued): Anti-infective compounds (TB)
9. 10-2016-7031006 (filed): Compounds for treating viral infections (HCV)

3.2 Publications

1. Kang S, Kim YM, Kim RY, Seo MJ, No Z, Nam K, Kim S, Kim J (2016). Synthesis and structure-activity studies of side chain analogues of the anti-tubercular agent, Q203. European Journal of Medicinal Chemistry. 125:807-815 (Published)
2. Kim HY, Kong S, Oh S, Yang J, Jo E, Ko Y, Kim SH, Hwang JY, Song R, Windish MP (2016). Benzothiazepinecarboxamides: Novel hepatitis C virus inhibitors that interfere with viral entry and the generation of infectious virions. Antiviral Research. 129:39-46 (Published)

3.3 Ongoing & new collaborations

1. DMPKT evaluation, Wuxi AppTec. China
2. Objectives or ongoing research activities for collaboration, Name of the collaborator(s), Position and Affiliation of the collaborator(s), Country

Chemistry Platform

1. Influenza drug discovery (collaboration with RVRL group)

1.1 연구 요약

본 연구팀은 신약개발 프로그램에 있어, hit-to-lead 및 lead optimization과정을 통한 후보물질 창출에 중점을 두었다. 기존의 작용점 기반 신약 개발 과정과 달리, 한국파스퇴르연구소에서는 세포기반 이미징 기법을 신약개발 연구에 활용함으로써 새로운 작용점을 발굴할 수 있는 역량을 키워왔다. 'target-free' 전략으로 수행된 110,000 화합물 라이브러리 스크리닝으로부터 THO (Thio-oxadiazole) 화합물군이 도출되었으며, 화합물의 구조-활성 상관관계 연구가 진행되었다. 화합물의 구조적 최적화 과정 동안, 화합물군의 취약한 대사 안정성은 human 및 mouse liver microsome 모두에서 증진되었으며 (t1/2 >60 min), 생체 내 적용 가능한 in vitro ADME 특성을 확보했다. 현재 THO lead들은2종의 A형 (H1N1, H3N2) 및 B형 바이러스 모두에 우수한 활성을 보이며, 경구투여 시 높은 혈중 약물노출도와 생체이용률로서 우수한 약물동력학 특성을 보인다. 이를 바탕으로 THO화합물에 대한 광범위한 선도화합물 최적화 작업이 진행 중이며, 생체 내 효능시험을 진행할 예정이다.

1.2 2016년 연구성과 요약

- 라이브러리 스크리닝으로부터 THO scaffold를 발굴하고, 최적화 연구가 진행됨.
- 선도화합물 창출 과정 동안 THO scaffold가 근본적으로 지니고 있던 대사안정성 및 세포독성이 개선되었으며, 현재 경구투여로 우수한 약물동력학 특성을 보이는 후보물질을 도출하였음.
- 구조적 최적화 과정 동안 화합물의 구조적 신규성을 확보하였으며, THO series를 포함한 3개 화합물종에 대한 국내 특허를 출원함.

2. Development novel plasmalema selective fluorescent dye (collaboration with Technology development Platform (TDP))

2.1 연구 요약

세포의 plasma membrane에 선택적으로 염색되는 compound S 화합물의 특성에 착안하여 새로운 형광 dye를 고안하고 합성하였다. Compound S는 평면적인 multi-fused ring 구조를 지녀 세포기반 실험에 수반되는 aqueous media 및 DMSO 용매조건에서도 매우 낮은 용해도를 보이며, 형광 강도가 크지 않다. 형광 intensity, sensitivity 및 용해도를 증진시킨 형광 dye 개발을 위하여 compound S의 구조를 모방한 다양한 화합물들이 고안 및 합성되었으며, 그 결과 높은 형광강도와 함께 plasmalema selective staining property가 개선된 화합물들이 도출되었다. 또한 이들 화합물은 높은 농도에서도 세포독성을 나타내지 않으며, DMSO 및 aqueous에서도 매우 좋은 용해도를 지님으로써 세포기반 assay에 활용될 수 있는 높은 가치를 지닌다. 이들 결과를 바탕으로 현재 국내 특허 출원을 진행 중이다.

2.2 2016년 연구성과 요약

- Plasmalema에 선택적인 fluorescence dye개발. (높은 형광강도로 증진된 staining property, 개선된 세포독성 및 용해도)
- 구조적 신규성 확보로 특허출원 진행 중.

3. 기타 연구 성과

- HCV 치료제 후보물질 (TU series)의 기술이전: 2015년, 새로운 작용기전과 우수한 항바이러스 활성을 지니는 TU 화합물의 최적화가 완료되었으며, 2016년 J2H biotech에 기술이전되어 후속연구가 진행 중임.
- TB-TTCA 및 HCV-TU 화합물의 작용기전 연구를 위한 다양한 탐침 화합물이 고안 및 합성됨. (conventional biotinylated probes, photo-reactive probes and click probes)
- HBV, EBOLA 및 MERS 프로그램의 스크리닝 hit 선별과정에 참여 및 leishmania 프로그램의 hit 선별과 소규모의 구조-활성 상관관계 연구 진행.
- IPK 라이브러리의 정도 관리 (QC).
- 국내 제약기업에 Open NMR service 제공.

Core Support Groups

Animal Facility

Group Head: Regis Grailhe, Ph.D.

Laboratory members: Sully Lee, D.V.M. (Junior researcher); Sinyoung Park, M.Sc. (Junior researcher); Seungbin Lim, B.Sc. (Senior assistant)

1. Research Summary

1. Efficacy experiment of new drug compound against hypercholesterolemia
2. Animal care and management of animal facility
3. IACUC (Institutional Animal Care and Use Committee) operation

2. Research Progress in 2016

2.1 Results

1. Animal care and management of animal facility
   A. Daily animal care
   B. Maintaining SPF (Specific Pathogen Free) condition
   C. Reporting to QIA (Animal and Plant Quarantine Agency) and MFDS (Ministry of Food and Drug Safety) regarding animal facility operation
   D. Response to MFDS (Ministry of Food and Drug Safety) animal facility investigation
   E. Development of SOP (Standard Operation Procedures)
   F. Monitoring animal facility microbial status; Health monitoring, Water monitoring, Environmental monitoring
2. IACUC (Institutional Animal Care and Use Committee) operation
   A. Review of Animal Protocols
   B. Holding an IACUC Regular meeting

3. Major Accomplishments

3.1 Presentations

1. Sully Lee, National-wide Survey on Laboratory Animal Facilities in Korea, Singapore/Singapore, November 8-10, 2016 (poster)

3.2 Ongoing & new collaborations

1. Offering animal care service, ABLbio, South Korea

Animal Facility

1. 연구 요약

- 고콜레스테롤혈증 효능 시험
- 동물 유지, 번식 및 동물 시설 관리
- 동물실험윤리위원회(IACUC, Institutional Animal Care and Use Committee) 운영

2. 2016년 연구성과 요약

1. 동물 유지, 번식 및 동물 시설 관리
   A. 동물 유지 및 관리
   B. 특정병원체부재(Specific Pathogen Free, SPF) 시설 유지 및 관리
   C. 정부기관(식품의약품안전처 및 농림축산검역본부)에 동물실 운영과 관련한 사항 보고
   D. 식품의약품안전처의 동물실험시설 점검 대응
   E. 동물실 표준작업지침서(SOP, Standard Operation Procedures) 작성
   F. 동물실 미생물수준 모니터링; 건강모니터링, 수질 모니터링, 환경모니터링
2. 동물실험윤리위원회(IACUC, Institutional Animal Care and Use Committee) 운영
   A. 동물실험계획서 검토
   B. 동물실험윤리위원회 정기회의 개최

2016 Research Outputs and Outcomes

Collaborations
공동 연구

53

- Research agreements: 11
- MTAs: 16
- Industry-sponsored research: 15
- MOUs: 11

Revenue
수입 (1)

~1bn KRW

- Licensing out & Industry-sponsored research

Intellectual Property
지식 재산 (2)

159 filed / 41 registered

- International: 129 filed / 30 registered
- Domestic: 30 filed / 11 registered

Publications
논문

42

- Published, in press and accepted

Active Grants
연구 과제

2,083m KRW

- 14 grants (domestic and international)

Scientific Exchanges
세미나/심포지엄

135

- International symposia, meetings and training(3): 6
- Seminar series(4): 25
- Oral presentations: 71
- Poster presentations(5): 33

Education & Training
교육

46

- UST professorships: 6 by 2017 spring semester
- Internship undergraduate students : 30
- Ph.D./Master students : 10

Science Communications
대외 활동

3,501

- Science programs: 62
- Students participated in science programs: 3,078
- Press releases: 24
- Interviews: 6
- Media coverage: 331

(1)

IPK Licenses Out a Hepatitis C Virus Drug Candidate to J2H Biotech

On 11 Oct, 2016, IPK and J2H Biotech signed an out-licensing agreement for a first-in-class hepatitis C virus (HCV) drug candidate. In 2013, IPK screened small molecule compounds libraries with the infectious HCV cell culture system to discover novel viral interventions. The project resulted in identifying a Thiophene Urea (TU) compound series. With financial support from the Korea Drug Development Fund, the TU small molecule series was refined further to be an optimized Lead, and final optimization was completed in 2015. TU inhibits HCV's ability to enter into hepatocytes by targeting a viral protein, and it prevents cell-to-cell spread of the virus, thereby inhibiting the major route of HCV transmission in the liver.

JaeSun Kim, Co-CEO, J2H, says, "Although HCV drug research has dramatically improved in recent years, there is still great unmet needs. IPK's HCV entry inhibitor will be an alternative way of satisfying the needs of many. And I expect there will be synergy in combining it with HCV protease inhibitors."

한국파스퇴르연구소, J2H 바이오텍에 C형 간염 치료 후보물질 이전

2016년 10월 11일 한국파스퇴르연구소는 혁신 신약(first-in-class) C형 간염 치료제 후보물질을 J2H 바이오텍에 이전하는 협약식을 가졌다. 2013년 한국파스퇴르연구소는 새로운 작용기전을 가진 바이러스성 치료제를 발굴하기 위해 C형 간염 바이러스 세포배양 시스템을 이용한 저분자 화합물 라이브러리를 검증하는 프로젝트를 진행하였고, 티오펜 우레아(TU: Thiophene Urea)라는 화합물질 계열을 발견하였다. 그리고 2015년 범부처신약개발사업단(KDDF: Korea Drug Development Fund)의 연구비 지원으로, 티오펜 우레아(TU) 저분자 물질계열의 선도물질(Lead)을 최적화하여 최종 후보물질을 도출하였다. 티오펜 우레아(TU)는 바이러스의 특정 단백질에 결합하여 C형 간염 바이러스가 간세포 내로 들어가는 것을 억제한다. 따라서 간(liver) 안에서 C형 간염 바이러스의 확산을 억제하게 된다.

김재선 J2H 바이오텍 공동 대표는 "C형 간염 치료제 연구 개발은 최근 수년 동안 비약적인 발전을 거듭했지만 여전히 미충족 수요가 존재한다"며 "한국파스퇴르연구소의 HCV entry inhibitor는 이러한 미충족 수요를 채워줄 의미 있는 대안이 될 수 있으며, 또한 HCV protease inhibitor와의 병용을 통해 상승효과가 있을 것으로 기대된다"고 말했다.

(2)

Summary of Intellectual Property

IPK is committed to the acquisition and development of advanced technology platforms. Our innovative inventions and proprietary technologies are protected for exploring commercialization funding opportunities.

In 2016, 39 patent applications were filed locally and internationally. New inventions include a novel biomarker and a screening method for superbacteria. Host factors that are crucial for the replication of influenza virus and novel compounds with anti-influenza activity were also protected. Two PCT applications went into national phase in 2016; one on anti-hepatitis C virus (HCV) molecules and the other one on anti-tuberculosis molecules.

Eight patents were granted in 2016 and these represented issue of patents which include methodology patents on our phenomic screening and target identification technologies along with patents on anti-tuberculosis therapeutics and influenza host factor. IPK is expecting more international and domestic issued patents 2017.

한국파스퇴르연구소는 첨단 기술 플랫폼 개발과 구축에 전념하고 있으며, 이러한 혁신적인 발명과 고유 기술들의 지식재산은 보호되고 있으며 상업화의 기회로 이어지고 있다.

2016년 39개의 국내 및 해외 특허가 출원되었으며, 슈퍼박테리아 치료제 발굴을 위한 새로운 스크리닝 방법과 바이오마커 기술이 특허로 보호되었다. 또한 인플루엔자 바이러스 복제에 중요한 역할을 하는 신규 인간 유전자 및 항인플루엔자 효능을 가진 화합물들도 새로운 특허에 포함된다.

2016년도에 등록된 8개의 특허들은 페노믹 스크리닝 분석 및 타깃 분석 기술을 대표하는 방법론적 특허와 항결핵 치료제, 그리고 새로운 인플루엔자 관여 인간 유전자에 대한 발명들이다. 2017년에는 더 많은 국내 및 해외 특허의 등록을 기대 중이다.

(3)

List of International Symposia, Meetings and Training

No.	Date	Name of Event
1	April 19.16	IPK-GE Collaborative Symposium
2	May 9-13, 2016	HCA training
3	May. 30. 16	French Tech Days
4	September 26..16	HBV Workshop
5	October14..16	KSMCB-IPK Satellite session
6	December 9.16	French Healthcare Day Symposium

(4)

List of Seminar Series

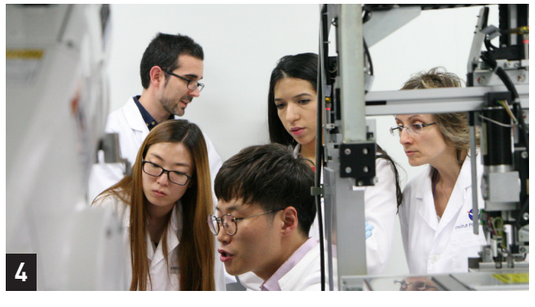
No.	Date	Name	Organization	Talk Title
1	16.1.26	Gabsang Lee	Johns Hopkins School of Medicine	Application of human pluripotent stem cells toward modeling genetic disorders of peripheral nervous system and skeletal muscle lineages
2	16.2.24	Moran Ki	Department of Cancer Control and Policy, GCSP, National Cancer Center	Epidemiology of MERS in the Republic of Korea
3	16.3.9	Seungyoon Nam	Department of Life Sciences, Gachon University & College of Medicine, Gachon University	Systems biology application in gastric cancer therapeutics
4	16.3.10	Noria Segueni	CNRS INEM UMR 7355	The role of IL-1 and TNF/TNFR1 pathways in the host response to Mycobacterium tuberculosis infection
5	16.4.20	Fredrik Sundberg	GE Healthcare LS	Drug Action with Innovative Label-free Technologies
6	16.4.27	Jeung Whan Han	Sungkyunkwan University	S6K1 Phosphorylation of H2B Mediates EZH2 Trimethylation of H3: A Determinant of Early Adipogenesis
7	16.5.4	Sun Kim	SNU (bioinformatics center)	Network-based Cancer Systems Biology
8	16.5.11	Joo Chun Yoon	Group head	Seminar topic: Innate immunity against viral infection
9	16.6.8	SoYeon Kim	Senior Research Scientist	Seminar topic: single molecule imaging
10	16.6.7	Joachim Son Forget	Neuroradiologist	Healthcare systems as today and cognitive sciences of tomorrow
11	16.6.16	Byeonghwa Jeon	Assistant Professor	Antibiotic growth promoters, a double-edged sword of antibiotic resistance and virulence
12	16.6.21	Guillaume Dumas	Research Fellow of Institut Pasteur & Visiting Researcher of Institut Pasteur Korea	Coordination Dynamics from Neurons to People
13	16.6.22	Kwangkook Lee	Assistant Professor	Functional study based on structural insights of Botulinum nerotoxin A complex
14	16.7.20	Stefan Hannus	CSO	Company Presentation Intana Bioscience and siTOOLs Biotech – new tools for target validation and drug discovery
15	16.8.31	Geraldine Wee	Asia Business Development Manager	Eurofins Pharma Discovery Services
16	16.9.8	Meehyein Kim	Principal Researcher	Antiviral Activity of KR-23502 Targeting Nuclear Export of Influenza B Virus Ribonucleoproteins
17	16.9.12	Aya Miura	Regional Marketing Development Manager	CRISPR-Cas9 Technology for Screening: LentiArray CRISPR Libraries
18	16.9.21	You-Hee Cho	Professor	Drosophila infection models to study bacterial pathogens
19	16.9.22	Jonathan M. Gershoni	Professor	Epitope-based vaccines: reconstitution of conformational discontinuous epitopes
20	16.10.10	Thomas Pietschmann	Professor	Clinically approved ion channel inhibitors close gates for hepatitis C virus and open doors for drug repurposing and more affordable therapy
21	16.10.12	Parag Kundu	Senior Research Fellow	Gut microbiome of ageing-host: Friend or foe?
22	16.10.26	Denis Kainov	Group Leader (FIMM-EMBL) / Professor in infectious diseases	Multi-omics studies towards novel modulators of influenza A virus-host interaction
23	16.11.9	Sangdun Choi	Professor	Developing Immunomodulators as Therapeutic Agents
24	16.11.24	Hak-Sung Kim	Professor	Repebody: Designed protein scaffold for therapeutic and biotechnological applications
25	16.12.14	Sang-Sun Yoon	Yonsei University College of Medicine	Host susceptibility to enteric infection is influenced by altered community of gut microbiota

(5)

List of Poster Presentations in 2016

1. Jo Eunji, Quantification of Infectious Hepatitis B Virus by Using an End Point Dilution Assay in Cell Culture, 2016 International HBV meeting, Seoul, South Korea, 22-Sep-2016
2. Yang Jaewon. Poster. Development of a shRNA-based Screening Assay to Identify HBV Restriction Factor, 2016 International HBV meeting, Seoul, South Korea, 22-Sep-2016
3. Song YH, Poster. CD133 confers cancer stem-like cell properties by stabilizing EGFR-AKT signaling in hepatocellular carcinoma, KSMCB, Seoul, Korea, 13-Oct-2016.
4. Kim SH, Poster. Secretome analysis of tumor spheroids for the discovery of HCC-specific biomarkers, KSMCB, Seoul, Korea, 13-Oct-2016.
5. Souheyla Benfrid, Kyu-Ho Paul Park et al. Poster. The dengue virus non-structural protein NS1 is a lipid-binding protein, 12th GERLI international lipidomics meeting 'microbe and host lipids', Toulouse, France, 23-Oct-2016.
6. F. Sonthonnax, B. Besson F. Larrous, J. Chamot-Rooke, R. Grailhe, H. Bourhy.. Poster. Title: Cooperation of Lyssavirus P and M proteins on Jak/Stat and NF-kB pathways regulation. 7th International Symposium on Emerging Viral Diseases. Wuhan, China. October 21
7. G. Dumas, N. Antoniou, S. Kim, R. Matsas, R. Grailhe. Poster. Title: Network Biomarker Extraction with High-Throughput calcium Imaging Microscopy on hiPSC-Derived Human Neurons. RIIP. November 29
8. Anastassia V. Komarova, Alice Meignie, Chantal Combrede, Thibaut Douche, Anna Zhukova, Mariette Matondo-Bouzanda, Benno Schwikowski, Regis Grailhe, and Frederic Tangy. Poster. Title: Measles virus C protein interplay with cellular apoptotic pathways RIIP. November 29
9. Sangchul Lee, Inhee Choi (in collaboration with CRL), Poster. Secretome analysis of tumor spheroids for the discovery of HCC-specific biomarkers, The Korean Society for Molecular and Cellular Biology (KSMCB) 2016, South Korea, 13-Oct-2016
10. Park I-S, Yang G, No J and Kim J. Poster, Development of live cell FRET/BRET screening platform to find an inhibitor of Leishmania donovani topoisomerase I dimer, 2016 KSMCB, Seoul, Korea, 14-Oct-2016.
11. Constantin Radu, Poster, The IPK Sample Management Experience of The NTD Drug Discovery Booster, International Conference of the Korean Society for Molecular and Cellular Biology 2016, Seoul, Korea, Oct.-14-2016
12. Sully Lee. Poster. National-wide Survey on Laboratory Animal Facilities in Korea, 7th Asian Federation of Laboratory Animal Science Congress, Singapore, Singapore, 09-Nov-2016.
13. Jihye Lee, Poster. Acid Phosphatase 2 (ACP2) is necessary for the membrane fusion of influenza virus entry, OPTIONS IX for THE CONTROL OF INFLUENZA (ISIRV), CHICAGO, USA, 08-26-2016.
14. Dongjo Shin, Poster. Double PHD Fingers 2 Promotes the Immune Escape of Influenza Virus by Suppressing Interferon-Production, OPTIONS IX for THE CONTROL OF INFLUENZA (ISIRV), CHICAGO, USA, 08-26-2016.
15. Seo HR, Poster. CD133 attenuates ROS accumulation via a steady increase in the expression of the cystine/glutamate transporter xCT: Consequence on chemoresistance in hepatocellular carcinoma, The American Association for Cancer Research Annual Meeting 2016, nest N. Morial Convention Center, New Orleans, Louisiana, 16-April-2016.
16. Song YH, Poster. Establishment of 3D tumor microenvironment in vitro for Hepatocellular Carcinoma Therapy. The American Association for Cancer Research Annual Meeting 2016, nest N. Morial Convention Center, New Orleans, Louisiana, 17-April-2016.
17. Song YH, Poster. TGF-independent CTGF induction regulates cell adhesion-mediated drug resistance by increasing collagen I in HCC spheroids. The 42th Annual meeting of Korean Cancer association with international cancer Conference. Seoul, South Korea, 16- June-2016.
18. Kim J, Eun H, Zhenzhen Y, Vandenberghe M, Delzescaux T and Grailhe R Poster. Three-dimensional histological imaging of mouse brain using High Content Microscope Analysis and 3D-HAPi, KSBMB International Symposium 2016 Seoul, South Korea, 19-May-2016.
19. Honggun Lee. Poster. Anticipating the Logistics of Infectious & Neglected Diseases: The Sample Management & Automation Perspective, Society for Laboratory Automation and Screening 2016, San Diego, USA, 01/24/2016
20. Kideok Kim. Poster. Expanding the Sample & Data Reporting Paradigm, Society for Laboratory Automation and Screening 2016, San Diego, USA, 01/25/2016.
21. Jihye Lee, Poster. Acid Phosphatase 2 (ACP2) is necessary for the membrane fusion of influenza virus entry, OPTIONS IX for THE CONTROL OF INFLUENZA (ISIRV), CHICAGO, USA, 08-26-2016.
22. Dongjo Shin, Poster. Double PHD Fingers 2 Promotes the Immune Escape of Influenza Virus by Suppressing Interferon-Production, OPTIONS IX for THE CONTROL OF INFLUENZA (ISIRV), CHICAGO, USA, 08-26-2016.

Events & Seminars



1_ **2016.04.19** IPK–GE Symposium / IPK–GE 심포지엄

2_ **2016.04.28** The inaugural meeting of LRIG Korea / LRIG 코리아 창립멤버 미팅

3_ **2016.05** Awarded for the DNDi's Project of the Year by DNDi / DNDi 올해의 프로젝트상 수상

4_ **2016.05.09-13** HCA training / HCA 교육 프로그램

5_ **2016.05.30** French Tech Days – Demo day session / French Tech Days – Demo day 세션

6_ **2016.07.05** Visit of Valerie Pecresse (the chairman of the regional council of Ile–de–France) / 발레리 페크레스(일드프랑스 지역 도지사) 방문

7_ **2016.08.18** Visit of President and CEO of Seoul National University Bundang Hospital / 분당서울대학교병원 병원장 방문

8_ **2016.09.26** HBV Workshop / B형 간염 바이러스 워크숍

9_ **2016.09.28** A signing ceremony with Gyeonggi Provincial Government & 5 Gyeonggi based organizations for the [IPK–Pharma–Biotech Drug Discovery Joint Research Project] / 한국파스퇴르연구소–경기도내 제약기업 신약공동개발연구사업 협약식

10_ **2016.10.11** A signing ceremony with J2H Biotech for an out–licensing agreement for a first–in–class hepatitis C virus (HCV) drug candidate / 한국파스퇴르연구소–J2H바이오텍 C형 간염 치료 후보물질 기술이전 협약식

11_ **2016.10.14** IPK session at 2016 KSMCB / 2016 한국분자세포생물학회 국제 컨퍼런스 – IPK 세션 운영

12_ **2016.10.24** Visit of Chair of Asan Institute for Life Sciences / 서울아산병원 아산생명과학연구원장 방문

13_ **2016.11.25** Visit of Dr. Francoise Barre–Sinoussi (Co–winner of the 2008 Nobel Prize in Physiology or Medicine) / 프랑수아즈 바레–시누시 박사 방문(2008 노벨생리의학상 공동수상자)

14_ **2016.12.09** French Health Care Day Symposium / 프랑스 헬스케어데이 심포지엄

Interviews

Dr. Anastasia KOMAROVA

Visiting Researcher at Dr. Regis Grailhe's lab | Affiliation: IP-France visiting professor

Q1. With 33 institutions in the Network, what made you choose IPK? Why did you choose to work with Dr. Grailhe's team? What did you hope to accomplish at IPK? And did you meet your research goals?

The Institut Pasteur International Network (IPIN), which includes 33 research institutes around the world, is a network of research and expertise aimed at fighting infectious diseases and advancing applied and fundamental research. In order to further add value to the overall Network, the IPIN regional office in Paris focuses on improving regional collaboration among nearby members. There are several key advantages derived from international collaboration among different countries, such as knowledge sharing, information transmission, access to resources and equipment, and higher quality of research. I was given a great opportunity to benefit from the IPIN and developed a collaborative project with Dr. Grailhe's team at the Institut Pasteur Korea.

IPK is recognized as a technology-driven Institut within the IPIN network, in particular with its unique cell-based phenotypic screening technology platform. IPK provides fundamental technologies to bridge basic and applied research complemented by alternative approaches, which are not found in European IPIN Institutes. Among potential collaborators working at IPK, I have identified the Technology Development Platform laboratory headed by the Dr. Regis Grailhe. This collaboration was a unique opportunity in the context of my research activity to study virus host protein-protein interactions in living cells by applying Bioluminescence Resonance Energy Transfer (BRET) screening technique. This technology, which is not developed on our campus in Paris, offers me the possibility to study and better understand the strategy developed by the measles virus in the course of evolution to infect human cells and to achieve efficient viral replication. During my project at the Institut Pasteur Korea, we analysed the interaction of measles virus proteins with the host cell innate immunity as well with the cell death protein network. We were able to quantify these interactions and validate some of my working hypotheses.

Although my working time at IPK was only three months long, it yielded significant scientific results. We accomplished for the first time to detect virus-host protein-protein interactions using BRET in the presence of viral infection. This collaborative work gives a solid base for the future studies. Indeed, we are currently applying for several funding sources to pursue experiments that I started at IPK last year.

Q2. What was the most valuable research experience you had at IPK? And how has this experience furthered your scientific career?

My three-month mission at IPK provided me with an extremely important working experience. Together with Dr. Regis Grailhe team we were able to complete in due time our scientific program that allows for the first time studying virus-host protein-protein interactions in a viral context using BRET technique. But more important, in addition to scientific accomplishments, I would like to emphasise that IPIN provides an exceptional opportunity for researchers to integrate and effectively work within the international scientific community. Finally, my three-month mission at IPK is an extremely important life experience from the intercultural communication point of view and will surely help me develop future scientific collaborations with different laboratories at the Institut Pasteur Korea as well as other institutes in Asia.

Q1. 파스퇴르연구소 네트워크 33개 연구소 중에서 한국파스퇴르연구소를 선택한 이유와 한국파스퇴르연구소의 여러 연구 분야 중 레지스 그레일 박사 연구실(기술개발 플랫폼)을 선택한 이유가 무엇인가요? 한국파스퇴르연구소에서 성취하고자 했던 바는 무엇이었으며, 목표를 이루셨는지 궁금합니다.

전 세계 33개 연구소로 구성된 파스퇴르연구소 국제 네트워크(IPIN, 이하 네트워크)는 전염병 퇴치와 기초 및 응용 연구의 발전을 목표로 하는 전문적인 연구 네트워크입니다. 본부는 프랑스 파리에 위치하고 있으며, 네트워크 회원사 간 협력 강화를 통한 연구 시너지를 창출을 위해 노력하고 있습니다. 이를 위해 다양한 협력 연구를 추진하여 지식 공유, 정보 교류, 연구 자원과 첨단 장비의 효과적 활용을 촉진하고 연구의 질적 향상을 이끌고 있습니다. 저는 파스퇴르 네트워크 간 국제 협력의 일환으로 한국파스퇴르연구소의 레지스 그레일 박사 연구팀과 함께 공동 프로젝트를 진행하는 의미있는 기회를 얻었습니다.

한국파스퇴르연구소는 파스퇴르 네트워크 내에서 기술 중심 연구소로 활약하고 있으며, 특히 연구소의 독특한 세포 기반 표현형 스크리닝 기술 플랫폼의 우수성이 잘 알려져 있습니다. 한국파스퇴르연구소는 기초 연구와 응용 연구를 연계하는 원천기술을 보유하고 있으며, 이러한 기술은 유럽 지역 네트워크에서는 찾아볼 수 없는 한국파스퇴르연구소만의 독자적인 기술입니다. 저는 한국파스퇴르연구소의 레지스 그레일 박사가 이끄는 기술개발 플랫폼 연구팀과 공동연구를 진행했으며, Bioluminescence Resonance Energy Transfer(BRET) 스크리닝 기술을 적용하여 살아있는 세포 내에서 바이러스 숙주의 단백질간 상호작용을 분석하는 특별한 연구 경험을 쌓았습니다. 프랑스 파리에서는 볼 수 없었던 해당 스크리닝 기술을 활용하여 홍역 바이러스가 인간 세포를 감염시키고 효율적인 복제를 진행하기 위해 진화하는 과정을 심층적으로 연구할 수 있었습니다. 또한, 숙주의 선천적 면역체계 및 세포 사멸 단백질과 홍역 바이러스 단백질 간 상호작용을 분석했으며, 이 중 일부를 정량화하여 연구 가설을 부분적으로 검증하는데 성공하였습니다. 비록 3개월이라는 길지 않은 기간이었지만 공동연구를 통해 상당히 의미있는 성과를 이룰 수 있었습니다. 특히, BRET를 사용하여 감염 상태에서 바이러스와 숙주의 단백질간 상호작용을 처음으로 밝혀 내는데 성공했으며, 이를 후속 연구로 연계할 수 있는 탄탄한 기반을 구축하였습니다. 실제로 현재 저희 연구팀은 한국파스퇴르연구소와 시작한 실험을 계속 진행하기 위해 다양한 연구과제에 지원하고 있습니다.

Q2. 한국파스퇴르연구소에서 근무하면서 얻은 가장 소중한 연구 경험은 무엇이었나요? 그리고 그러한 경험이 본인의 과학적 커리어를 어떻게 발전시킨다고 생각하십니까?

한국파스퇴르연구소에서 3개월에 걸친 연구 프로젝트를 수행하며 저는 매우 중요한 연구 경험을 쌓았습니다. 레지스 그레일 박사 연구팀과 함께 BRET 기술을 사용해 바이러스 환경에서 바이러스 및 숙주의 단백질간 상호작용을 연구하는 신규 프로젝트를 계획에 따라 진행 및 완료하였습니다. 하지만 더욱 중요한 점으로 파스퇴르 네트워크가 글로벌 과학 커뮤니티 내에서 연구원들이 효과적으로 교류하고 연구할 수 있는 특별한 기회를 제공한다는 점을 꼽고 싶습니다. 저에게 있어 한국파스퇴르연구소에서 보낸 3개월의 시간은 문화간 커뮤니케이션의 관점에서 매우 중요한 삶의 한 부분이며, 앞으로 파스퇴르 네트워크 내 한국 및 다른 아시아 지역 연구소와 협력하는데 도움이 되리라 확신합니다.

Thibault Rosazza

Visiting Researcher at Leishmania Research Laboratory

Q1. With 33 institutions in the Network, what made you choose IPK? Of all the research areas at IPK, why did you choose to work with Dr. No's team? What did you hope to accomplish at IPK? And did you meet your research goals?

I worked at IPK in the context of an international collaboration between three laboratories of the Pasteur's network (Paris, Seoul and Shanghai). Our goal was to expand and enhance this collaboration directly by transferring technologies, learning and teaching new scientific approach to improve leishmania drug discovery. More precisely, we wanted to develop an in vivo model specific to Leishmania parasite at IPK to test new active compounds. And it's now something that is handle by Animal Facility and Dr. No's team.

Q2. What was the most valuable research experience you had at IPK? And how has this experience furthered your scientific career?

The most valuable research experience that I had at IPK was the possibility to interact directly with the different partners involved in this scientific project. We could discuss and manage it together and this permit us to progress quickly and successfully.
I also appreciated the professionalism of IPK's members and the modernity this Institute.

Q1. 파스퇴르연구소 네트워크 33개 연구소 중에서 한국파스퇴르연구소를 선택한 이유와 한국파스퇴르연구소의 여러 연구 분야 중 노주환 박사 연구실(리슈만편모충연구실)을 선택한 이유는 무엇인가요? 한국파스퇴르연구소에서 성취하고자 했던 바는 무엇이었으며 목표를 이루셨는지 궁금합니다.

저는 파스퇴르연구소 국제네트워크 중 3개 연구실(파리, 서울, 상하이) 간의 국제 협력 프로젝트에 참여하며 이곳에서 근무하게 되었습니다. 해당 프로젝트의 목표는 기술 교류와 새로운 과학적 접근 방법을 배우고 가르침으로써 리슈만편모충증 신약 개발 연구를 위한 직접적인 연구 협력을 확장하고 향상시키는 것이었습니다. 특히, 본연구소에서는 리슈만편모충을 활용한 생체 내 실험모델 개발하여 치료 효능을 가진 화합물을 찾기 위한 연구를 진행했고, 본 연구는 현재 노주환 박사팀과 동물실험실의 협력으로 후속연구가 활발히 진행되고 있습니다.

Q2. 한국파스퇴르연구소에서 근무하면서 얻은 가장 소중한 연구 경험은 무엇이었나요? 그리고 그러한 경험이 본인의 과학적 커리어를 어떻게 발전시킨다고 생각하십니까?

해당 연구 프로젝트에 참여하는 여러 파트너들과 직접 교류할 수 있었던 것이 가장 소중한 연구 경험이었습니다. 프로젝트에 참가한 연구진들과 연구 과제에 대해 함께 논의하며 관리할 수 있었고, 이를 통해 우리는 신속하고 성공적으로 연구 목표를 향해 나아갈 수 있었습니다. 또한 한국파스퇴르연구소 직원들의 전문성과 첨단 연구 환경에 대해 감사하게 생각합니다.

Phuong Hong Nguyen

Ph.D. Student at UST-IPK campus

Q1. Why did you choose UST-IPK campus to get your Ph.D. degree? Is there any reason?

I chose the UST-IPK campus because it has a large selection of research projects, good scientists, active collaborations, as well as an international environment. Additionally, it offered various possibilities for study in my research field of interest, such as Hepatitis, Zika, and Ebola, which are limited at other universities. The wide range of research topics aligns with my career aims.

Q2. What do you hope to accomplish throughout UST-IPK's Ph.D. course?

My goal is to improve my background knowledge and research skills through the course work and lab research activities. Also by graduation, I would like to have a number of high quality publications.

Q3. What is your future plan after UST-IPK Ph.D. course?

Based on experience and knowledge from my study and research, I would like to be a project manager that sets up the research plan and manages the whole processes and progress of the project with researchers. My long-term goals involve setting up a new lab where I can continue to research on my current topic of interest virology.

Q1. UST-IPK 캠퍼스의 박사학위 과정을 선택한 이유가 무엇인가요? 특별한 이유가 있나요?

저는 연구 분야의 다양성, 훌륭한 연구진, 활발한 연구협력, 그리고 국제적인 업무 환경 등을 고려해 UST-IPK 캠퍼스를 선택하게 되었습니다. 특히 UST-IPK 캠퍼스는 저의 관심 연구 분야(간염, 지카, 에볼라 등)에 대한 다양한 연구 기회를 제공한다는 것과 연구 주제들이 박사학위 취득 후 제가 생각하는 진로 방향과 부합한다는 점이 본 캠퍼스를 선택하는데 중요한 요소로 작용했습니다.

Q2. UST-IPK 캠퍼스의 박사학위 과정을 통해 성취하고자 하는 것은 무엇입니까?

학위 과정과 연구실 연구 활동을 통해 저의 경력과 전공 지식, 연구 기술을 향상시켜 졸업 후에도 꾸준히 우수 논문을 게재할 수 있었으면 좋겠습니다.

Q3. 학위 취득 후에는 어떤 계획을 가지고 있나요?

연구원으로서의 경험을 바탕으로 다양한 연구 과제를 기획하고 관리하는 연구 책임자가 되고 싶습니다. 개인적으로 세운 장기적인 목표는 바이러스학 분야에서 현재 흥미를 가지고 있는 연구 주제들에 대한 연구를 계속해 나갈 수 있는 새로운 실험실을 만드는 것입니다.

UST-IPK CAMPUS

Founded in 2004, University of Science and Technology (UST) aims to foster highly qualified R&D professionals in the field of new convergence technology through on-site R&D education. IPK became a member campus in 2014 and is the only non-profit research organization on the 33 member campus roster.

UST-IPK 캠퍼스

과학기술연합대학원대학교(UST)는 지난 2004년, 연구현장 중심 교육으로 신생융합기술 분야의 석 · 박사 인력 양성을 목적으로 개교했습니다. 한국파스퇴르연구소는 2014년 참여 기관으로 선정되었으며, 현재 총 33개 UST 캠퍼스 중 유일한 공익법인 민간연구소입니다.

DONATE TO INSTITUT PASTEUR KOREA AND JOIN US IN PROTECTING THE LIVES OF MILLIONS OF PEOPLE AFFECTED BY INFECTIOUS DISEASES

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